STUDIES THE AMELIORATIVE EFFECT OF NANO - CURCUMIN AGAINST NICOTINE INDUCED COMPLICATIONS OF FEMALE RATS UNDER NORMAL PROTEIN DIETARY CONDITION

Thesis Submitted for the
Degree of Doctor of Philosophy (Science)
of Jadavpur University

Under the Supervision of
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Dedicated to
My Beloved
Parents

JADAVPUR UNIVERSITY

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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled "**Studies the ameliorative effect of nano - curcumin against nicotine induced complications of female rats under normal protein dietary condition**" submitted by Anwesha Samanta, who got her name registered on 20.07.2017 for the award of Ph. D. (Science) degree at Jadavpur University, is absolutely upon her own work under the supervision of Dr. Brajadulal Chattopadhyay, Professor, Department of Physics, Jadavpur University and that neither this thesis nor any part of it has been submitted for either any degree/diploma or any other academic award anywhere before.

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DECLARATION

I do hereby declare that the work embodied in this thesis entitled "**Studies the ameliorative effect of nano-curcumin against nicotine induced complications of female rats under normal protein dietary condition**" which is being submitted for the Degree of Doctor of Philosophy (Science) has been carried out by me in the Biophysics Laboratory, Department of Physics, Jadavpur University, Kolkata. Neither the thesis nor any part thereof has been presented anywhere earlier for any degree/diploma or academic award whatsoever.

Date: 13.02.2023

Kolkata, India

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List of Symbols and Abbreviations

- **Ach:** Acetylcholine
- **ALP:** Alkaline phosphatise
- **ALT:** Alanine aminotransferase
- **AST:** Aspartate aminotransferase
- **CAT:** Catalase
- **CRT:** Chemoradiotherapy
- **CVD:** cardiovascular disease
- **DSBs:** Double-strand breaks
- **EMT:** Epithelial-mesenchymal-transition
- **FMO3:** Flavin-containing monooxygenase 3
- **GGT:** Gamma-glutamyl transferase
- **GR:** Glutathione reductase
- **GXp:** Glutathione Peroxidase
- **H_2O_2:** Hydrogen peroxide
- **HDL:** High density lipoprotein
- **IFN-γ:** Interferon gamma
- **IL-4:** Interleukin-4
- **IL-6:** Interleukin-6
- **LDH:** Lactate Dehydrogenase
- **LDL:** Low density lipoprotein
- **MDA:** Malondialdehyde
- **nAChRs:** Nicotinic acetylcholine receptors
- **NK:** Natural killer
- **NKT:** Natural killer T
- **NNK:** 4-(metylnitrosamino)-1-(3-pyridyl)-1-butanon
- **NNN:** N′-nitrosonornicotine
- **NRT:** Nicotine replacement therapy
- **NTS:** Nucleus tractus solitaries
- **PAH:** Poly cyclic hydrocarbons
- **PFC:** Prefrontal cortex

- **ROS:** Reactive Oxygen Species
- **RT:** Radiotherapy
- **SOD:** Superoxide Dismutase
- **SSBs:** Single strand breaks
- **TC:** Total cholesterol
- **TG:** Triglyceride
- **TNF- α:** Tumour necrosis factor alpha
- **TSNA:** Tobacco-specific N-nitrosamines
- **UGT:** Uridine diphosphate-glucuronosyltransferase
- **VP:** ventral pallidum
- **VTA:** ventral tegmental area
- **µg:** Microgram
- **µL:** Micro-litre
- **µM:** Micro Molar

Research Proceedings

Conferences and Seminars attended:

- Poster presentation on "Nanocurcumin adjudicated genotoxic repair of nicotine induced female rats under normal protein- diet condition." at International Conference on Nanotechnology (ICNT-2018) Brajalalchak, Haldia, Purba Medinipur, 16th – 17th November, 2018.
- Poster presentation on "Rapid synthesis of Bio-inspired Nanoparticles for various biological Applications" at Seminar on Twists and Turns in Physics Research: Special Emphasis on Condensed Matter and Biophysics (TTPR-2017) Jadavpur University, Kolkata-700032, 21st -22nd February

List of Publications

- **Anwesha Samanta**, Krishna Chattopadhyay, Somashree Biswas, Bhola Nath Paul, Brajadulal Chattopadhyay. Promising Efficacy of Nanocurcumin in Comparison against Nicotine- Induced Complications. Journal of Biologically Active Products from Nature. 2022;12(4):366-377.
- **Anwesha Samanta**, Trinath Chowdhury, Brajadulal Chattopadhyay, Krishna Chattopadhyay. Studies on therapeutic efficacy of nanocurcumin against nicotine induced damage of blood cells. International Journal of Pharmaceutical Research and Innovation.2021;14:12-26.
- Krishna Chattopadhyay, **Anwesha Samanta**, Subrata Mukhopadhyay, Brajadulal Chattopadhyay. Potential amelioration of nicotine induced toxicity by Nanocurcumin. Drug Development Research. 2018.
- Samadrita Sengupta, **Anwesha Samanta**, Jayati Bhowal. Effect of different type of coagulating agent on physico-chemical and organoleptic properties of non dairy rasgulla (cheese ball). Journal of Microbiology, Biotechnology and Food Sciences. 2017;6(4):1107-1111.

Chapter: 1

Introduction

1. INTRODUCTION

Nicotine a plant alkaloid predominantly present in tobacco leaves. It is the second most causative agent used as psychoactive substance. An estimated 1.14 billion people consume nicotine daily, mainly in the form of cigarette smoking worldwide. Annually around 7 million deaths occurred associated either directly or indirectly with cigarette smoking (GBD 2019, 2021). During last two decades prevalence of smoking declined significantly among high income countries but it increased in low- and middle-income countries in the same time. For this reason, the multinational companies manufacturing cigarette moved to developing and under developing countries. More interestingly the number of female smokers is increasing steadily throughout the World (Allen et al., 2014).

Female smokers are more susceptible to different pathological changes like colorectal cancer, breast cancer, cardiovascular diseases compared to male smoker (Huxley et al., 2011). Nicotine also affects female sex hormones leading to still birth, premature infants with low birth weight (Brand et al., 2011)

Nicotine showed its toxic effects in the form of bio-chemical changes, immunological disorders, respiratory problems, cellular changes and genetic disorders (Maiti et al., 2015; Banerjee et al., 2010; Marcy and Merrill, 1987) Cigarette smoking alone is the major risk factor for burden of cancer worldwide and responsible for more than one third cancer related deaths (Jung and Yoon, 2022). Nicotine has toxic effects on the vital organs like liver, kidney, lungs and heart with a special emphasis on DNA damage and mitochondrial dysfunction (Banerjee et al., 2010). The risk of adverse effects on kidney was found to be higher with long term nicotine intake (Lee et al., 2021) which enhances renal oxidative stress (Arany et al., 2011) leading to kidney failure (Ramalingam et al., 2019). In 21st century cigarette smoking is one of the most important causes for Cardio vascular diseases around the world. Nicotine intake significantly enhances the risk of severe coronary and

cerebro-vascular diseases like myocardial infarction, cerebral stroke and sudden mortality. Tobacco smoking is also influences the progression and / or aggravation of cardiac failure among individuals with chronic kidney diseases and increases the chances of arterial fibrillation (Benowitz and Burbank, 2016). Regular consumption of nicotine is responsible for increasing the rate of oxidative damage and at the same time reducing the potentiality of antioxidant defence mechanism. Many of the health consequences due to long term inhalation of cigarette smoke may be mediated through its adverse effects on the immune system. Nicotine decreases the levels of certain antibodies and total immunoglobulin (IgG, IgM, IgA and IgD), also helps in increasing IgE antibody in serum. Thus, nicotine showed its ability in changing immunological status leading to various inflammatory diseases (Nouri-Shirazi and Guinet, 2003).

Curcumin is another plant product chiefly found in turmeric has been consumed as a dietary spice and also as an herbal medicinal agent against different health associated problems since more than 1000 years back. Curcumin is a biologically active compound having anti-bacterial properties (Schraufstatter and Bernt, 1994), cholesterol-lowering capacity (Patil and Srinivasan, 1971), antidiabetic (Srinivasan, 1972), anti-inflammatory (Srimal and Dhawan, 1973), and anti-oxidant (Sharma, 1976) activities. The ameliorative effect of curcumin against nicotine induced toxicity has been well documented. Role of curcumin against nicotine induced hepato-toxicity, renal toxicity, cardiac and respiratory diseases, cancer; immunological impairments have been well established.

Despite of its many beneficial effects, a major problem in using curcumin is its low bioavailability. It has been confirmed by various studies that curcumin shows very poor bioavailability. Several works also documented very low or even undetectable concentrations of curcumin in body fluids and extra intestinal tissues. The major reasons may be due to its poor absorption, faster rate of metabolism, molecular instability, and speedy systemic elimination (Anand et al., 2007). Animal experiment studies demonstrated that the

maximum amount of curcumin (≤90%) ingested orally is excreted along with faces (Metzler et al., 2013). Clinical trials of curcumin are restricted and unwarranted due to its above-mentioned properties (Nelson et al., 2017). To overcome these problems, various attempts have been undertaken using modern technology.

Development of nanotechnology is one of the great achievements in the 21st century. The application of nanoparticles in the field of medical sciences is becoming an important area of interest. Nanoparticles have several advantages with better drug loading rate, higher biocompatibility, and targeted delivery, that provides the greater possibility for the effective treatment of different diseases compared with traditional drug molecules. Nanomaterials, with a large surface area, improved the solubility of indissolvable drugs with high absorption rate, reduces the total drug dosage. This may reduce the adverse side effects of the traditional molecules (Jiabo, 2022).

Nanocurcumin showed protective effect against several toxicities like lead induced toxicity acrylamide induced liver damage, arsenic-induced immune dysfunction, excessive copper induced reproductive toxicity, aluminium induced toxicity (Flora et al., 2013; Atia et al., 2022; Sarawi et al., 2022; Alghriany et al., 2022). But such role of nanocurcumin against nicotine induced toxicity has not so far been explored. The present work was designed to study the ameliorative effects of nanocurcumin against nicotine induced under normal protein diet condition. In my study nanoparticles of curcumin was prepared by ultra-sonication and characterised by FE-SEM, UV-Visible spectrophotometer, FTIR and XRD. This prepared nanocurcumin used against nicotine induced female albino rats under the dose of 4mg/kg body weight. Various parameters of different groups of animas were analysed like SGOT, SGPT, ACP, ALP, Urea, creatinine, MDA, SOD, Catalase, GPX, GSH, IL-4, IL-6, IFN-γ, TNF-α, BCL-2, BAX, Estrogen, Progesterone. The DNA damage study and the DNA content also checked in my study.

1.1. AIMS AND OBJECTIVES

Tobacco use has reached the proportion of a global epidemic. In all tobacco products nicotine present in a substantial quantity and which has been internationally recognized as a drug of addiction. It is a major public health problem. Curcumin is a natural product used against nicotine toxicity but some of its drawback limits its use. Nanoparticles have been proposed as nano-drug for the oral administration of poorly bio-available molecules.

So, the aims and objectives of my study are

1. Preparation and characterization of nano-curcumin to increase its bio-availability and bio-distribution.
2. Use of nano-curcumin and its ameliorative action against various nicotine-induced physicochemical stresses (hepatotoxicity, oxidative, inflammatory and genotoxicity) under normal protein diet condition.
3. To compare the ameliorative action of nanocurcumin against various nicotine-induced physicochemical stresses to that of native curcumin.

Chapter: 2

Literature Review

2. LITERATURE REVIEW

Nicotine is a natural nitrogenous alkaloid predominantly present in tobacco plants and also in eggplants, tomatoes, green pepper and potatoes. All these plants belong to the family nightshade. The quantity of nicotine varies from plant species to species with the highest amount in tobacco plants. Nicotine is a highly addictive stimulant and is taken mainly by cigarette smoking and with other tobacco products like beedi, hookah, cigar, and kretak. Nicotine intake is highly injurious to human health. The tobacco epidemic is one of the greatest public health threats that the world has faced ever. More than 6.8 million deaths were recorded annually among active smokers and around 1.2 million deaths among passive smokers (Global Burden of Disease, 2019). According to the World Health Organization, 22.3% of the global population used tobacco which includes 36.7% of the world's male and 7.8% of the female population. More than 80% of tobacco users (1.3 billion) live in low and middle-income countries with the highest burden of tobacco-associated mortality and morbidity (WHO, 2020).

2.1. Overview of Nicotine

2.1.1. Chemistry of Nicotine

Nicotine is a dinitrogenous alkaloid present in leaves of the tobacco plants with concentrations of 3% in *Nicotiana tabacum* and 14% in *Nicotiana rustica.* The IUPAC name of nicotine is 3-(1-methyl-2-pyrrolidinyl) pyridine (Figure 2.1). It has two nitrogen-containing heterocycle rings, pyridine, and pyrrolidine with an asymmetric carbon. It exists in two configurations (S)- nicotine and (R)-nicotine. In tobacco, the proportion of (S)-nicotine is higher than (R)-nicotine. The chemical formula of nicotine is C10H14N2 with molecular weight 162.234 g.mol-1, the melting point is -79°C, the boiling point is 247°C, rotatory index (S) is aD = -168 at 20° C, density=1,010, the refractive index is n=1,530. Nicotine is a hygroscopic, colourless yellow-brown, oily liquid that is readily

soluble in alcohol, light petroleum, or ether. It is soluble in water in its neutral amine base form at 60°C to 210°C (ACS, 2018; Benowitz et al., 2009).

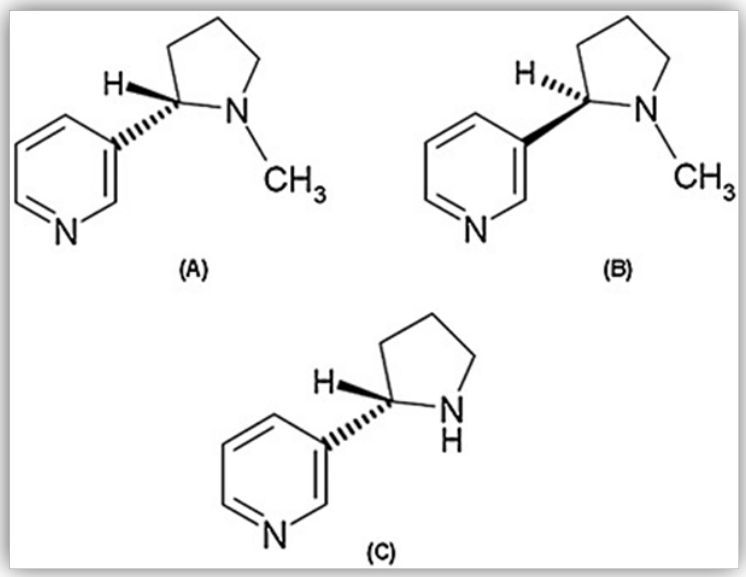

Figure 2.1: The Chemical structure of (A). (S)- (−)-nicotine, (B). (R)- (+)-nicotine (C). (S) (−)-nornicotine

2.1.2. Absorption of nicotine

Nicotine is a weak base (pH-8.0). In acidic environment, it persists in ionized state which can cross the biological barriers very poorly. Most of the flue-cured tobacco is acidic (pH-5.5-6.0), so a little amount of nicotine absorbed through buccal mucosa. Air-cured tobacco products are alkaline (pH-6.5 or higher) in which nicotine persists in nonionized form, which can readily be absorbed through the mouth cavity (Hegde et al., 2017). After inhalation of tobacco smoke nicotine reaches the alveoli of the lungs. The larger surface area of the alveoli and suitable pH promote the quick absorption of nicotine. The concentration of nicotine in the blood increases immediately following a puff and reaches the brain within 10-20 seconds which is faster than intravenous administration (Rostami et al., 2022). Nicotine is also absorbed through oral mucosa (Benowitz et al., 2009) by chewing gum, and snuff. The most frequent route of nicotine intake is inhalation due to tobacco smoking. Nicotine some time absorbed through the skin due to some occupational risks of handling nicotine (green tobacco sickness) (Weizenecker and Deal, 1970). The dermal

release rate of nicotine depends upon the permeability of the skin, diffusion rate through the polymer matrix, and rate of passage through a membrane. Dermal nicotine release and concentration in plasma vary due to different transdermal systems (Fant et al., 2000). Nicotine gum, nasal spray, transdermal patch, lozenges, inhalers, and sublingual tablets are used as nicotine replacement therapy (NRT). NRTs are responsible for slower rate of nicotine absorption through cell membrane. The blood level of nicotine increases gradually by the use of NRTs than smoking (Wadgave et al., 2016).

2.1.3. Distribution and accumulation of nicotine

After entering into the bloodstream (pH 7.4) about 69% of the nicotine persists in ionized and 31% in nonionized form but its affinity with plasma proteins is low (5%) (Catassi et al., 2008). By examining human autopsy materials, the highest accumulation of nicotine was recorded in the kidney, liver, lung, and spleen, and the lowest in adipose tissue. The concentration of nicotine in skeletal muscle is nearer to that in whole blood (Aoki et al., 2020). The affinity of nicotine binding with neural tissues was found to be high among smokers than non-smokers, due to presence of a higher number of nicotinic cholinergic receptors (Breese et al., 1997). Significant accumulation of nicotine was also noticed in gastric juice and saliva (Lindell et al., 1996) due to ion-trapping of nicotine. Nicotine also accumulates in breast milk with a milk/plasma ratio of 2.9 (Chinnala and Konda, 2017). Nicotine can pass through the placental barrier and enters into the foetal serum and amnionic fluid (Suter and Aagaard, 2020). The rate of nicotine accumulation in different organs and its resultant pharmacological effects are depending upon the route and the rate of dosing. Nicotine rapidly reaches the pulmonary venous circulation following cigarette smoking and is quickly delivered to the brain via the left ventricle and systemic arterial circulation. It takes only 10–20 sec for reaching the brain following a puff of a cigarette. Following smoking a cigarette, concentration of nicotine in arterial blood may be high up to 100 ng/ ml with a range between 20 and 60 ng/ ml (Henningfield and Keenan, 1993; Gourlay and Benowitz, 1997; Rose et al., 1999; Lunell et al., 2000). The rapid delivery of nicotine within a small

interval by smoking or intravenous injection results in high levels of nicotine in the central nervous system. It helps for determining the dose of nicotine required for any pharmacological outcome among smokers and in experimental animals.

2.1.4. Metabolism of Nicotine and role of different enzymes

Nicotine metabolism takes place in the liver. It metabolized to six primary metabolites. 70– 80% of nicotine is converted to cotinine in humans. This reaction occurs in two steps; firstly, nicotine is converted to nicotine-Δ1′ (5′)-iminium ion and equilibrium with 5′-hydroxynicotine. This reaction is catalysed by CYP2A6. In the second step, the iminium ion is oxidized to cotinine by cytoplasmic aldehyde oxidase and P4502A6 (Murphy, 2021).

About 4–7% of nicotine metabolized to another metabolite Nicotine N′-oxide (Benowitz et al., 1994) mediated by a flavin-containing monooxygenase 3 (FMO3), which results in the formation of both the di-astronomers, the 1′-(R)-2′-(S)-cis and 1′-(S)-2′-(S)-trans-isomers in animals (Perez-Paramo et al., 2019). Nicotine N′-oxide remains unmetabolized, except it may reduce back to nicotine in the intestine and lead to the recycling of nicotine. There are two nonoxidative pathways in nicotine metabolism: methylation of nicotine-to-nicotine isomethonium ion (also called N -methylnicotinium ion) and glucuronidation of about 3-5% of nicotine to N-quaternary glucuronide (Benowitz et al., 1994) in the presence of uridine diphosphate-glucuronosyltransferase (UGT) enzyme(s). Another important but minor pathway is oxidative N-demethylation responsible for the production of nornicotine from nicotine. Hecht et al. (2000) described a new cytochrome P450-mediated metabolic pathway for nicotine metabolism. Here, 2′-Hydroxylation of nicotine leading to production of 4-(methylamino)-1-(3-pyridyl)-1-butanone with an intermediate form of 2′-hydroxynicotine. 2′-Hydroxynicotine also yields nicotine-Δ1′ (2′)-iminium ion. 4-(methylamino)-1-(3-pyridyl)-1-butanone is further metabolized to 4-oxo-4-(3-pyridyl) butanoic acid and 4- hydroxy-4-(3-pyridyl) butanoic acid. This pathway is important, 4-(methylamino)-1-(3-pyridyl)-1-butanone can be converted to carcinogenic

NNK. But such endogenous NNK production from nicotine metabolism is yet to be detected in humans or rats (Hecht et al., 1999). Cotinine again metabolizes to six primary metabolites: 3′-hydroxycotinine (Tanner et al., 2015), 5′-hydroxycotinine (Tan et al., 2021), cotinine N-oxide, cotinine methonium ion, cotinine glucuronide, and norcotinine. The main nicotine metabolite detected in smokers' urine is 3′-Hydroxycotinine. Different biochemical reactions of nicotine metabolism have been demonstrated in Figure 2.2.

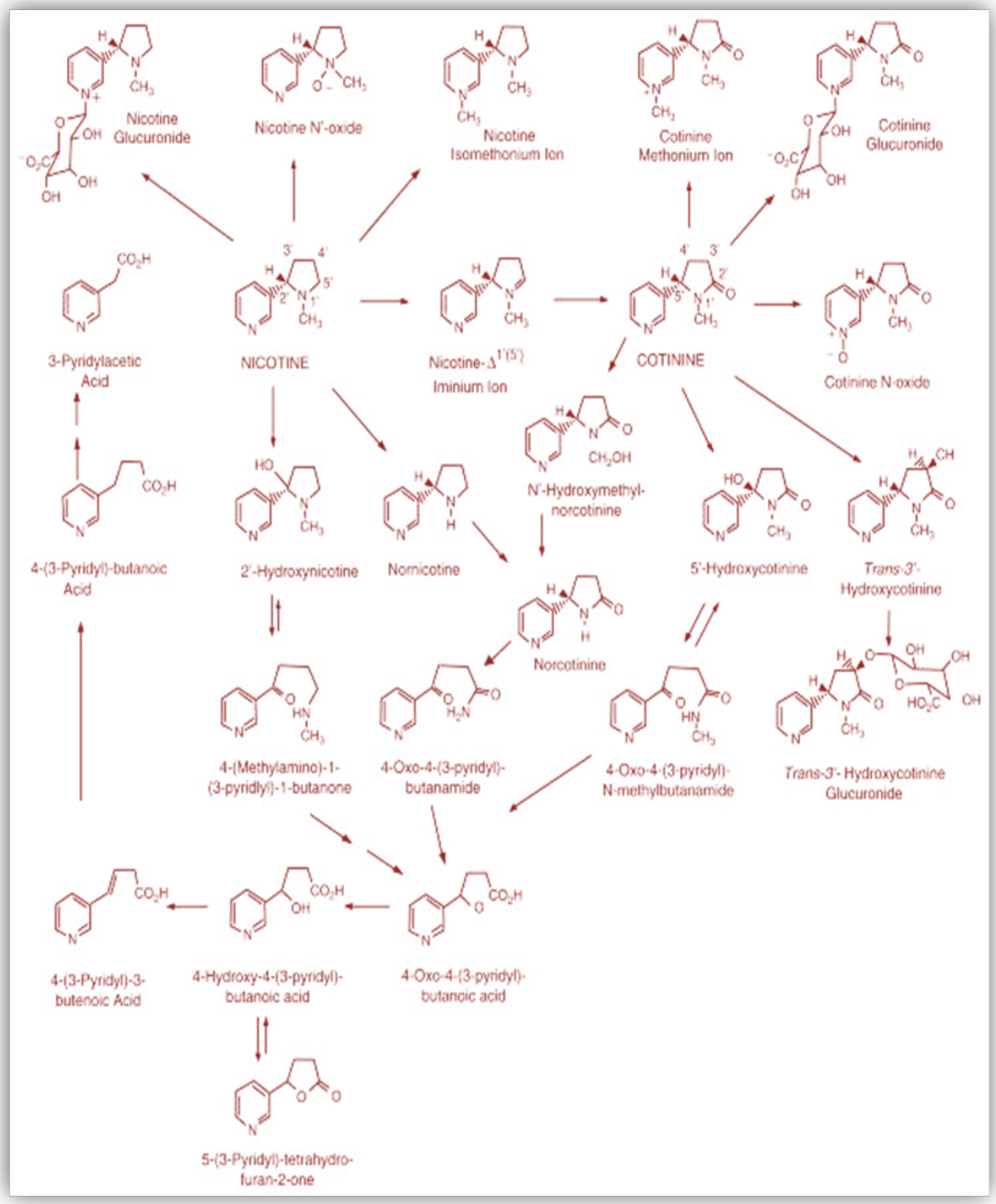

Figure 2.2: Bio-chemical pathways for nicotine metabolism

2.1.5. Patho-physiology of nicotine

Nicotine is the psychoactive agent present in tobacco. It functions as an agonist at nicotinic acetylcholine receptors (nAChRs), localized throughout the brain and peripheral nervous system (Wittenberg et al., 2020). nAChRs are pentameric ionic channels, made up of α2–α7 and β2–β4 subunits in different combinations. Acetylcholine (ACh) acts as an endogenous ligand (Boulter et al., 1987; Couturier et al., 1990; Picciotto et al., 2008). nAChR undergoes conformational changes by binding with nicotine and leading to the opening of internal pores that allow an influx of sodium and calcium ions (Changeux, 2018). Activated nAChR produces action potential firing at postsynaptic membranes and downstream modulation of expression of a gene by a calcium-mediated second messenger system (McKay et al., 2007). The presynaptic membrane contains nAChRs and modulates neurotransmitter release (Tomàs et al., 2017). Nicotine binding affinity and efficacy of nAChRs varies depending upon different combinations of α and β-subunits. Expression of nAChRs also depends on the localization in the brain and neural cell type (Wooltorton et al., 2003; Gipson and Fowler, 2020; Hamouda et al., 2021). For instance, a faster rate of desensitization of nicotine was recorded by β2-containing nAChRs than that by α7-containing nAChRs (Wooltorton et al., 2003). Localization in the brain and the type of nAChR plays a crucial role in mediating the various degrees of nicotine effects (Jensen et al., 2020).

2.1.6. Neurocircuitry of nicotine addiction

Nicotine showed both rewarding effects (‘buzz’ or ‘high’) as well as adverse effects (nausea and dizziness), depending upon the dose and associated factors like inter-individual sensitivity and tolerance (Carstens et al., 2022). The addictive properties of nicotine are expressed by the integration of divergent signals from different regions of the brain that lead to reward and aversion (Figure 2.3). Among initial smokers’ nicotine exhibits both reinforcing as well as aversive effects, those together showed a similar expression of continuous use. When individuals are habituated to frequent smoking, nicotine-induced

pharmacodynamic changes occur in brain circuits. That leads to reduced sensitivity of the drug-induced aversive effects. Nicotine is a strong reinforcer leading to conditional secondary effects related to drug-using experiences. It may enhance the affinity of more drug intake.

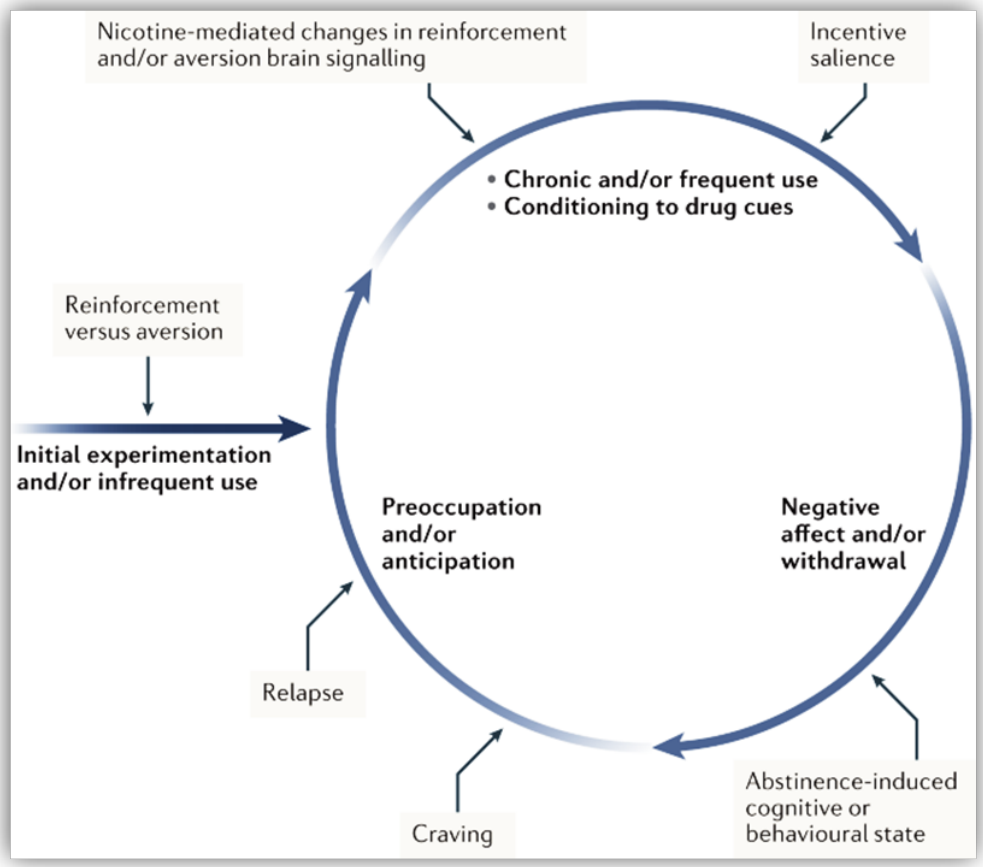

Figure 2.3: Cycle of tobacco/nicotine use (Source: Le Foll et al., 2022)

When such individuals enter into a phase of abstinence, nicotine withdrawal symptoms like irritability, agitation, deficits of memory, absent-minded, and anxiety are experienced. Such negative emotional symptoms lead to an increased urge of retaking nicotine (Le Foll et al., 2022). The pleasurable effect of nicotine is accredited through the mesolimbic routs made up of dopaminergic nerve cells situated at the ventral tegmental area (VTA) and prefrontal cortex (D'Souza et al., 2011) (Figure 2.4). Various types of nAChR on glutamatergic, dopaminergic, and GABAergic neurons are expressed by VTA (Mameli- Engvall et al., 2006; Picciotto et al., 2012). By the administration of nicotine, the level of dopamine is enhanced by raising the dopaminergic

neurons. This may involve in rewarding effect and is mainly controlled by the action of nicotine on α4 and β2-riched nAChRs (Wills and Kenny, 2021).

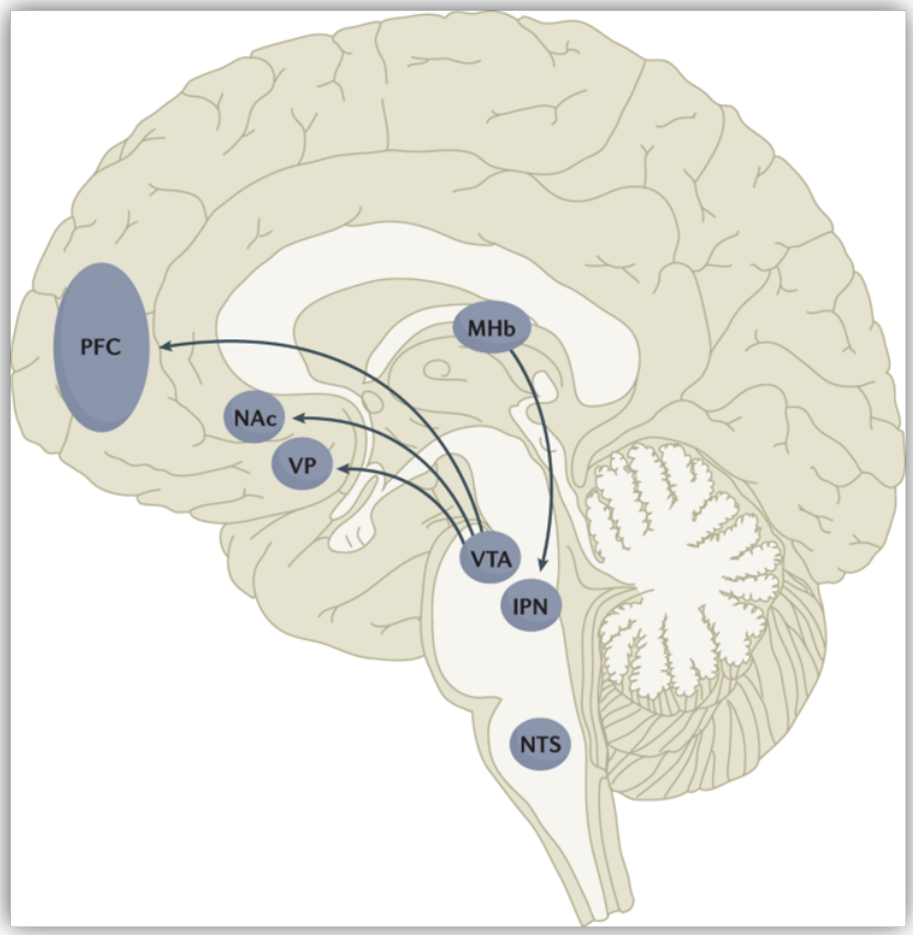

Figure 2.4: Brain regions involved in nicotine addiction

(Source: Le Foll et al., 2022)

On the contrary, the undesirable effects mediated by nicotine limiting drug intake and alleviation of withdrawal symptoms associated with fasciculus retroflexus projection. Other areas of the brain: the prefrontal cortex (PFC), nucleus tractus solitaries (NTS), ventral pallidum (VP), and insula are also found to be interconnected with various aspects of nicotine dependence. These regions of the brain are directly or indirectly associated and control drug-seeking and drug-taking behaviours. In animal models nicotine withdrawal may be measured by observing several physical symptoms like scratching, shaking, head nods and affective signs by noticing augmented anxiety-related behaviours (Salas et al., 2004; Salas et al., 2009).

2.1.7. Harmful effects of nicotine

Nicotine is a unique active component of tobacco that affects almost all organ systems by the activation and binding with nicotinic-acetylcholine (nACh) receptors distributed in various regions of the brain. Nicotine reduces RBC counts and haemoglobin concentration in the blood due to which the body loses its ability for carrying oxygen to several organs in rats (Banerjee et al., 2010). Nicotine exposure affects cardiovascular, neurological, neuromuscular, respiratory, excretory, immunological, and gastrointestinal systems. Animal model studies showed nicotine's capability to enhance existing tissue injury and several diseases like cancer, cardiovascular diseases, stroke, pancreatitis, alteration of liver enzymes, peptic ulcer, renal injury, reproductive and developmental abnormalities (Sanner and Grinsrud, 2015; Benowitz Burbank, 2016; Jena et al., 2016; Lai et al., 2017; Wannamethee and Shaper, 2010; Li et al., 2014; Zheng et al., 2020; Laldinsangi, 2022).

2.1.7.1. Effect of nicotine on liver

Nicotine showed adverse effects on the liver both directly as well as indirectly. By direct effects, it increases oxidative stress that activates stellate cells, leading to fibrosis development occurs (El-Zayadi, 2006). Pro-inflammatory cytokines that are related to liver cell injury are induced by nicotine (Zhang et al., 2022). Previous studies showed that patients suffering from chronic hepatitis C with a habit of smoking have an increased level of fibrosis score and histological activity index leading to HBV-related cirrhosis (Tsochatzis et al., 2009).

Secondary polycythaemia is an indirect toxic effect of smoking. Nicotine is responsible for increased level of carboxyhaemoglobin thus reducing the oxygen-carrying capacity (Malenica et al., 2017). It increases the production of erythropoietin and resulting in increased red cell mass. Increased red cell destruction is responsible for rising catabolic iron and erythropoietin. It enhances the iron absorption from the intestines (Bhoopalan et al., 2020). Macrophages scavenged the iron and finally accumulate in liver cells which

promote oxidative stress. Increased oxidative stress is responsible for liver injury (Galaris et al., 2019). Histopathological changes like mononuclear cell infiltration, hyperchromatic nucleus, and enlarged sinusoids were noticed in the liver of nicotine-induced rats (Singh and Singh, 2019; Iranloye and Bolarinwa, 2009; Marzouk et al., 2022; Amer and Mahmoud, 2014; Syed and Shangloo, 2020).

Nicotine also affects the synthesis of different metabolic enzymes in the liver. Cigarette smoking was significantly found to be associated with increased levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) and was inversely associated with increased aspartate aminotransferase (AST) (Wannamethee and Shaper, 2010). Increased levels of alanine aminotransferase (ALT) following nicotine intake among HCV-positive patients was also been reported (Rutledge and Asgharpour, 2020; Wang et al., 2002). An elevated level of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were reported among smokers with reduced total bilirubin compared to non-smokers (Abdul-Razaq and Ahmed, 2013; Khaitan et al., 2019; Setyawati and Anggraeni, 2018; Alsalhen and Abdalsalam, 2014). Previous studies demonstrated that nicotine causes a significant reduction of total protein (Jain and Jaimes, 2013; Alsalhen and Abdalsalam, 2014).

2.1.7.2. Effect of nicotine on kidney

Previous studies reported that nicotine develops chronic kidney diseases, especially those who are suffering from diabetes, hypertension, polycystic kidney disease, and post-kidney transplantation. Nicotine activates non-neural nicotinic acetylcholine receptors (nAChRs). Several nAChR subunits are expressed in the kidney but the α7-nAchR subunit is a special one; it is over-expressed in the proximal and distal tubes of kidney and acts as a mediator for the progression of renal disease. Nicotine increases the formation of reactive oxygen species and activates the pro-fibrotic pathway thus progress acute kidney injury, diabetes, acute nephritis, and subtotal nephrectomy. Nicotine

also reduces the glomerular filtration rate and renal plasma flow and increases blood pressure. In this way, nicotine induces the development of chronic kidney diseases (Jain and Jaines, 2013). Alteration of renal function was recorded by several workers. Increased level of blood urea nitrogen and plasma creatinine was reported (Salahshoor et al., 2019; Halimi et al., 2000; Begum et al., 2018) among smokers. Histopathological changes in the kidney showed some vacuolated tubular cells, dilated tubules, and intraluminal casts in some tubules (Singh and Singh, 2019; Menshawy et al., 2019; Chen et al., 2019; Salahshoor et al., 2019). Nicotine also induces renal injury causes a decrease in total protein (Yacoub et al., 2010).

2.1.7.3. Effect of nicotine on cardiovascular diseases & lipid profile

Nicotine enhances the risk of cardiovascular diseases (CVD). About 140,000 deaths occur annually due to CVD by cigarette smoking. The past report showed that in United States 33 % of deaths from CVD and 20 % from ischaemic heart disease were due to smoking. Acute coronary and cerebrovascular diseases like myocardial infarction, stroke, and sudden death are increasingly influenced by smoking. Various cardiovascular effects including angina pectoris, intermittent claudication, vasospastic angina, and angioplasty of coronary and peripheral arteries are aggravated by smoking (CDC, 2010).

Higher levels of triglycerides (TG), total cholesterol (TC), and LDL- cholesterol in contrast to decreased HDL-cholesterol were reported among tobacco smokers compared to non-smokers (Sinha et al., 1995; Nath et al., 2022; Szkup et al., 2018; Hasan et al., 2022, Singh et al., 2016, Rao and Subash, 2013; Gheni and Nanakali, 2020). A significantly elevated level of VLDL-cholesterol was noticed by Singh et al. (2016).

2.1.7.4. Nicotine and cancer

Tobacco products are responsible for cancer especially nicotine, polycyclic hydrocarbons (PAH), and tobacco-specific N-nitrosamines (TSNA). Nicotine

acetylcholine receptors (nAChRs) are activated by nicotine and nicotine binds with these receptors (Yershova et al., 2016). After oral administration of nicotine, two carcinogenic compounds NNN (N′-nitrosonornicotine) and NNK (4-(metylnitrosamino)-1-(3-pyridyl)-1-butanon) are formed (Sanner et al., 2015). Increased cell proliferation and cell survival are happened by the interaction between nicotine and nAChRs (Dasgupta et al., 2009). Epithelial-mesenchymal-transition (EMT) that is related to the acquisition of malignant phenotype was observed in nicotine-exposed cells (VU et al., 2016). Nicotine also plays an important role in the inhibition of anticancer immune response. Previous studies reported that dendrite cells that are responsible for anticancer immunosurveillance are badly affected by nicotine. It reduces the response of radiotherapy (RT) or chemoradiotherapy (CRT) in mice (Sanner and Grimsrud, 2015).

2.1.7.5. Effect of nicotine on Antioxidants Level

Nicotine induces the rate of formation of Reactive Oxygen Species (ROS). These are free unstable radicals and have the capability to interact in several biological systems, leading to oxidative stress (Gandhi et al., 2009). Antioxidants protect the biological system from the adverse effects of such free radicals by scavenging them. Nicotine is a source of oxidants with the capacity of destroying the antioxidant defence system (Chundru and Ram, 2013). The enzyme Lactate Dehydrogenase (LDH) is present in many organs and tissues. The blood level of LDH increases rapidly with increased oxidative stress in the body (Chernecky and Berger, 2013). The high blood level of LDH is an indicator of acute or chronic cell damage like heart attack, stroke, blood flow deficiency, hepatitis, and tissue death (Masella et al., 2005). Catalase (CAT) is another antioxidant enzyme present in all organisms, that dissociates hydrogen peroxide into water and oxygen. Catalase is produced and located in Hydrogen peroxide (H_2O_2) producing cellular environments like peroxisomes and mitochondria (Chelikhani et al., 2004; Yang et al., 2014). Superoxide Dismutase (SOD) is another important antioxidative enzyme that catalyses the transformation of the superoxides to oxygen and hydrogen peroxide thus

playing a crucial role in the antioxidant defence mechanism at the cellular level (Maier and Chan, 2002). The action of SOD varies and maintains a very low concentration of superoxides at the tissue level (Cases et al., 2017). Glutathione Peroxidase (GXp) is an enzyme of the peroxidase family that protects from oxidative damage (Yang et al., 2014). GPx reduces lipid hydroperoxides to their corresponding alcohols and also reduces hydrogen peroxide to water: $2GSH+H2O \rightarrow GS-GS+2H2O$ (Bhabak and Mugesh, 2010).

The levels of superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx), and glutathione reductase (GR) were significantly decreased among nicotine-treated albino rats (Oyeyipo et al., 2014; Thirumalai et al., 2010). A similar observation was also reported by Aspera-Werz et al., 2018; Raddam et al., 2017; among cigarette smokers. In contrast decreased levels of SOD, Catalase, and increased levels of GPx were among tobacco smokers and chewers (Agarwal, 2019). Nicotine-induced elevated levels of SOD, and CAT declined GPx activity in cell culture were noticed by Delijewski et al. (2014). Nicotine-induced free radicals, damages lipids and produce malondialdehyde (MDA) by peroxidation. The MDA level is a marker of lipid peroxidation. A significantly higher level of MDA was measured in nicotine-induced rat granulose cells (Sezer et al., 2020). A similar observation was also reported in human primary endometrial cells (Khademi et al, 2019), in the liver and erythrocytes of rats (Ben Saad et al., 2018), in murine plasma (Chattopadhyay and Chattopadhyay, 2008).

2.1.7.6. Genotoxic effect of nicotine

Nicotine provokes DNA damage in various forms such as single-strand breaks (SSBs) (Moktar et al., 2009) and may be double-strand breaks (DSBs) (Ishida et al., 2014). SSBs are the initial DNA damage and are the biomarker of nicotine exposure (Moktar et al., 2009). DSBs are biologically important as they may be responsible for chromosome translocation leading to cancer (Cannan et al., 2016).

The comet assay is a standard method for genotoxicity testing (Cordelli et al., 2021). Nicotine enhances the formation of micronucleus in human gingival fibroblasts indicating DNA damage (Argentin and Cicchetti, 2004). Nicotine-induced DNA damage was assessed by the comet assay in human leucocytes (Sobkowiak et al., 2014), human neuroblastoma cells (Dalberto et al., 2020), human buccal cells (Mohanapriya et al., 2021), and in-vitro culture of peripheral rat blood (Sudheer et al., 2007). A higher rate of DNA damage was demonstrated among female smokers than male smokers (Soylemez et al., 2011). DNA damage was also estimated by micronucleus assay in different types of cells. A higher frequency of micronuclei formation was reported among smokers than non-smokers (Smart et al., 2019; Mohammed et al., 2020; Bansal et al., 2012) and in exfoliated buccal cells of tobacco users (Gopal and Padma, 2018).

2.1.8. Nicotine induced regulation of inflammatory cytokines, Pro-apoptotic markers and female hormones

Tobacco smoking may influence the rate of production of different cytokines associated with several inflammatory and autoimmune diseases (Sokolowska et al., 2019; Durazzo et al., 2014; Shein et al., 2019; Bergstrom, 2004). Nicotine induces inflammation by increasing the production of pro-inflammatory cytokines. It enhances the production of pro-apoptotic protein and inhibits the production of anti-apoptotic protein and thus influences the inflammation of the body (Zhang et al., 2022). Nicotine also alters the hormonal level and it is responsible for abortion during pregnancy (Adeyemi et al., 2018).

2.1.8.1. Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine (Figure 2.5) that affects the immune system and also operates many biological and physiological activities like cell growth regulation, gene activation, proliferation, and cellular differentiation. It is synthesized in various types of cells such as monocytes, fibroblasts, endothelial cells, macrophages, T and B lymphocytes, mast cells, and granulocytes. It regulates the production of different acute-phase proteins in the liver. The

most important role of IL6 is the differentiation of B-cells into plasma cells producing antibodies (Tanaka et al., 2014). The production of IL6 is influenced by nicotine. IL-6 expression is unregulated by nicotine through AP-1 and STAT-3-activated pathways in human endothelial cells (Ung et al., 2019). A higher level of IL-6 was detected among current smokers than that of non-smokers (Carpagnano, 2003; Jamil et al., 2017; Al-tameemi et al., 2022).

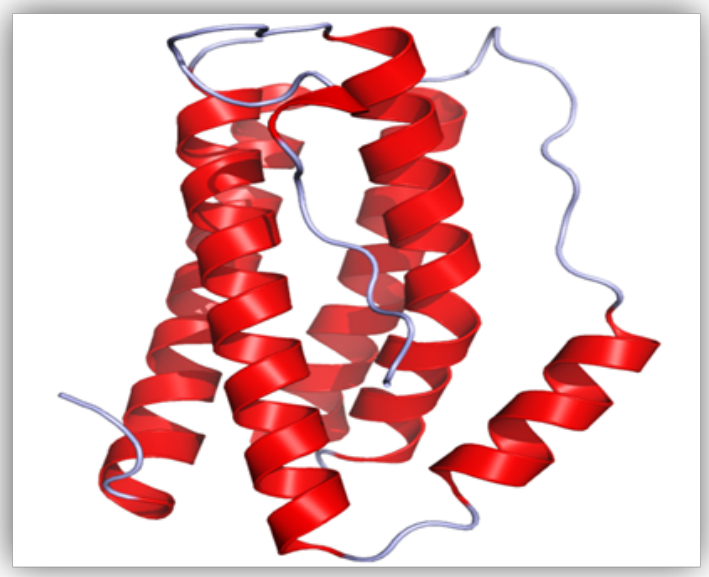

Figure 2.5: Crystallographic structure of IL-6

2.1.8.2. Tumour Necrosis Factor- α (TNF- α)

TNF- α is a pro-inflammatory cytokine (Figure 2.6) that plays an important role in inflammatory response both locally as well as in circulation. Expression of vascular endothelial cells is triggered by it and also enhances the leukocyte adhesion molecules which stimulate the infiltration of immune cells. The crucial role of TNF- α is in early response against different microbial infections by influencing the lymphocyte infiltration to the infection site (Cooper et al., 2009; Hartel et al., 2005). Serum level of TNF- α was found to be higher among smokers than the non-smoker control (Petrescu et al., 2010; Olsson et al., 2018). In contrast decreased level of TNF- α was noticed in nicotine treated group of rats (Kizildag et al., 2021). No such variation of TNF- α was also found in the serum of smokers (Wang et al., 2022).

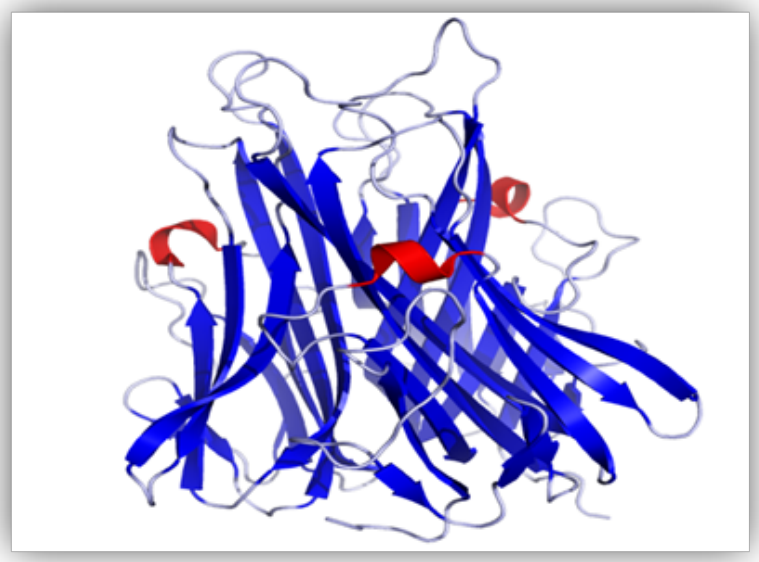

Figure 2.6: Crystallographic structure of TNF- α

2.1.8.3. Interleukin-4 (IL-4)

Interleukin (IL)-4 (Figure 2.7) is the most important cytokine controlling allergic inflammation. IL-4 is also involved in unfolding of many biological events like the way of T cells responds dynamically to changing environment. It is the signature cytokines along with IL-13 for type II inflammatory response against any invading microbe or allergen. Basophils, eosinophils, CD4 T cells, mast cells, and NK T cells are the source of IL-4 (Junttila, 2018). The genomic locus of IL-4 is situated on chromosome 5 of humans and chromosome 11 of mice (Zhu, 2015; Ansel et al., 2006). Production of IL-4 is dependent on calcineurin (Guo et al., 2008). Nicotine-induced enhanced production of IL-4 was observed in rheumatoid arthritis patients (Wu et al., 2018). Similarly higher level of IL-4 was recorded among smokers than among non-smokers (Abed et al., 2020; Elisia et al., 2020; Byron et al., 1994; Merghani et al., 2012). No difference in serum IL-4 levels was noticed among never and ever smokers (Olsson et al., 2018).

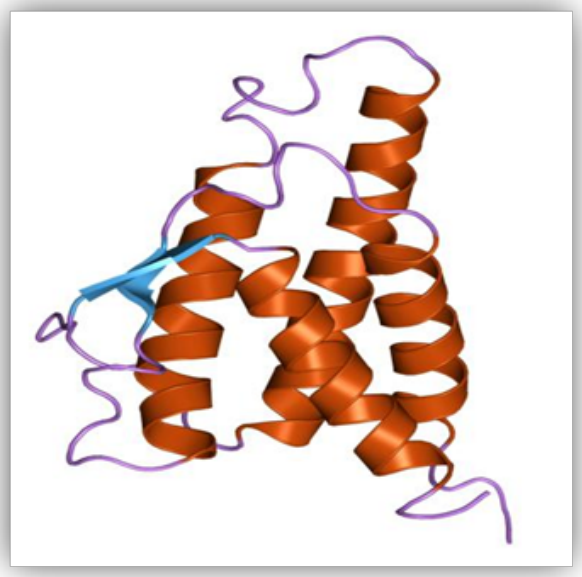

Figure 2.7: Crystallographic structure of IL-4

2.1.8.4. Interferon- γ (IFN-γ)

Interferon-γ (IFN-γ) is the only member that belongs to the type II interferon family. It is a protein consisting of two polypeptide chains arranged in an antiparallel fashion (Figure 2.8) is encoded by the IFNG gene (Zaidi and Merlino, 2011). Three different types of IFN-γ are present in human blood with different molecular weights: one is active free form and the other two are mature IFN-γ molecules (Alspach et al., 2018; Lilkova et al., 2019). It is a pleiotropic cytokine with antiviral, antitumor, and immunomodulatory activities. It coordinates both innate and adaptive immune responses (Mendoza et al., 2019). It showed both pro-tumorigenic and antitumor immunity (Tau et al., 2001; Maimela et al., 2018). Natural killer (NK) and natural killer T (NKT) cells regulate the synthesis of IFN-γ in innate immunity but CD8 and CD4 T-cells are the major sources of the adaptive immune system (Burke and Young, 2019). An elevated level of IFN-γ was found in the gingival tissue of chronic periodontitis patients (Cesar-Neto et al., 2006), a similar observation was also made among nicotine smokers (Lee et al., 2017; Rahimi et al., 2018; Singh et al., 2019). Reduced IFN-γ level was also recorded in dendritic cells (Tao et al., 2019; Raymond et al., 2021).

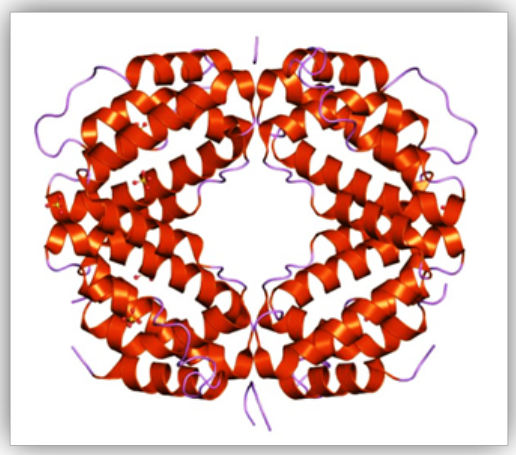

Figure 2.8: Crystallographic structure of IFN-γ

2.1.8.5. BCL-2 and BAX

The proteins belonging to the Bcl-2 family play a pivotal role in programmed cell death or apoptosis. Structurally there are two subgroups that exist in the Bcl-2 family: multi-domain with three or four domains and BH3-only Bcl-2 family proteins. The multi-domain Bcl-2 family proteins consist of both pro- and anti-apoptotic members, whereas BH3-only proteins are proapoptotic (Igaki and Miura, 2004).

Proteins under the Bcl-2 family are divided into three subfamilies depending upon their sequence homology and functions (i) A subfamily consisting of Bcl-2, Bcl-xL, and Bcl-w that exert anti-cell death activity with sequence homology in four regions (BH1- Bh4). (ii) A second subfamily represented by Bax and Bak share sequence homology at BH1, BH2, and BH3 but not at BH4. All these proteins exert pro-apoptotic activity. (iii) Third subfamily includes Bik and Bid, which are pro-apoptotic and share sequence homology only within BH3 (Figure 2.9) (Tsujimoto, 1998). The role of Bcl-2 in cancers and other important conditions makes it an attractive target for pharmaceutical intervention. Bcl-2 expression was downregulated following cigarette smoking was recorded (Zeng et al., 2016, 2020). In contrast, increased Bcl-2 protein expression was noticed in nicotine induced adult rat spleen (Ahmed et al., 2014).

Figure 2.9: Homology of Bcl-2 family proteins. Sequence homology observed at four regions and designated as BH1 to BH4. Bik subfamily members share sequence homology within the BH3 region only. (Source: Tsujimoto, 1998)

Higher levels of BAX and lower levels of Bcl-2 were observed among the smoker group (Biswas et al., 2015) whereas the reverse thing was recorded in breast cancer cell line (Aali and Motalleb, 2015). Increased BAX expression in the smoker group was observed by Shaik et al., 2019. The ratio of BAX and Bcl-2 was increased in nicotine-induced human renal proximal tubular epithelial cells (Kim et al., 2016).

2.1.8.6. Oestrogen and progesterone

Two steroidal hormones estrogen and progesterone play a vital role in regulating mammalian reproductive system. One primary action of these hormones is to regulate the development and function of the uterus. The transcription of specific uterine genes is regulated by these hormones (DeMayo et al., 2002). Nicotine smoking inhibits the activity of aromatase enzyme that catalyses the transformation of androgens to estrogens. Consequently, nicotine reduces circulating estrogen levels and leads to early onset of menopause in women (d'Adesky et al., 2018). Nicotine alters the function of the endocrine system and affects the release of female hormones which are very important agents for the protection of women's health (Maiti et al., 2015). Deficiency of

estrogens results in irregular periods, infertility, bone weakness, hot flashes, depression, and urinary tract infections (Jandikova et al., 2017). Similarly, a deficiency of progesterone affects the menstrual cycle, pregnancy, and embryogenesis of humans and other species (Jandikova et al., 2017). Nicotine reduces circulating estrogen levels in female rats (d'Adesky et al., 2018). Smoking may reduce or neutralize completely the efficacy of orally administered estrogens (Mueck and Seeger, 2005). Cigarette smoking reduced the efficacy of both endogenous and exogenous estrogen (Ruan and Mueck, 2015). Nicotine-induced declined estradiol and progesterone in the serum of Wistar rats has been documented by Adeyemi et al., 2018; Ahmadi et al., 2013; Khalid et al., 2017. The urge of smoking is inversely related to the ratio of estrogen and progesterone (Pang et al., 2018).

2.1.9. Nicotine intake

Tobacco products are used in various ways smoking, chewing, and sniffing.

2.1.9.1. Direct smoking

Different nicotine products are used directly by people as direct sources.

Cigar: Cigars are prepared by rolling bundles of dried and fermented tobacco leaves. Cigar contains three parts filler, binder leaf, and wrapper leaf. Daily cigar smoking is responsible for various diseases like cancer, and cardiovascular diseases (Chang et al., 2015).

Blunts: Blunts are stubby cigars, wide that have had the tobacco removed and replaced with marijuana. It can also fill up with rolls of tobacco leaf. Adolescents and young adults attractive to blunt because it is inexpensive, available in the market, has an attractive flavour and its wrapper is easily opened (Kong et al., 2018). Its use is not risk-free. Even though all of the filler tobacco is removed from the blunt but its users are still exposed to nicotine by the cigar wrappers. This wrapper contains 1.2 mg to 6.0 mg of nicotine (Peters et al., 2016).

Cigarettes: Cigarette is a narrow cylindrical body made up of thin papers containing burnable tobacco remains rolled within it. Most cigarettes contain

reconstituted tobacco products like recycled tobacco stems, stalks, scraps, collected dust, and floor sweepings and mixed glue, chemicals, and fillers, the product is then sprayed with nicotine extracted from tobacco scraps (Rabinoff et al., 2007). Cigarette smoke leads to various diseases like cancer, pulmonary disease, cardiovascular disease, and various health problem that affect every organ of the body (Mishra et al., 2015).

Bidi: Bidis are small, thin, hand-rolled tobacco products. It is mostly available in India and Southeast Asia. Bidis are relatively cheaper than cigarettes. A higher level of bidi smoking is found among the lower socio-economic status group. Bidis are also a cause of tobacco-related diseases and deaths in India. The previous report shows that bidis contain nicotine and cancer-causing chemicals higher than cigarettes. It is responsible for lung and oral cancer, respiratory disease, and tuberculosis (Mbulo et al., 2020).

Kretek: In Indonesia kretek is originated. It is called an unfiltered cigarette. Kretek is made by blending tobacco; cloves and other flavours. The 90% smoker of Indonesia prefers kretek for smoking (Braun et al., 2022). Kretek has many brands like Dji Sam Soe, Bentoel, Minak Djinggo, Djarum, Gudang Garam, and Wismilak. (Dji Sam). Djarum cigarette contain 7.4 mg nicotine. It has fewer bad effects on our health than conventional cigarettes due to the presence of eugenol in clove. It gives taste satisfaction, and an aromatic odour (Nuryunarsih et al., 2021).

Chewing tobacco: Chewing tobacco is one type of smokeless tobacco. It is placed between the cheek and lower limb. It is crushed manually by teeth and releases the flavour and nicotine. It is carcinogenic and responsible for oral cancer (Warnakulasuriya and Straif, 2018).

Snuff: Snuff is a smokeless tobacco made from grounded tobacco leaves. It is inhaled or snuffed into the nasal cavity and delivers nicotine. Long-term use of nasal snuff causing progress of chronic rhinitis results in the block and stuffy noses. Previous studies reported that moist snuff causes cancer (Narake and Gupta, 2014).

Snus: The origin of snus is Sweden and other Scandinavian countries and it is one type of smokeless tobacco product. Snus is not like moist snuff or chewing tobacco because it is not made from fire-cured tobacco leaves, it is made from steam-cured tobacco leaves. It develops a lower rate of cancer and other tobacco-related disease than cigarettes (Clarke et al., 2019).

Water pipe: It is also called a hookah. It passes tobacco smoke through the water before inhalation by the smoker. Compared to cigarette smoker's hookah smokers are exposed same toxic compounds but at higher levels. So, hookah smokers are affected by various negative health impacts like cancer, cardiovascular disease, infections, and lungs disease more than conventional cigarette smokers (Qasim et al., 2019).

2.1.9.2. Passive smoking

Passive smoking is the intake of smoke through breathing by a person other than the direct smoker. When someone smokes tobacco, the smoke is releases to the surrounding and is inhaled by other people present in the same environment. It is also called second-hand smoke. Previous studies reported that both active and passive smoking equally induces some types of diseases like breast cancer, allergic rhinitis, allergic dermatitis, and food allergy. Passive smoking is responsible for developing various health problems especially diseases among children (Cao et al., 2015). Hirayama, 1981 reported that lung cancer is seen among non-smoking wives of smoking husbands.

2.1.10. Epidemiology of Tobacco smoking

Historically, tobacco was used as part of traditional ceremonies but its use was limited. The use of commercial cigarettes increased dramatically in the early twentieth century due to large-scale manufacturing and promotion by media and advertising (CDC, 2014). The Global Burden of Disease Project (GBDP) estimated that about 1.14 billion people smoked globally in 2019 (GBD 2019, 2021). A declining trend in smoking was noticed between 1990 and 2019. Thereafter it increases among the adult population. Smoking-associated death

occurs approximately one in every 0.8–1.1 million cigarettes smoked / per year (Jha, 2020), reflecting the estimated worldwide consumption is about 7.4 trillion cigarettes. Around 7 million smoking-associated deaths were estimated in 2019 (GBD 2019, 2021). In most populations, the prevalence of smoking was found to be much higher among lower-income groups with lower levels of education (Palipudi et al., 2012) and also among people with mental health disorders along with other co-addictions (Goodwin et al., 2011; Weinberger et al., 2018). Male smokers were more frequent than that of female (Figures: 2.10 – 2.12). The regional prevalence of smoking varies significantly throughout the globe and is also in different ethnic groups. Rates of smoking were found to be higher in some regions of Asia, including China and India, but lower in North America and Australia. The prevalence of mental health disorders and other co-addictions are higher among smokers than non-smokers (Goodwinet al., 2011; Weinberger et al., 2018; Hassan and Le Foll, 2021).

Almost 90% of smokers start smoking during their adolescence, between 15 and 25 years of age (Akel et al., 2022). Prevalence of smoking declined among youths between 1990 and 2019 (Warner, 2019).

Severe effect of smoking was noticed among smokers who initiates smoking in early adolescence and persists throughout their life (CDC, 2014; Jha and Peto, 2014). About 320 million smoking-related deaths occur in the younger age group (within 30 years) those, who did not quit smoking (Jha et al., 2013). The actual number of smoking-related deaths might be greater than the estimated numbers (Jha, 2020; Jha and Peto, 2014; Banks et al., 2015; Pirie et al., 2013).

A model-based study has estimated 7.7 million smoking-related deaths globally in 2020, of which 80% were in men among which 87% were current smokers (GBD 2019, 2021). Cancer, heart attack, emphysema, and stroke are the major causes of tobacco deaths (CDC, 2014; Jha and Peto, 2014). Tuberculosis and other lung disease are common associate diseases among smokers (Jha, 2020).

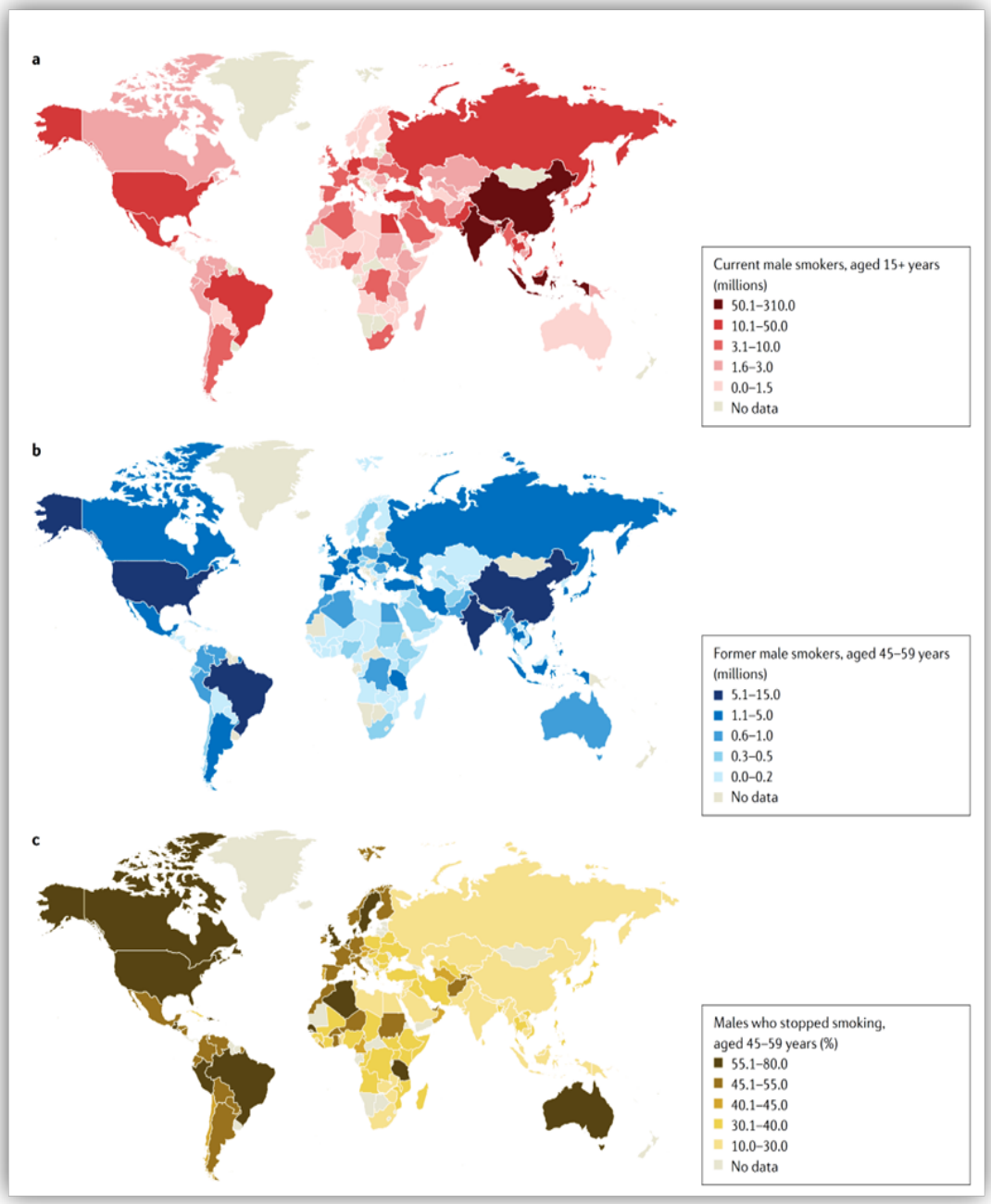

Figure 2.10: World-wide distribution of male smokers: (a). Current male smokers above15 years of age- expressed in millions (b). Previous male smokers 45–59 years of age / country (in millions). (c) . Percentage of former male smokers 45–59 years of age / country stopped smoking. The data presented are for male smokers during 2015–2019 from different countries surveyed directly. (Source: GBD 2019, 2021)

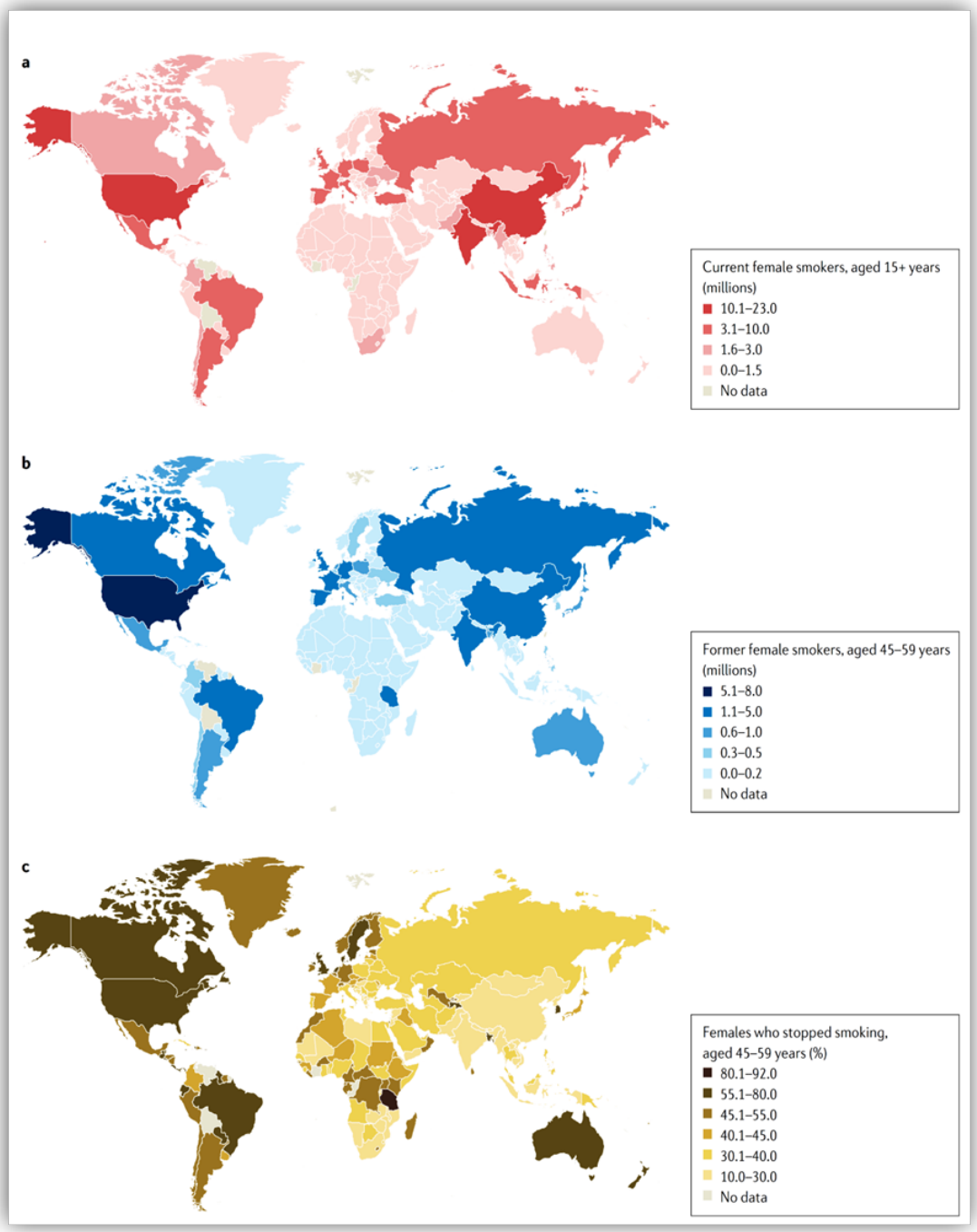

Figure 2.11: World-wide distribution of female smokers: (a). Current female smokers above15 years of age- expressed in millions. (b). Previous female smokers 45–59 years of age / country (in millions). (c) . Percentage of former female smokers 45–59 years of age / country stopped smoking. The data presented are for female smokers during 2015–2019 from different countries surveyed directly. (Source: GBD 2019, 2021)

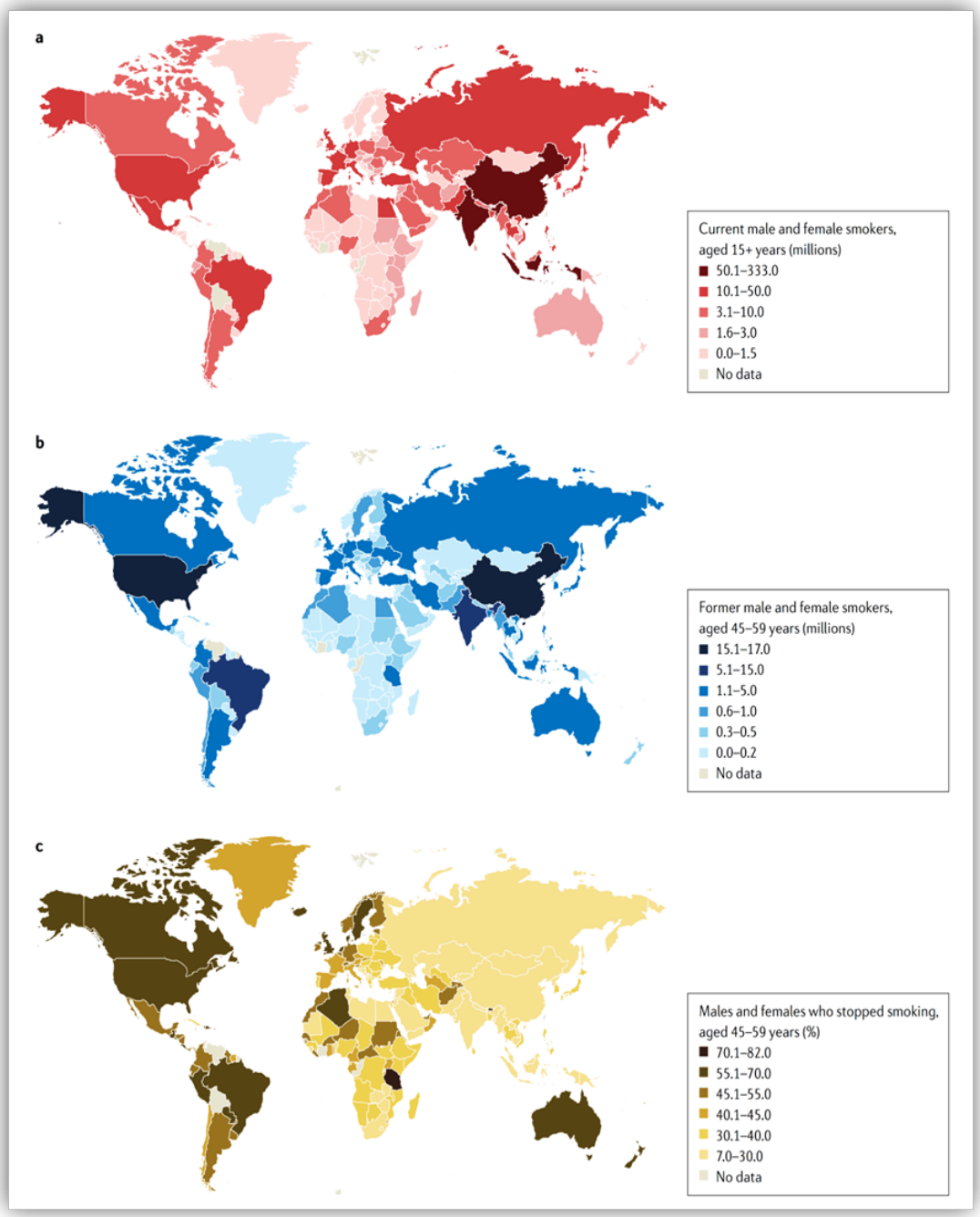

Figure 2.12: World-wide distribution of male and female smokers (combined): (a). Current male and female smokers above15 years of age- expressed in millions. (b). Previous male and female smokers 45–59 years of age / country (in millions). (c) . Percentage of former male and female smokers 45–59 years of age / country stopped smoking. The data presented are for male and female smokers during 2015–2019 from different countries surveyed directly.

(Source: GBD 2019, 2021)

2.1.11. Indian scenario of smoking

India has a long history of smoking, since 2000 BC as mentioned in the "Atharvaveda". Fumigation (dhupa) and fire offerings (homa) were prescribed in the Ayurveda for medical purposes since 3,000 years back. Tobacco was introduced to India in the 17th century and it later merged with existing practices of smoking (mostly cannabis). The World Health Organization has published a report in 2011 that "Nearly 80% of the more than one billion smokers worldwide live in low- and middle-income countries including India, where the burden of tobacco-related illness and death is the heaviest" (WHO, 2011). According to the Indian Heart Association (IHA), India accounts for 60% of the world's heart disease burden. They have identified a reduction in smoking as a significant target of cardiovascular health prevention efforts. Smoking is the predominant habit among males in India constituting more than 50% of tobacco users (Gupta et al., 2017).

2.1.12. Effect of nicotine on the female population

In recent years the prevalence of cigarette smoking somewhat increased among the female population Worldwide. The various attractive advertisement given by the tobacco companies influences the smoking percentage among women in high and low-income countries. Among adolescents, the percentage of cigarette smoking among men was 4.5 and women were 4, in the case of adults 18.5 % of women, and 24.5 % of men. In older age the percentage of smoking is higher in the female population (9.3 %) than in males (8.9 %) (Allen et al., 2019). Due to lower immunity power, women were more susceptible than men to smoking-related mortality and morbidity. The risk of some specific cancer by smoking has a stronger effect on women than men. Previous studies reported that women smokers had a greater risk of colorectal cancer than men smokers (Gram et al., 2020). The risk of breast cancer shows greater among smoker women who have a family history of breast cancer and started smoking in adolescent and pre-menarcheal age than their non-smoker peers (Jones et al., 2017). Smoker women have a 25% increased risk of

cardiovascular diseases than their male counterparts (Gallucci et al., 2020). Smoker women who suffered from PCOD have increased levels of fasting blood sugar and free testosterone level that was the risk factors for coronary heart disease (Pau et al., 2013). Previous studies reported that nicotine influence abdominal obesity among women. Nicotine enhanced the testosterone level and reduced the estradiol level. Smoker women have a higher waist-hip ratio than their non-smoker peers. Thus, smoking reduced sexual attractiveness among women (Pölkki and Rantala, 2009). Nicotine is known as a reproductive toxicant and smoking women suffer from various reproductive disorders like infertility, subfecundity, and menstrual disorder (Oboni et al., 2016). A higher rate of asthma was observed among smoker women in irrespective of body weight but a higher rate of asthma level showed among normal weight and underweight smoker men (Chen and Mai, 2011). Women who smoke have overactive bladder symptoms and incontinence than male smokers (Kawahara et al., 2020). During smoking cessation, women gain more weight than men. Perkins et al. (2013) reported that abstinence and negative mood enhanced the craving to smoke with a higher degree in females than their male counterparts.

2.2. Curcumin

Turmeric is an important spice that is used both in the medicinal and culinary fields. It belongs to the ginger family of rhizomatous herbaceous perennial plants that are called *Curcuma longa*. It is mostly cultivated in tropical and subtropical regions of the world, primarily grown in Asian countries like India, China, Indonesia, Japan, and Bangladesh. The active ingredient presents in turmeric which makes a magical spice that is curcumin. It is also known as "wonder drug of life" (Verma et al., 2018). There is a historical background about the importance of Curcuma species, since 5,000 years back as mentioned in Ayurveda and 2,000 years back in Atharveda. Long back, curcumin was popularly known as a "substance that gives the yellow colour". During the middle of the 19th century, curcumin was considered a biologically active molecule having antibacterial activity and used effectively against different

species of Staphylococcus, Salmonella, and Mycobacterium (Kocaadam and Sanlier, 2017).

2.2.1. Chemistry of curcumin

Curcumin was first isolated by Vogel and Pelletier in 1815, but not in its pure form. In 1913 Lamp and Milobedeska confirmed its synthesis and chemical structure. Its chemical name is 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione. It is a lipophilic polyphenol and also known as diferuloylmethane. The molecular weight of curcumin is 368.379 and the chemical formula is $C_{21}H_{20}O_6$. Its melting and boiling point is 183^0C and 591.4^0C respectively. The structure of the curcumin contains three chemical entities, two aromatic systems of o-methoxy phenolic groups, attached with a seven-carbon connector consisting of an α, β-unsaturated β-diketone moiety (Figure 2.13). This chemical structure of curcumin is responsible for its insolubility in water at a neutral and acidic pH but makes it soluble in ethanol, alkali, ketone, methanol, acetic acid, chloroform, dimethyl sulfoxide (DMSO), and acetone (Sohn et al., 2021).

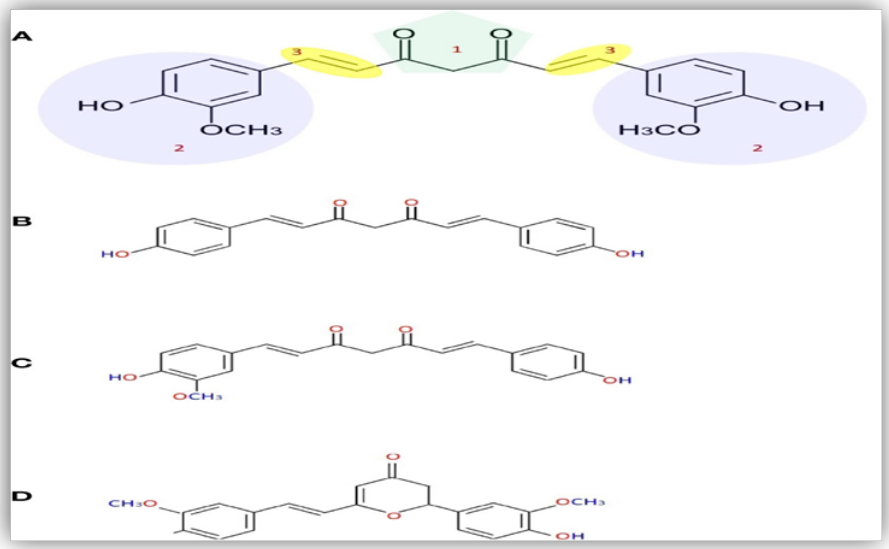

Figure 2.13: Chemical structure of curcumin (A) (1). b-diketone or keto-enol, (2) phenolic, (3) alkene linker, demethoxycurcumin (B), bisdemeth-oxycurcumin (C) and cyclocurcumin (D). (Source: Karthikeyan et al., 2020)

2.2.2. Biological properties of Curcumin

Curcumin is a pleiotropic molecule and it has various biological properties. First In 1937, curcumin was first used as a drug against human diseases (Fadus et al., 2016). Directly and indirectly, curcumin is able to bind and hamper various proteins, metals, growth factors, transcription factors, receptors, enzymes, and other important biomolecules. Over the last 60 years, around 3000 studies indicated that curcumin has antioxidant, anti-inflammatory, antineoplastic, anti-diabetic, hepato-protective, nephroprotective, neuroprotective, immunomodulatory, anti-HIV, anti-cancer properties (Menon and Sudheer, 2007; Wilken et al., 2011; Den et al., 2019; Hashish and Elgaml, 2016; Prasad and Taygi, 2015). The absorption rate of curcumin is poor in the gastrointestinal tract. The biological activities of curcumin are summarised in Figure 2.14.

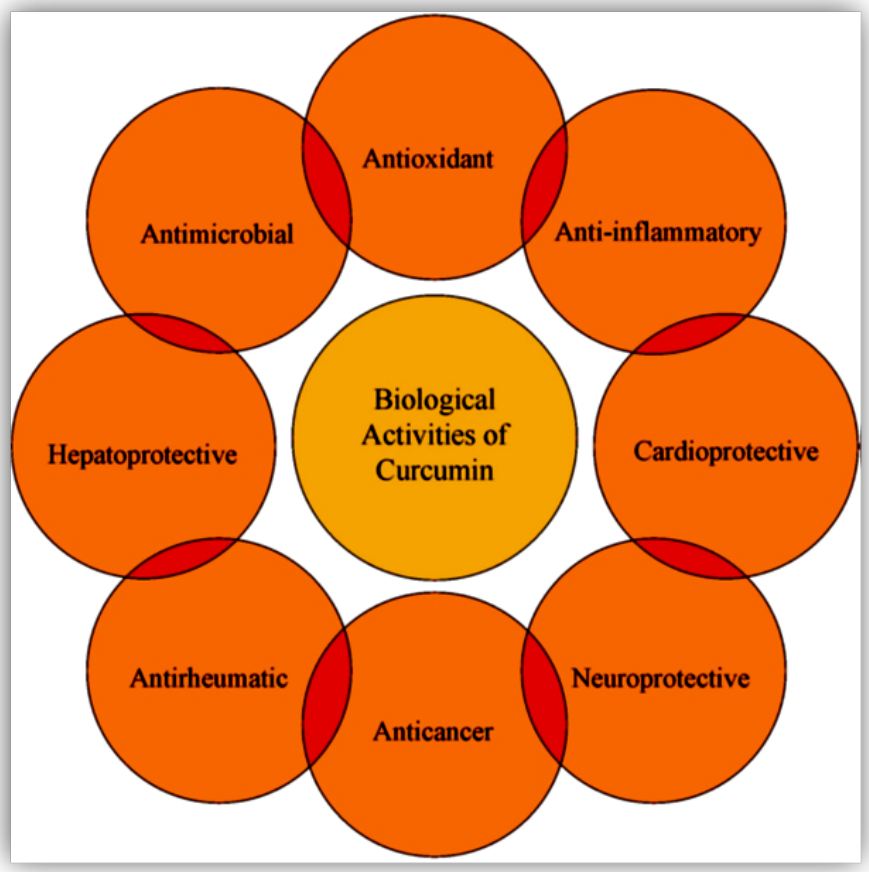

Figure 2.14: Schematic representation of biological activities of curcumin (Source: Sharifi-Rad et al., 2020)

2.2.2.1. Hepato-protective effect of curcumin

There are several reasons responsible for liver diseases like non-alcoholic fatty liver disease, alcoholic fatty liver disease, and liver diseases by virus (Hepatitis B virus, Hepatitis C virus) (Haga et al., 2015). In chronic liver disease, reactive oxygen species (ROS) are increased and oxidant, antioxidant imbalance occurs that contributes to oxidative stress. It hampers liver fibrogenic response and leads to ischemia, necrosis, and apoptosis. It is responsible for altered gene expression and progressed liver damage (Cichoż-Lach and Michalak, 2014). Previous studies reported that curcumin prevents methotrexate-induced oxidative stress related to liver disease. It lowers the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes level and increases glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), SOD and CAT level and treat liver toxicity (Moghadam et al., 2015). Another study reported that sodium fluoride-induced oxidative stress that induces hepatic toxicity is treated by curcumin (Al-Harbi et al., 2014). Curcumin also prevents malathion-induced oxidative stress, curcumin helps the decrement of MDA and nitric oxide levels and increment of GSH levels (Alp et al., 2012). Cadmium-induced liver oxidative damage is also corrected by curcumin (Mohajeri et al., 2017). Carbon tetrachloride (CCl_4)-induced liver injury is ameliorated by curcumin. Curcumin exerts its action by inhibiting the TGF-β1/Smad signalling pathway and CTGF expression (Zhao et al., 2014). Nicotine-induced liver damages were found to be corrected by Curcumin by lowering aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum nitric oxide levels (Salahshoor et al., 2015). Curcumin repaired hepatic damages due to other factors were also reported by Al-Rubaei et al., 2014; Farzaei et al., 2018; Jarhahzadeh et al., 2021.

2.2.2.2. Nephroprotective activities of curcumin

Curcumin is a polyphenol used to treat renal diseases. In developed and underdeveloped countries chronic kidney disease is a serious health problem.

The inflammation, oxidative stress, and apoptosis are the pathophysiological basis of this disease. This does not occur only in humans but is also seen in other animals. Plasma levels of certain inflammatory cytokines, nitrosative markers, and oxidative stress are increased in chronic kidney disease patients. The plasma urea and creatinine level are increased in this condition (Rapa et al., 2019). Curcumin has nephroprotective activities, it protects kidneys from toxic factors. In copper sulphate exposed condition kidney did not function properly due to declined urea and creatinine excretion. Curcumin showed its nephroprotective activities and normalizes plasma levels of urea and creatinine (Hashish and Elgaml, 2016). High blood pressure, elevated levels of urinary albumin, and various inflammatory cytokines like IL-1β, IL-6, and TNF-α were reported in adenine-induced conditions. Curcumin, a natural product helps to minimize their levels (Ali et al., 2017). Curcumin also shows its reno-protective effect against cisplatin-induced nephrotoxicity (Ugur et al., 2015). Curcumin shows its therapeutic effect against nicotine renal toxicity by lowering serum urea and creatinine level in albino rats (Azab et al., 2021).

2.2.2.3. Anti-lipidemic effects of Curcumin

Hyperlipidaemia with increased cholesterol, free fatty acids, and triglycerides was significantly reduced; especially triglycerides, which returned to their normal levels by curcumin supplementation (Zingg et al., 2013; Jalali et al., 2020; Baziar and Parohan, 2020). Curcumin significantly reduced the low-density lipoprotein cholesterol (LDL-C) and triglyceride (Den Hartogh et al., 2020; Pivari et al., 2019). Curcumin showed beneficial effects in cardiovascular diseases by normalizing LDL-C, VLDL-C, IDL-C, total cholesterol, and HDL-C (Rafiee et al., 2021). Nicotine-induced hyperlipidaemia was corrected by this natural ingredient in Wistar rats (Kalpana et al., 2005).

2.2.2.4. Antioxidant activities of curcumin

Curcumin showed both antioxidant and anti-inflammatory activities in various conditions (Lin et al., 2007; Marchiani et al., 2014). Curcumin showed improvement in systemic markers for oxidative stress (Sahebkar et al., 2015).

Experiments proved that it can increase the activities of antioxidants like superoxide dismutase (SOD) (Banach, et al., 2014; Menon and Sudheer, 2007; Panahi et al., 2016). A significant effect of supplementation of curcuminoids on all associated parameters with oxidative stress including plasma activities of SOD and catalase, serum concentrations of glutathione peroxidase (GSH), and lipid peroxides (Sahebkar et al., 2015). It affects free radicals by different mechanisms. It scavenges free radicals like ROS and RNS (Menon and Sudheer, 2007) and activates different enzymes GSH, catalase, and SOD for the neutralization of free radicals (Lin et al., 2007; Marchiani et al., 2014). Besides this curcumin can inhibit enzymes responsible for ROS generation like lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase (Lin et al., 2007). Lipophilic nature makes curcumin an efficient scavenger of peroxyl radicals and thus acts as a chain-breaking antioxidant (Priyadarsini et al., 2003).

2.2.2.5. Anti-inflammatory activities of curcumin

Several experimental studies documented that curcumin is a potential agent for treating various inflammatory diseases (Aggarwal and Sung, 2009; Cianciulli et al., 2016; Edwards et al., 2017; Dai et al., 2018). It was evident that curcumin showed anti-inflammatory effects in various ways. Curcumin inhibits pro-inflammatory transcription factors like NF-kB and AP-1. It decreases the production of proinflammatory cytokines e.g., TNF-alpha, IL-2, IL-6, CRP, etc. It also suppresses the enzymes like 5-lipoxygenase and COX-2 and -5 and hampered the mitogen-activated protein kinases pathways (Aggarwal and Sung, 2009; Panahi et al., 2014a; Panahi et al., 2014b; He et al., 2015; Machova et al., 2015).

2.2.2.6. Antigenotoxicity properties of curcumin

Various physical and chemical agents are responsible for chromosomal damage and such damage is repaired by supplementing curcumin (Acar et al., 2022). Curcumin prevents DNA breakage due to changes in its nitrogenous bases by oxidative stress, thus maintaining genomic stability (Sahebkar et al.,

2015). Mendonça et al. (2015) reported that curcumin ameliorated cisplatin-induced chromosomal and DNA damage and is determined by comet assay and micronucleus assay. Previous studies reported that curcumin shows beneficial effects against copper-induced genotoxicity in BalbC mice (Corona-Rivera et al., 2007). Radiation-induced increased percentage of micronuclei formation was also reduced by curcumin supplementation (Jagetia, 2015). Curcumin also exerts its antigenotoxic effect by reducing the micronuclei induction by chromium trioxide (Prasad et al., 2017). Banerjee et al. (2010) reported that curcumin helps to reduce nicotine-induced DNA damage to blood cells.

2.2.2.7. Anticancer activities of curcumin

The positive effects of curcumin have been demonstrated against several diseases including cancer (Devassy et al., 2015). Curcumin may restrict the development of cancer in many phases. It suppresses the transformation, development, and invasion of tumours, angiogenesis, and metastasis. Curcumin is supposed to suppress the growth of tumour cells through various pathways like cell proliferation pathway (cyclin D1, c-myc), cell survival pathway (Bcl-2, Bcl-xL, cFLIP, XIAP, and cIAP1), caspase activation pathway (caspase 8, 3, and 9), tumour suppressor pathway (p53, p21), death receptor pathway (DR4, DR5), and many other cells signal pathways (Ravindran et al., 2009). Evidence showed that curcumin is effective against various types of cancers including multiple myeloma, lung, pancreas, colon, prostates, and breast cancers (Anand et al., 2008; Devassy et al., 2015). The effectiveness of radiotherapy is also found to be increased by curcumin and thus shortens the duration of treatment (Akpolat et al., 2010). Previous studies reported that curcumin helps to modulate the antiapoptotic protein Bcl-2 and proapoptotic protein Bax ratio in testicular cells of diabetic rats and inhibits apoptosis (Zhao et al., 2017). Liang et al. (2018) reported that the alteration of EMT in lung tissue of mice due to tobacco smoke is also ameliorated by curcumin through downregulating of the MAPK pathway.

2.2.2.8. Effect of curcumin on the reproductive system

Curcumin is a phytochemical has various essential properties like antioxidant, anti-inflammatory, and apoptotic properties, it is useful for maintaining a healthy reproductive system. Previous studies reported that cadmium-induced toxicities in mice cause a decrement in testosterone and progesterone level in mice leads the reproductive disorder. Curcumin helps to increase this essential hormonal level and showed its beneficial effect (Abu-Taweel, 2016). Sadoughi. (2016) reported that curcumin helps in increasing estrogen and progesterone level among alloxan-induced diabetic rats and maintain a healthy reproductive system. Shah and Shrivastava (2021) reported that letrozole-induced polycystic ovarian syndrome which is a combination of endocrine metabolic abnormalities and various types of inflammation is treated by curcumin.

2.2.3. Drawbacks of curcumin

2.2.3.1. Toxicity of curcumin

In spite of its several beneficial effects, curcumin has some toxicity which is dose-dependent. Higher doses of curcumin alter enzyme activities and may lead to adverse effects (Nelson et al., 2017). Recent studies reported that high doses of curcumin influence DNA damage and inactivate P53 by reacting with thiol groups of cysteine residue (Jantawong et al., 2021). In some cases, it exerts its cytotoxic effect against some cancer cell lines (Martínez-Castillo et al., 2018). Intake of curcumin up to 8 gm per day for three months by healthy human subjects showed no toxicity (Sharifi-Rad et al., 2020). But the same dose is responsible for abdominal pain among pancreatic cancer patients. It might be due to the curcumin-induced inhibition of prostaglandin synthesis and inhibition of COX (Hassanzadeh et al., 2020).

2.2.3.2. Limitations of curcumin

The pharmacokinetics of any compound depends on its rate of gastrointestinal absorption, metabolism, and bioavailability. Several studies reported that curcumin is poorly absorbed in oral administration (Jäger et al., Owery, 2014;

Lopresti, 2018; Dei and Ghidoni, 2019). Curcumin is mostly water-insoluble and low bioavailable compound (Priyadarsini, 2014; Jamwal, 2018; Tabanelli et al., 2021). The bioavailability of curcumin is very low in different tissues and blood following its supplementation. Very low curcumin concentration in human plasma (0.051µg/mL) was detected following 2 g /kg curcumin treatment (Dei and Ghidoni, 2019). Similarly, 0.22 µg/mL plasma concentration of curcumin was estimated following the administration of 1 g curcumin/kg in mice (Hassanzadeh et al., 2020). The concentration of curcumin in liver and kidney tissues was found to be very negligible or undetectable. Due to its poor stability curcumin is metabolized and excreted rapidly. Curcumin is degraded within 30 min in an alkaline medium (pH > 7) and produces ferulic acid, vanillin, feruloyl methane, and Trans-6-(40-hydroxy-30-methoxyphenyl)-2 (Zheng and McClements, 2020). The metabolism of curcumin rapidly occurs within the liver. Curcumin is transferred into the form of its water-soluble metabolites and excreted through urine. The majority of curcumin is excreted along with faces (≤90%) (Sharifi-Rad et al., 2020).

Several approaches have been introduced for greater bioavailability, high plasma concentration, and to increase the cellular permeability and delayed metabolism of curcumin (Ishida et al., 2002; Selvam et al., 2005; Sun et al., 2006; Ohori et al., 2006).

2.3. Nanocurcumin and its overview

Synthesis of nano curcumin overcomes the problems associated with its delivery, bioavailability, high rate of metabolism and fast expulsion. Various techniques are adapted for the preparation of nanocurcumin.

2.3.1. Different curcumin nano formulations & their therapeutic role

During last two decades many curcumin nano formulations (Table 2.1) have been prepared to overcome problems associated with its delivery and medicinal activities. The role of different curcumin nano formulations is listed in Table 2.1.

Table 2.1: Role of nanocurcumin against different diseases

(Source: Karthikeyan et al., 2020)

Sl. No	Curcumin nano-formulation	Description	Models used	Major outcomes	References
1	Liposomes	Liposomes are a spherical vesicle consisted of single or multiple phospholipid bilayers surrounding aqueous units that very closely resemble the cell membrane structure. It solubilizes curcumin in the phospholipidic bilayer and allows curcumin to be distributed in aqueous medium and increases the effect of curcumin.	Malaria, melanoma, Renal ischemia, colorectal cancer, and lung cancer	Increased the antimalarial and antimelanoma effects, greater encapsulation efficiency, excellent bioactivity, and anticancer activity	Basnet et al., 2012; Rogers et al., 2012; Tefas et al., 2017; Sesarman et al., 2018; Chen et al., 2019; Huang et al., 2019; Reddy et al., 2019; Vetha et al., 2019
2	Polymers	Polymers are another widely used effective drug delivery system for curcumin. It can able to improve the oral bioavailability and solubility of curcumin.	Wound healing and colorectal cancer	Exhibited strong wound healing and long blood circulation, suppression of tumor growth, higher growth inhibition in cancer cells than free curcumin, and increased the cellular uptake and better anticancer activity	Li et al., 2012; Anitha et al.,2014; Chaurasia et al., 2016; Xie et al., 2017 Nasery et al., 2020
3	Gold nanoparticles	Gold nanoparticles have own unique physical and chemical properties and various surface functionalities. It offers versatalite platform in drug delivery (curcumin)	Prostate and colorectal cancer cells	Improved antioxidant activity, extended blood circulation, better solubility and stability, enhanced biocompatibility, and considerable anticancer activity	Singh et al., 2013b; Rejinoldet al., 2015; Nambiar et al.,2018; Elbialy et al., 2019
4	Magnetic nanoparticles	Magnetic nanoparticles used for multiple purposes including drug delivery (curcumin), hyperthermia, and quality imaging	Cancer and inflammatory cells	Improved cellular uptake, potent targeting capability of curcumin, magnetic resonance imaging, effective protection against inflammatory agent, controlled curcumin delivery, excellent bio-compatibility, & anticancer activity	Yallapu et al., 2012b; Bhandari et al., 2016; Saikia et al., 2017; Aeineh et al., 2018; Ayubi et al., 2019
5	Solid lipid	SLNs possess a lipid core	Allergy, colitis	Extended	Yadav et al., 2009;

	nanoparticles (SLNs)	matrix that can solubilize drug (curcumin) and the lipid core is steadied through emulsifiers. Normally SLN is spherical in shape.	and cerebral ischemia, and breast cancer lines	circulation of blood, increased anti-inflammatory effects, targeted and enhanced drug release in brain, and better anticancer activity	Kakkar et al., 2011; Wang et al., 2012; Kakkar et al., 2013; Bhatt et al., 2018; Wang et al., 2018; Fathy Abd-Ellatef et al., 2020
6	Conjugates	The complex formed from the joining together of two or more molecules, especially by covalent bond is referred as conjugates. Curcumin conjugation with small molecules and hydrophilic polymers increase its solubility and oral bioavailability	Fibroblast cells, breast cancer, and amyloid fragments	Increased the solubility, stability and bioavailability, strong anti-cancer activity, higher stability and bioavailability, and anti-amyloid effects	Manju & Sreenivasan, 2011; Singh et al., 2013a; Brahmkhatri et al., 2018
7	Cyclodextrins	Cyclodextrins are the bucket shaped oligosaccharides and well-known solubilizing and stabilizing agent. It can solubilize the curcumin in a lipophilic cavity, and the outer hydrophilic surface assists in greater dispersion of the formulation.	Bowel disease, lung, pancreatic, breast, colorectal cancer, and prostate cancer cells	Developed bioavailability and increased solubility, improved antiproliferation, anticancer and anti-inflammatory effects, increased the solubility, and formulated as eye drops.	Yallapu et al., 2010; Yadav et al., 2012; Dandawate et al., 2012; Abruzzo et al., 2016; Ntoutoume et al., 2016; Maria et al., 2017; Guo, 2019
8	Solid dispersions	Solid dispersions are referred as one or more active component in an appropriate matrix. It can improve the bioavailability of poor water-soluble drugs like curcumin.	Breast tumor, rat paw edema, and wound healing	Prolonged survival, anti-tumor and anti-metastasis activity and prolonged survival, enhanced stability, bioavailability and anti-inflammatory, anti-bacterial and improvement of vaginal wound healing	Liu et al., 2013; Teixeira et al., 2016; Silva et al., 2019; Zhang et al., 2019
9	Micelles	Micelles (20–100 nm) are normally colloidal dispersions made from amphiphilic molecule. It assists better solubilization and targeted delivery to curcumin.	Lung tumor and colorectal cancer	Bioavailability and solubility improved, prolonged life, targeted drug delivery, great chemical stability, and better antitumor and anticancer effects	Adhikary et al., 2010; Podaralla et al., 2012; Raveendran et al., 2013; Yang et al., 2015; Chang et al., 2016; Javadi et al., 2018; Chen S. et al., 2020
10	Nanospheres	Nanospheres are known as solid matrix particles where in the main component (drug) is mixed, but microcapsule contains internal core	*E. coli*, *Staphylococcus aureus*, *Vibrio vulnificus*, and *Candida albicans*.	Exhibited strong antimicrobial and anti-cancer effects, effective target delivery and anti-amyloid effect	Arunraj et al., 2014; Liang et al., 2017; Huo et al., 2019; Masoule et al., 2019; Kim et al., 2020

		and outer polymeric shell.	Breast cancer cells, melanoma cells, and Alzheimer's		
11	Nanogels	A nanogel is a nanoparticle composed of a hydrogel synthesized by either physical or chemical cross-linking of polymers under controlled conditions. Cross linked structure of nanogels offer a strong base for drug storage and release. It is a possible technique to prepare and release active types of drugs like curcumin to cells for remaining activity, improving stability, and prevent drug immunogenicity	Pancreatic cancer, colorectal cancer and skin cancer cells	Targeted and controlled drug release, prolong circulation, enhanced bio availability, and better anticancer activity	Mangalathillam et al., 2012; Wei et al., 2013; Madhusudana Rao et al., 2015; Amanlou et al., 2019; Wang et al., 2019; Priya et al., 2020
12	Nanodisks	Nanodisks are disk-shaped bilayers, apolipoprotein-stabilized and self-assembled. They boost the solubility and targeted release of curcumin	Mantle cell lymphoma	Improved biological activity and apoptosis to mantle cell lymphoma and anticancer activity	Ghosh et al., 2011; Singh et al., 2011; Subramani et al., 2017

2.3.2. Methods of nanocurcumin preparation

Nanotechnology helps to the formulation of the nanoparticle of curcumin and enhances its solubility, bioavailability, stability, and pharmacological activity. Various approaches are adapted to the preparation of the nanocurcumin. These are (Rajalakshmi and Dhivya, 2018).

- Ultrasonication
- Ionic gelation method
- Antisolvent precipitation method
- Fessi method
- Thin film hydration method
- Solid dispersion method
- Emulsion polymerization method
- Coacervation techniques
- Nanoprecipitation method

- Single emulsion method
- Solvent evaporation method
- Spray drying method
- Single emulsion method
- Microemulsion

2.3.3. Role of physicochemical properties of curcumin nanoparticles influencing its therapeutic efficacy

2.3.3.1. Particle size and its distribution

Particle size plays an important role in determining the biodistribution of the drug within specific organs, biodistribution throughout the body, attachment, rolling, phagocytosis, firm adhesion of nanoparticles, and accumulation. The appropriate particle size enhances the rate of absorption, permeation, bio-distribution, and circulation time as well as the bioavailability increases in the manifold. The excretion of a larger particle from the body is greater than a smaller particle. The rate of aggregation under the physiological condition of the larger particle is higher than the smaller particle (Hoshyar et al., 2016). Manikandan et al. (2022) reported that the reduction of the particle size of the curcumin up to the nano range (1-100nm) makes it more water soluble and an efficient anti-oxidant component than native curcumin. Ubeyitogullari and Ciftci (2019) revealed that the reduced particle size of curcumin is responsible for low crystallinity and maximum bio-accessibility which makes it a better therapeutic agent. Setthacheewakul et al. (2010) reported that the prepared nanocurcumin exhibits 10-40 folds higher absorption rate than the same oral dose of free curcumin administered to the Wister strain.

2.3.3.2. Surface properties of nanoparticles

Surface area, surface charge, and surface hydrophilicity are the important characteristic features of surface properties. Reduced particle size increases the surface area to volume ratio. The huge surface area to volume ratio of nanoparticles enhanced reactivity between the particle and its near

environment thus helping to increase the biodistribution in the body tissues and organs (Gatoo et al., 2014). The surface charge represents the electric potential of the nanoparticles and it is correlated with the nanoparticle's chemical composition. Due to the presence of zeta potential, no aggregation is found in water-soluble nanocurcumin and it forms a stable suspension (Shao et al., 2015). Previous studies reported that a curcumin nanoparticle that is positively charged exerts superior antimicrobial activity against Listeria monocytogenes than free curcumin (No et al., 2017).

2.3.3.3. Shape of particle

The shape of the particle is another important factor that plays an important role in various biological processes. The shape of the nanoparticle affects cell uptake, rate of drug delivery, and site of the drug delivery from the systems. Appropriate interaction is achieved by proper shape of the nanoparticle (Ankamwar, 2012). The spherical shape of the nanocurcumin whose average diameter is 28 nm helps to enhance the cellular uptake and showed a better cytotoxic effect against human laryngeal cancer cells than native curcumin. (Hanna and Saad, 2020). Rahimi et al. (2016) reported that the spherical shape of the prepared nanocurcumin is more stable and soluble and displayed more anticancer efficiency than free curcumin.

2.3.4. Therapeutic application of Nanocurcumin

2.3.4.1. Hepatoprotective effect of nanocurcumin

The efficacy and the bioavailability of the nanocurcumin are greater than native curcumin. This better efficacy has broad applications in therapeutics. For example, nanocurcumin exhibits greater bioavailability and anti-oxidant efficacy in the mouse brain compared to curcumin. Nanocurcumin reduces the oxidative stress indicators and also reduces the rising level of AST, and ALP. Nanocurcumin shows a better hepatoprotective effect against acrylamide-induced stress than native curcumin (Atia et al., 2022). Mohammed et al., 2020 reported that due to the better stability, bioavailability, and absorption,

nanocurcumin used for the treatment of diethyl nitrosamine-induced hepatic carcinoma by increasing serum albumin, glutathione peroxidase (GPx), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) level and decreasing malondialdehyde (MDA) level. Nanocurcumin helps to reduce the diethyl nitrosamine-induced hepatocarcinogenic effect of nitrosamines by decreasing the heterocyclic aromatic amine toxicity. Kheiripour et al. (2021) reported that nanocurcumin shows a better effect than native curcumin against paraquat-induced hepatotoxicity by modulating oxidative stress and expression of genes in the Nrf2 signalling pathway. The regular arrangement of the hepatic cell and central vein is distorted by paraquat and nanocurcumin exposure helps to rearrangement of the hepatic cell and central vein.

2.3.4.2. Nephroprotective effect of nanocurcumin

The concentration of the nanocurcumin was found to be higher than native curcumin in the kidney tissues. The greater bioavailability of the nanocurcumin showed better renoprotective activity than native curcumin. Nanocurcumin exerts its nephroprotective effect against cisplatin-induced nephrotoxicity by decreasing the serum creatinine, and blood urea nitrogen levels and increasing serum albumin levels. Cisplatin changes the histopathological features of kidney tissues and was normalized by the use of nanocurcumin (Sandhiutami et al., 2019). The better dissolution and deliverable capacity of nanocurcumin show a better renoprotective effect than curcumin against streptozotocin-induced diabetes by reducing the serum urea and creatinine level (Jie et al., 2021). Nanocurcumin improves kidney function by suppressing the expression of MMP-9 and minimizing fibrosis area in interstitial and tubular atrophy of rat kidneys with unilateral ureter obstruction (Karthikeyan et al., 2020). El-Desoky et al. (2022) reported that tartrazine induced elevated levels of serum creatinine, urea, and uric acid and changes in the histopathology of the kidney tissues were ameliorated by nanocurcumin.

2.3.4.3. Antilipidemic effect of nanocurcumin

Nanocurcumin maintains better cardiometabolic health than native curcumin by regulating glycaemic index, lipid profiles, inflammation, and systolic blood pressure (Ashtary-Larky et al., 2021). Previous experiments showed that doxorubicin hydrochloride-induced apoptosis and ROS production in rat embryonic cardiomyocytes decreased by both curcumin and curcumin nano formulation. Due to the better pharmacokinetic properties of curcumin nano formulation showed better cardioprotective activity than unformulated curcumin (Salehi et al., 2020). Dastani et al. (2022) reported that systematic inflammation and disordered lipid metabolism lead to the progression of coronary diseases in diabetic patients. Nanocurcumin supplementation reduces highly sensitive C reactive protein, and lipoprotein levels and reduced the chances of atherosclerosis progression in diabetic patients. Ray et al., 2016 reported that ultra-low doses of nanocurcumin can exert regression of cardiac hypertrophy in rat model. Rachmawati et al. (2016) showed that nanocurcumin has better antihypertensive and antihypercholesterolemic activity than native curcumin by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and Angiotensin Converting Enzyme (ACE).

2.3.4.4. Antigenotoxic effect of Nanocurcumin

According to the literature, the antigenotoxic activities of nanocurcumin is more effective than free curcumin because of better solubility and bioavailability. Better efficacy of nanocurcumin in tartazine-induced genotoxicity measured by comet assay was recorded in rats (El-Desoky et al., 2020). Al-Bishri (2017) showed a protective effect of nanocurcumin in hyperthyroidism by downregulating the oxidative stress, inflammation and DNA damage in rat. Curcumin nano formulation has a better outcome than native curcumin against cyclophosphamide-induced testicular genotoxicity (Poojary et al., 2021). Yadav et al. (2016) reported that arsenic and fluoride co-exposure induced DNA damage repaired by nanocurcumin. Chromosomal

damage due to Iodine-131 in peripheral lymphocytes was also repaired by nanocurcumin (Farhadi et al., 2018).

2.3.4.5. Anti-inflammatory activities of nanocurcumin

The better pharmacokinetic properties of nanocurcumin made it as a superior anti-inflammatory agent than native curcumin. Lower doses (15-fold) of nanocurcumin are equally effective as native curcumin in decreasing the production of inflammatory cytokines (IFNγ and TNFα) and chemokines (CXCL9 and CXCL10), chemokines receptor (CXCR3), inflammatory markers (Granzyme B) in cerebral malaria (Dende et al., 2017). Valizadeh et al. (2020) documented anti-inflammatory activities of nanocurcumin in covid-19 patients by decreasing the secretion of inflammatory cytokines like IL-1β and IL-6 mRNA expression. The better absorption property of nanocurcumin made it an effective therapeutic agent than free curcumin by modulating cytokine profile that enhances tumour cell growth and migration. Nanocurcumin reduces the cytokine secretion in activated T cells like TNF-α, IL-8, IL-6, IL-10, and IL-1β (Milano et al., 2013). Nanocurcumin showed a good effect in the treatment of central nervous disease by decreasing the expression of pro-inflammatory cytokines and increasing the expression of anti-inflammatory cytokines (Yavarpour-Bali et al., 2019).

Chapter: 3

Materials & Methods

3. MATERIALS AND METHODS

3.1. Preparation of Nanocurcumin and its characterization

3.1.1. Reagents

Curcumin: Curcumin was purchased from Sigma Chemicals Company, St. Louis

3.1.2. Procedure

For synthesis of nanocurcumin, 0.018 M curcumin solution was prepared by dissolving curcumin in dichloromethane (DCM). The solution was added to warm (50^0C) Milli-Q ultrapure water with a flow rate of 0.2 mL/min for 10 min under ultrasonic-pulse sonication subjected to constant stirring (Hielscher Ultrasonic Processor UP100H, Germany). The solution containing nanocurcumin turned yellow and separated by centrifugation. It was then sterilized by autoclaving, freeze-dried at - 80^0C followed by lyophilization (Eyela-FDU- 2000, Japan). A dry orange coloured powder of nanocurcumin was obtained.

The surface plasmon resonance of the laboratory synthesized nanocurcumin was analysed optically using UV-Vis spectrophotometer (UV-3101PC, Shimadzu, Japan). The nanocurcumin was morphologically characterised and size was measured by Field Emission Scanning Electron Microscopy (FE-SEM, FEI INSPECT F50, The Netherlands). The prepared nanocurcumin was characterized by Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer FTIR-1600, USA) by mixing dried powder of nanoparticles with KBr. Spectra were taken of 4 cm^{-1}. Existence of different functional groups was detected by FTIR. The crystalline nature of the prepared nanocurcumin was detected by XRD (Bruker AXS, Inc., Model D8, WI). The crystalline phase was detected by running the sample through 40 kV voltage and 40 mA current with 0.028 min^{-1} scanning rate within a range of 2Θ (5 to 40°).

3.2. Animals and Treatments

Female albino rats of Wistar strain (Rattus norvegicus), 30 in number, 60 - 75 days old, weighing 140-150 g were procured from the Animal Housing Facility and maintained with normal protein diet according to the guidelines of the Institutional Animal Ethics Committee of the Jadavpur University, Kolkata, India (Ref. No.: AEC/PHARM/1502/14/2015, Dated: 112 30/07/2015)24. The animals were maintained with normal protein diet containing 18% casein, 70% carbohydrate, 7% fat, 4% salt mixture, and 1% vitamin mixture (Hawk, Oser, & Summerson, 1954). Animals were divided into 6 groups, each containing 5 animals as described below –

i. Control group: These rats were fed with normal diet only.

ii. Nicotine group: The animals of this group were treated with nicotine (injected subcutaneously) with a dose of 2.5 mg/kg body weight for three weeks and maintained under normal protein diet.

iii. Nicotine and curcumin group: The animals of this group were treated with nicotine (injected subcutaneously) with a dose of 2.5 mg/kg body weight followed by supplementation of curcumin (80 mg/kg body weight) orally for three weeks and maintained under normal protein diet.

iv. Nicotine and nanocurcumin group: The animals of this group were treated with nicotine (injected subcutaneously) with a dose of 2.5 mg/kg body weight followed by supplementation of nanocurcumin (4 mg/kg body weight) orally for three weeks and maintained under normal protein diet.

v. Curcumin supplemented group (CS): These rats were fed with normal protein diet followed by supplementation of effective dose of curcumin (80 mg mg/kg body weight) orally for 21 days

vi. Nanocurcumin group: The animals of this group were supplemented with nanocurcumin (4 mg/kg body weight) orally for three weeks along with normal protein diet.

3.2.1. Administration of nicotine

A solution of nicotine hydrogen tartrate salt (2.5 g nicotine salt dissolved in one mL normal saline) was prepared as stock solution. It was diluted with normal saline to obtain a final concentration 2.5 µg/µL. Required volume of nicotine solution as per body weights of the animals was taken in a syringe and was injected subcutaneously daily for 21 days to the respective animals.

3.2.2. Administration of curcumin and nanocurcumin

Both, curcumin and nanocurcumin were dispersed separately in sterile distilled water and administered orally with gavages following three hours of nicotine administration in respective groups of animals. The doses of nicotine (2.5 mg/kg bodyweights/day), curcumin (80 mg/kg bodyweights/day) and nanocurcumin (4 mg/kg bodyweights/day) were administered for 21 consecutive days to the respective groups of animals as designed. Animals were kept in starvation for 12 h after 21 days of treatment and sacrificed on the next day following mild anaesthesia. Blood samples were collected immediately from the heart and stored in plain vial without any anticoagulant. Sera were separated and stored at - 20 ^{0}C for future analysis. Vital organs like liver, kidney, and pancreas were dissected out and stored in vacuum desiccators at - 20 ^{0}C to prevent auto-oxidation.

3.3. Biochemical Assays

3.3.1. Acid Phosphatase (ACP) Assay

The serum Acid Phosphatase level was determined by the process explained by Bessey et al., 1946 using commercially available kit (Arkray Healthcare Pvt. Ltd, India).

3.3.1.1. Reagents

- Acid buffer-substrate solution
- NaOH (0.1N)

3.3.1.2. Procedure

Two dry sterile glass tubes were taken and marked as “sample” and “control”, respectively. Acid buffer- substrate solution (1ml) was taken in each test tube and incubated for 37°C; 5 mins). The tubes were again incubated (37°C; 30 mins) after serum (0.2ml) was added in “sample”. Sodium Hydroxide (0.1N; 4ml) was added to each tube; serum (0.2ml) was added to “control” and mixed well. The O.D. measured was the difference of “Sample” with corresponding “Control”, against 405 nm in Spectrophotometer.

3.3.2. Alkaline Phosphatase (ALP) Assay

Serum Alkaline Phosphatase level was estimated using commercially available kit (Arkray Healthcare PVT. Ltd., India) following the method described by Kind and King, 1954.

3.3.2.1. Reagents

- Buffered substrate (pH 10)
- Chromogen reagent
- Phenol standard (10 mg %)
- Distilled Water

3.3.2.2. Procedure

Four dry sterile glass tubes were taken and marked as “Sample”, “Control”, “Blank”, and “Standard,” respectively. Distilled water (1.5ml) and 0.5 ml buffered water (pH 10) were taken in each tube, mixed thoroughly and incubated at 37°C for 3 mins Serum (0.05 ml) was taken in the “sample,” and 0.05ml of Phenol standard (10 mg %; 0.05 ml) was taken in the “standard” tube and incubated at 37°C for 15 mins. Chromogen reagent (1ml) was added in each test tube. Serum (0.05ml) was taken in “Control”. O.D. was measured against distilled water at 510 nm in the Spectrophotometer.

3.3.3. Aspartate aminotransferase (AST) or SGOT Assay

The level of AST was estimated as mentioned by Reitman and Frankel's (1957) using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.3.1. Reagents

- Alanine α Keto Glutarate substrate (pH-7.4)
- DNPH (2,4- dinitrophenylhydrazine) colour reagent
- NaOH solution (4N)
- 6mM working pyruvate standard (150IU/L)
- Deionized water

3.3.3.2. Procedure

Four dry, sterile glass tubes were taken and marked as Test, Control, Standard and Blank, respectively. 0.25 ml buffered alanine α keto glutarate substrate (pH 7.4) was taken in each test tube. Then 0.05 ml serum sample was added in the "test"; 0.05 ml pyruvate standard (6mM;) was taken in the "standard" tube, and incubated at 37°C; for one hour. Colour reagent 2,4-DNPH (0.25ml) was added in each tube. Then 0.05 ml deionized water was added in "blank"; and 0.05ml serum in "control" tube and incubated for 20 mins at 15°C to 30°C. Finally, 2.5ml NaOH (4N) solution was added in each test tube. O.D. was taken using a filter of 505 nm against "Blank".

3.3.4. Alanine aminotransferase (ALT) or SGPT Assay

The level of ALT was estimated as mentioned by Reitman and Frankel's (1957) using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.4.1. Reagents

- Alanine α Keto Glutarate substrate (pH-7.4)
- DNPH (2,4- dinitrophenylhydrazine) colour reagent
- 8mM working pyruvate standard (IU/L)
- Deionized water

3.3.4.2. Procedure

Four dry, sterile glass tubes were taken and marked as Test, Control, Standard and Blank, respectively. 0.25 ml buffered alanine α keto glutarate substrate (pH 7.4) was taken in each test tube. Then 0.05 ml serum sample was added in the "test"; 0.05 ml pyruvate standard (8mM;) was taken in the "standard" tube, and incubated at 37°C; for 30mins. Colour reagent 2,4-DNPH (0.25ml) was added in each tube. Then 0.05 ml deionized water was added in "blank"; and 0.05ml serum in "control" tube and incubated for 20 mins at 15°C to 30°C. Finally, 2.5ml NaOH (4N) solution was added in each test tube. O.D. was measured against distilled water at 505 nm.

3.3.5. Creatinine Assay

The estimation of creatinine in serum was done by the Alkaline Pictrate Method described by Moss et al., 1975 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.5.1. Reagents

- Picric Acid
- 0.75 (N) NaOH solution
- Stock Creatinine Standard (150 mg%)

3.3.5.2. Procedure

Serum sample (0.5ml) was taken in a glass tube, 0.05 ml distilled water and 3ml picric acid added with it and placed in a boiling water bath for 1min for deionization. Then the tube was cooled immediately and filtered using Whatman filter paper. Filtrate was used for the assay. Four dry, sterile glass tubes were taken and marked as Test, Control, Standard and Blank, respectively. Two ml of filtrate was taken in the "test" tube, 0.5 ml creatinine (150 mg%) in the "standard" tube, and 0.5ml double- distilled water in "Blank" tube. In "Blank" and "Standard", 1.5ml of picric acid was added. Finally, 0.5ml

of NaOH (0.75 N) was added in each tube mixed well and incubated at15°C to 30°C for 20 mins. O.D. was measured against double distilled water at 520 nm.

3.3.6. Urea Assay

The estimation of urea in serum was done by the Diacetylmonoxide (DAM) method described by Friedman and Young, 2000 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.6.1. Reagents

- Urea reagent
- Diacetylmonoxime (DAM)
- Working urea standard (30 mg%)

3.3.6.2. Procedure

Four dry, sterile glass tubes were taken and marked as Test, Control, Standard and Blank, respectively. Urea reagent (2.5 ml) was taken in each test tube. Serum sample (0.01ml) in the "Test"; 0.01 ml of urea standard (30mg %;) was in the "standard" tube was taken and mixed well. DAM was added in each tube and placed in boiling water bath for 10mins. After that tubes were cooled immediately under running tap water. O.D. was measured against "Blank" at 525 nm.

3.3.7. Estimation of Blood Urea Nitrogen (BUN)

The estimation of BUN in serum was done by calculation as described by Wallker et al., 1990 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

Calculation BUN= A x 0.467

Here, A= Urea (mg/dl) calculated as above.

3.3.8. Total Cholesterol Assay

Total cholesterol was estimated as described by Allain et al.,1974 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.8.1. Reagents

- Cholesterol mono reagent
- Cholesterol Standard

3.3.8.2. Procedure

Three dry, sterile glass tubes were taken and marked as Test, Standard and Blank, respectively. One ml of cholesterol mono reagent was taken in each tube. Then 10 µl of cholesterol standard added in "Standard" and 10 µl of serum in the "Test". Tubes were incubated at37°C for 5 mins. was taken using a filter of 505 nm against "Blank".

3.3.9. High-Density Lipoprotein (HDL- Cholesterol) Assay

Total cholesterol was estimated as described by Grundy, 1993 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.9.1. Reagent

- Cholesterol mono reagent
- Precipitating Reagent
- HDL- Cholesterol Standard

3.3.9.2. Procedure

In a dry test tube 200 µl serum and 200 µl precipitating reagent was taken and incubated for 10 mins at 15°C to 30°C then centrifuged at2000 x g for 15 mins for separation of HDL-Cholesterol (HDL-C). The supernatant was used for estimation of HDL. Three dry glass tubes were taken and marked as "Sample," "Blank," and "Standard," respectively. One ml of cholesterol mono reagent was taken in each tube. HDL- Cholesterol Standard (100 µl) was added in "Standard"; 100 µl sample in "Sample," mixed well and incubated at 37°C for 10 mins. The O.D. was taken using a filter of 505 nm against "Blank".

3.3.10. Triglycerides Assay

Serum triglyceride was estimated following the method described by Werner et al., 1981 using commercially available kit (Arkray Healthcare Pvt. Ltd., India).

3.3.10.1. Reagents

- Triglyceride mono reagent
- Triglyceride Standard solution

3.3.10.2. Procedure

Three dry glass tubes were taken and marked as "Sample," "Blank," and "Standard," respectively. One ml of triglyceride mono reagent was taken in each tube. 10 µl of triglyceride standard solution was taken in "Standard" and 10 µl serum in "Sample", all tubes were incubated at 37°C for 10 mins. O.D. was taken using a filter of 505 nm against "Blank".

3.3.11. Estimation of Very Low-Density Lipoprotein (VLDL) Cholesterol

VLDLC was calculated by the formula described by Suchanda, 2008.
VLDLC = Triglycerides (mg/dl)/ 5

3.3.12. Estimation of Low-Density Lipoprotein (LDL)

LDL cholesterol was calculated by the using the formula described by Oliveira et al., 2013
LDL cholesterol= Total cholesterol (mg/dl) – Triglycerides (mg/dl)/5 – HDL-C (mg/dl).

3.3.13. Estimation of Lipid Peroxidation

The level of lipid peroxidation was estimated by thiobarbituric acid test procedure as described by Chatterjee and Agarwal, 1988.

3.3.13.1. Reagents

- Phosphate buffer
- 20% trichloroacetic acid solution
- Thiobarbituric acid (TBA)

3.3.13.2. Tissue preparation

0.5 gm of liver tissue was homogenized in 5 ml phosphate buffer (0.1M; pH 7.5) and centrifuged at7000 x g for 20 mins. The assay was done using the clear supernatant.

3.3.13.3. Procedure

Two ml of homogenate, 1 ml TCA and 2 ml TBA were taken in a Stoppard tube mixed thoroughly and heated for 10 mins in boiling water bath. Then cooled immediately and centrifuged at 5000 x g for 10 mins.) The OD was taken at 530 nm against blank.

Calculation

Lipid peroxidation level was calculated by the malondialdehyde (MDA) level produced by degradation of hydro-peroxide. Extinction coefficient of MDA is 1.56 x 105 / M/ cm (Sinnhuber et al., 1958). The results were expressed as nano mole MDA/mg of protein.

3.3.14. Estimation of Catalase (CAT)

Estimation of catalase was done by the enzyme-catalysed decomposition of H2O2 as described by Cohen et al., 1970.

3.3.14.1. Reagents

- Phosphate buffer (0.01 M; pH 7.0)
- H_2O_2 (6 mM)
- $H2SO_4$ (6 N)
- $KMnO_4$ (0.01 N)

3.3.14.2. Tissue preparation

0.5 gm of liver tissue was homogenized in 5 ml phosphate buffer (0.01M; pH 7.5) and centrifuged at 7000 x g for 20 mins. The assay was done using the clear supernatant.

3.3.14.3. Procedure

Three dry glass tubes were taken and marked as Sample, Blank, and Standard. Cold sample, buffer and distilled water; 0.5 ml were taken in three different tubes respectively. The enzymatic reaction was started by thorough mixing in 5 ml cold H_2O_2 for 3 mins. Then reaction was stopped by adding 1 ml H_2SO_4 (6N). O.D. was measured at 480 nm immediately following addition of 7 ml $KMnO_4$ (0.01N)

Calculation

The catalase activity was expressed by the rate of decomposition of H_2O_2 using the formula: K=log (S0/S3) X 2.3/T

K = (Log S0/S3) x 2.3/ T
K = first-order reaction rate constant
T = time interval for which reaction was carried out (3mins)
S0 = substrate concentration at zero time
S3 = substrate concentration after 3mins
Catalase activity is expressed in nmol H_2O_2 decomposed / min/mg protein

3.3.15. Estimation of Superoxide Dismutase (SOD)

Estimation of SOD activity was done by the enzymatic reduction of nitro tetrazolium to pharmazone by superoxides as described by Beauchamp and Fridovich, 1971.

3.3.15.1. Reagents

- mM Phosphate buffer (pH 7.8)
- Methionine (650 mM)

- Riboflavin (900 UM)
- Nitro-blue tetrazolium (NBT) (7.5 mM)
- EDTA (200mM)

3.3.15.2. Tissue preparation

Liver tissue (5gm) was homogenized in 5 ml phosphate buffer (0.01M; pH 7.8) and centrifuged at 7000 x g for 20 mins. The assay was done using the clear supernatant.

3.3.15.3. Procedure

50 µl tissue homogenate, 1.7 ml phosphate buffer, 150 µl EDTA, 600 µl methionine, and 300 µl NBT taken in a glass tube. A tube devoid of the tissue homogenate was used as "Control". Riboflavin (200 µl) was added in each tube and kept under tube light. O.D. was measured at 560 nm at various time intervals.

Calculations:

$$Z = (X - Y)/ X *100$$

Z= SOD activity in nmol O2 decomposed / min / 100 mg protein

X= Optical Density at zero mins

Y= Optical Density at 2 mins.

3.3.16. Estimation of Glutathione Peroxide (GPx)

GPx activity was measured on the basis of oxidation of the selenol by H_2O_2 (Levander et al., 1983).

3.3.16.1. Reagents

- 50 mM Phosphate buffer (pH 7.0)
- 50 mM EDTA
- 200 mM Glutathione (GSH)
- NADPH

Reaction mixture: Phosphate buffer 11.808 ml was added with EDTA, 20 µl glutathione reductase, 64.8 µl GSH, and 1.2 mg NADPH. Then 108 ml of Phosphate buffer was added and pH adjusted to 7.0.

3.3.16.2. Tissue preparation

Liver tissue (5gm) was homogenized in 5 ml phosphate buffer (50mM; pH 7.8) and centrifuged at 7000 x g for 20 mins. The assay was done using the clear supernatant.

3.3.16.3. Procedure

Three ml of reaction mixture was taken in a glass tube then 50 µl of homogenate mixed with it and O.D. was measured at 340 nm. After that, 10 µl of H_2O_2 was added. OD was measured following 0, 30, and 60 secs of H_2O_2 addition. Another tube containing reaction mixture (3 ml), 50 µl phosphate buffer, and 10 µl H_2O_2 were mixed, and O.D. was taken at 340 nm and used as 'Blank'.

3.3.16.4. Calculation

Units/ml enzyme = sample optical density x 2 x total volume of assay x dilution factor (6.22) x volume of sample used.

GPx activity = Units/ml enzyme amount of protein value were expressed as n mole/ min/ mg protein.

3.3.17. Calculation of Superoxide and peroxide handling capacities (SPHC)

Superoxide and peroxide handling capacities (SPHC) were calculated as described by Nayak et al., 2014.

GSH-independent SPHC= CAT/SOD

GSH-dependent SPHC = GPx/SOD

3.3.18. Total protein estimation from tissues

The total protein content of various tissues like; liver, kidney and ovary were estimated by Lowry method (Lowry et al., 1951).

3.3.18.1. Reagents

- Phosphate buffer (pH 7.0)
- Na_2CO_3 (2%) in 0.1 N NaOH
- NaK Tartrate (1%) in H_2O
- $CuSO_4$. 5 H_2O (0.5%) in distilled H_2O
- Reagent I: A -48 ml + B – 1ml + C-1ml
- Reagent II- Folin-Phenol [2 N]: water [1:1]
- BSA Standard - 1 mg/ ml

3.3.18.2. Tissue preparation

0.5 gm tissue (liver, kidney, ovary) was homogenized in phosphate buffer (5 ml; 50mM; pH 7.0) and centrifuged (7000 x g; 20 mins.). The clear supernatant was utilized for the assay.

3.3.18.3. Procedure

A standard graph was drawn using known concentrations of BSA. The amount of protein in the test sample was determined from the standard graph drawn.

The amount of protein in samples was measured from the standard graph according to the respective OD values.

3.3.19. Protein Oxidation Assay

3.3.19.1. Reagents

- Phosphate buffer (pH 7.4)
- Tri-chloro acetic Acid
- DNPH (10mM)
- HCl (2N)
- Ethanol

- Guanidine hydrochloride (6 M)

3.3.19.2. Procedure

Protein oxidation was estimated following the method of Levine et al., (1994) by measuring the carbonyl content of serum. Serum was diluted with phosphate buffered saline with the ratio of 1: 40, and centrifuged for 10 mins at 12,000 rpm. The serum protein was precipitated by centrifugation for 5 mins following addition of cold trichloroacetic acid (TCA, 20% final concentration). Solution 10 mM DNPH in 2 N HCl was mixed with precipitated protein and kept in the dark for 1 h with regular vortexing in 15 mins interval. The protein solution then centrifuged at 12000 rpm for 5 min following mixing with cold TCA (final concentration 10%). The supernatants were discarded and protein pellets were washed with 10% TCA by centrifugation. One ml of ethanol/ethyl acetate (in the ratio of 1:1, v/v) was added to eliminate any free DNPH. Samples were suspended in 6 M guanidine hydrochloride and incubated at 37 °C for 15 min with thorough mixing. OD was estimated against 366 nm.

3.3.20. Estimation of Haemoglobin

Haemoglobin% was estimated by the Sahli's Haemoglobinometer (Chatterjee, 1994).

3.3.20.1. Reagents and accessories

- Haemoglobinometer
- HCl (0.1N)

3.3.20.2. Procedure

The Haemoglobinometer consists of two tubes, 20 mm3 of haemolysed with same volume of 0.1N HCl and saturated C.O. gas; the colour of this tube was taken as the standard. An equal volume of HCl (0.1N) was mixed with a blood sample in another tube, and colour development was observed. The colour intensity of the sample adjusted with that of standard by adding HCl (0.1N).

3.3.21. DNA extraction from blood

The DNA was extracted from blood sample and content was estimated by slightly modified method of the National Institute of Health protocol, 2004, as described by Banerjee et al., 2012.

3.3.21.1. Reagents

- RBC lysis buffer
- Nuclei lysis buffer
- SDS (Sodium Dodecyl Sulphate) (10%)
- Proteinase K
- NaCl solution (6 M)
- Ethanol
- Tris-EDTA (T.E.) buffer (pH 8)

3.3.21.2. Procedure

DNA was extract by salting-out procedure. RBC lysis buffer (200µl) was added with 200µl EDTA blood and allowed for 20 minutes at room temperature. The tube was centrifuged at 1000 x g for 10 mins at 4°C. The pellet was separated by discarding the supernatant. Nuclei lysis buffer, proteinase K and SDS was added and the pellet was missed thoroughly and incubated at 65°C for 2-3 hrs. Ice-chilled ethanol (100%) was added and the contents were mixed by inverting the tube 10-20 times. The tube was centrifuged at 10K for 10 mins for DNA precipitation. The DNA was spooned with a wide-mouthed l tip and transferred to a micro-centrifuge tube containing 70% ethanol. The tube was centrifuged at 13K for 10 mins at 4°C. The supernatant was discarded precipitated DNA was dissolved in T.E. buffer (pH-8). The concentration of DNA and its purity were measured at A230, A260, and A280 using the Elico spectrophotometer.

Calculation

Concentration of DNA (µg/ml) = (OD at A260 – OD at A320) X dilution factor X 50 µg/ml.

Total DNA isolated (µg) = Concentration of DNA X Final sample volume (ml).
Purity of DNA = A260/A280

3.3.22. DNA Extraction from Tissue Cells

The DNA was extracted from the tissue cell and content was estimated by slightly modified method as described by Gupta, 1984.

3.3.22.1. Reagents

- Homogenizing buffer (1% SDS, 50 mM EDTA)
- Tris HCl (1 mM; pH 7.4)
- Proteinase K (100 µg/µL)
- Phenol (Distilled)
- Sevag (chloroform: isoamyl alcohol; 24:1)
- Ethanol
- NaCl (5 M)
- Tris-HCl (50 mM; pH 4)
- RNAse (100 µg/ml)
- Tris-EDTA

3.3.22.2. Procedure

Liver tissue (50 mg) and homogenizing buffer were taken in a tube and homogenized with tissue rafter (REMI-RQ127A), proteinase K (100 µg/µL) was then added and incubated at 45ºC for 45 mins). After incubation 0.5 ml 1 mM Tris HCl (pH- 7.4) was added and centrifuged at 4000 x g for 10 mins at 4ºC). Supernatant was discarded sequentially with 1 volume of phenol, 1:1 mixture of phenol/Sevag and, Sevag was mixed and allowed for 5 mins, 3 mins and 3 mins respectively. At the separation phase was done by centrifugation at 14000 x g for 25 mins. at 4ºC). NaCl (5 M) 0.1 volume and 1 ml ethanol (100%) was added and kept at -20ºC for one hour. Finally, the DNA was precipitated by centrifugation at14000 x g; for 15 mins. The precipitated DNA was rinsed twice with 70% ethanol and dissolved in T.E. buffer (0.5 ml). RNAse (100 µg/ml) in Tris-HCl was added and incubated at 38ºC for 30 mins for removal of RNA.

DNA was dissolved in T.E. buffer (0.2 ml). The concentration, amount and purity were measured spectrophotometrically at A230, A260, and A280 (Elico Spectrophotometer).

Calculation:

Concentration of DNA (µg/ml) = (OD atA260 – OD at A320) X dilution factor X 50 µg/ml.
Total DNA isolated (µg) = Concentration of DNA X Final sample volume (ml).
Purity of DNA = A260/A280

3.3.23. Comet Assay: Estimation of DNA damage

The DNA damage was estimated by comet assay as described by Singh et al., 1988 and Kido et al., 2006 with minor modifications.

3.3.23.1. Principle

Comet Assay for measuring DNA damage in eukaryotic cells was first described by Ostling and Johanson in 1984. The assay is based on single cell gel electrophoresis (SCGE) technique to determine the nature of breakage in single and/or double-strand in DNA. This method immobilizes the cells on a double-layered gel slide of low and high melting agarose. The immobilised cells are then lysed with alkaline solution and allowed for electrophoresis to detect the type of comet shape following electrophoresis. The damaged and fragmented DNA will lose its organized and compact structure and will move out towards the anode due to its overall negative charges. On the other hand, the undamaged DNA will not leave their tightly associated nuclear association. This migration of damaged and breakage DNA will form a unique pattern resembling to that of a comet following ethidium bromide staining.

3.3.23.2. Reagents

- High melting agarose (Melting point 40 – 42°C) (2%;)
- Low melting agarose (0.5%)
- Lysis buffer (Tris- EDTA) pH 10

- Alkaline Electrophoresis Buffer (300 mM NaOH in 1 mM EDTA) pH 13
- Neutralizing buffer (0.4 M Tris-HCl) pH 7.5
- Homogenizing Buffer (0.075M NaCl; 0.024M EDTA)
- Ethidium bromide (20 µg/ml)

From Blood:

3.3.23.3. Procedure

Kido et al., 2006 procedure was slightly modified for the comet assay.

High melting agarose (200 µL; Genei, India) was layered on a pre-cooled frosted glass slide, covered with a cover slip and allowed for casting. Low melting agarose (Genei, India) was melted and 80 µL of it was taken in a micro-centrifuge tube, 20 µL whole blood was mixed with it. The cover slip from the previously-prepared slide was removed thus creating a groove and the low melting agarose blood mixture layered gently in the groove. It was covered with a cover slip and allowed to cast for 15 min.). The cover slip was removed cautiously and incubated at 4^0C for 2 hrs. in freshly prepared cold lysis solution. The slides were placed in alkaline electrophoresis solution at 4^0C, on a horizontal gel electrophoresis apparatus and placed in a dark place for15 mins. Electrophoresis was started (1 v/cm; 250 mA) in the dark for15 mins. Following electrophoresis, the slides were washed in neutralizing buffer. The slides were examined and photographed with Leica fluorescent microscope Model 300 FX at 40 x magnification following Ethidium bromide staining.

3.3.23.4. Measurement of comet parameter

The measurement of the comet head diameter, tail length, and percentage of DNA damage was accomplished by the process explained by Uhl et al., 2000. 50 cells per animal were screened and examined under a fluorescent microscope Model 300 FX at 40 x magnification. Quantification of DNA damage for each cell was determined by Image J software as:

Total amount of DNA in the comet = (Total comet Area) × (Mean DNA intensity)

Total DNA in the comet head = (Total Head area) × (Mean DNA intensity)

% Of DNA damage = (Total DNA in comet) – (Total DNA in comet head) × 100
(Total DNA in Comet)

§ Tail length (lT): Distance between the end of the comet head and the end of the DNA migration

§ Head Area (A.H.): The area covered by comet head.

§ Comet Area (A.C.): The area covered by the total comet.

§ Mean Head intensity (I.H.): The mean intensity of the pixels located in the head area.

§ Head DNA (DNAH): The sum of the intensities located in the head area

DNAH = AH x IH

§ Tail Area (AT): The area covered by the comet tail.

AT = AC - AH

§ Tail DNA, DNAT: The difference between total comet DNA and head DNA.

DNAT = DNAC - DNAH

§ Percent Tail DNA (% DNAT): The percentage DNA in the tail.

% DNAT = 100 DNAT / (DNAT+ DNAH)

§ Tail Moment (M.T.): The product of tail length and fraction of DNA in the tail.

MT = lT x % DNAT

3.3.24. Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA is based on solid-phase enzyme immunoassay (EIA) to estimate the lowest range of ligand/antigen (ng/mL to pg/mL) in a liquid sample. It is an important diagnostic tool in medicine for quality-control and detection of specific protein in solutions (Savige et al., 1998). The ligand is measured by a ligand-specific antibody. Antigen (Ag) is coated on a solid surface (polystyrene microtiter plate), either non-specifically or specifically ("sandwich" ELISA). The targeted antibody (Ab) is immobilized with the antigen and forms a complex of antigen-antibody. Antibody may be detected by any secondary antibody is bio-conjugated by an enzyme. The aggregation of both the antibodies (primary and secondary) responsible for changing the colour of the product which indicates the presence of Ag: Ab complex. It is a diagnostic

method for detection of either antigen of antibody both qualitative and quantitatively.

In my study, the quantification of Insulin, Cytokines (IL-4, IL-6, TNF-α, and IFN-γ), Apoptotic Markers (BAX and Bcl-2), and hormones (Estradiol and Progesterone) were done by commercially available ELISA kit Genetix Puregene (India). Sandwich ELISA method was used for quantification of above-mentioned parameters. The Specific monoclonal antibody pre-coated microplates were used. Respective standard, control and sample were taken in the wells of microtitration plate specific for any particular parameter. The unbound materials were washed out, and an enzyme-linked polyclonal antibody specific to the parameter was put in the wells. Unbound antibodies, if any, were washed out and specific substrate solution was added. Stop solution was added to stop the reaction. Different intensity of colour depending upon products was measured. Quantification of studied parameters was done by calculating the ODs of samples and standard and control from the standard curves.

3.3.25. Molecular Docking

The mode of interaction of DNA, Cyt-C, haemoglobin, α-LA and serum proteins with nicotine and nano-curcumin was done by UCSF CHIMERA 1.13.1 molecular modeller system using GEMDOCK program for computing ligand conformation and orientation in respect to active site of receptor. Protein Data Bank (PDB ID: 1TUP) for tumour suppressor P53 complex with DNA, PDB ID: 1IRD for haemoglobin, PDB ID: 1A4V for α-LA protein and PDB ID: 5EXQ for Cyt-c protein was used as receptor. For docking, PubChem CID: 89594 for nicotine and PubChem CID:969516 for nanocurcumin was taken as ligand molecule. Human serum protein showed 73% homology with that of *Rattus norvegicus* and was used for molecular docking study. The chemical structure of nanocurcumin and curcumin was same Bhawana et al., 2011 so the PubChem ID of curcumin was considered for further studies.

3.3.25.1. Interaction Studies of Nicotine vs. Haemoglobin and Nanocurcumin

A solution of nicotine hydrogen tartrate (10 mM) in haemoglobin (3 mg/ml normal saline) was prepared. One ml of haemoglobin solution was placed in a quartz cuvette and absorbance spectrum was noted (300 nm – 500 nm) against normal saline as blank by an UV-Visible spectrophotometer. Different concentrations of nicotine solution (50 to 500 μM) were added to the haemoglobin solution and mixed well and the spectrum of each mixture was taken. The nicotine - Hb interaction was varied in different concentration of nicotine and it was increased till the complete suppression of Hb absorbance peak. Then, freshly prepared nanocurcumin was added gradually to nicotine + Hb solution till the final concentration of nanocurcumin reached to 10 to 50 μM in the solution. The absorbance was recorded at different concentration of nanocurcumin at 300 nm – 500 nm against normal saline as blank.

3.3.25.2. Interaction Studies of Nicotine vs. DNA and Nanocurcumin

One ml of DNA solution (30 μg/mL) in TE buffer was taken in a quartz cuvette and absorbance spectrum was noted (240 nm – 320 nm) against TE buffer as blank. Nicotine solution with different concentrations was added with DNA solution till the final concentration of nicotine from 50 to 250 μM, mixed well and the spectrum at different concentration of nicotine was taken. The nicotine concentration increased gradually till the suppression of DNA absorbance peak sufficiently. Freshly prepared nanocurcumin was added gradually to nicotine - DNA solution till the final concentration of nanocurcumin was reached 10 to 50 μM in the solution. Absorbance spectrum was recorded at 240 nm – 320 nm against TE buffer as blank at different concentration of nanocurcumin.

3.3.25.3. Interaction Studies of Nicotine vs. Protein and Nanocurcumin

From our previous studies it was observed that 500 μM nicotine concentration can suppress the absorbance peaks of different cellular proteins completely (50 μg/ml saline) (Zeng et al., 2016). So, the recording of nicotine (500 μM) absorbance spectrum was initiated from 230 nm to 300 nm. Freshly prepared nanocurcumin was gradually added with nicotine solution till the final concentration of nanocurcumin reached from 10 μM to 100 μM, incubated at room temperature for 15 minutes and the absorbance spectrum in different concentration of nanocurcumin was recorded.

To study nicotine-treated α–LA protein vs. nanocurcumin interaction, absorbance spectrum of α–LA protein (50 μg / ml water) was recorded (230 nm to 500 nm). Nicotine (500 μM) was then added to the α–LA protein and absorbance spectrum was recorded (230 nm to 500 nm). Various concentrations of nanocurcumin were added gradually to the nicotine-treated protein solution till the final concentration of nanocurcumin in the solution reached from 10 μM to 100 μM and absorbance spectra were recorded. Similar experiments were performed by using nicotine-treated Cyt-c protein vs. nanocurcumin.

3.3.26. Histo-pathological study of the different tissues

The histological studies of liver, kidney of treated and untreated rats was done following proper fixation and staining.

3.3.26.1. Material / Accessory Required

- Compound microscope
- Glass Slides
- Cover Slip
- Absolute alcohol
- Haematoxylin
- Eosin

- Bouin's fluid (for fixation)

a. Fixation: Soft tissues of vital organs like liver, kidney was cut into pieces put in a considerable amount of Bouin's fluid for 24 hrs. Tissues were washed with 70 % and 80% alcohol for several times to remove the fixative fluid and kept in 70% alcohol.

b. Embedding: The tissue specimens were dehydrated gradually by increasing concentrations (50%, 70%, 80%, and 95%) of alcohol. After that the tissues were kept in a mixture of absolute ethanol and chloroform for 4 hrs and then to chloroform for additional 4 hours. After that, the specimens were shifted to a saturated solution of paraffin and chloroform. The temperature of the solution was maintained by placing in a paraffin bath at 56^0C -58^0C. The specimens were kept in melted paraffin for 3 hrs. The specimen was then transferred into an embedding frame comprises of two glass plates and two L-shaped metal bodies by a spatula. The paraffin was cooled quickly by dropping the frame in cold water. The glass plate and metal frames were removed from the solid paraffin.

c. Cutting and Handling of Sections: Sectioning of the tissues was done by a rotary microtome. The specimen block was mounted over a special metal disc by heating. By up and down movement of the block against the knife several sections of tissue were made and remained stick to one another, forming the ribbon. The ribbons were placed on glass slides sequentially. A mixture of egg white and glycerine was used adhesive. Before staining, paraffin was removed by immersing in xylol for 5mins.

d. Staining (Haematoxylin and Eosin staining): Two different stains were used: Haematoxylin, a basic dye staining of nucleus and eosin an acidic dye for staining of cytoplasm. First, the slides with fixed sections were passed through different grades (95%, 80%, 70%, and 50%) of alcohols then to Water. After proper hydration, slides were kept in haematoxylin solution for 5mins or till the sections were turn to deep blue by examining under the microscope. Excess stain was removed by placing the slides in water for 15-30 mins. After that, the sections were passed through different concentrations (50%, 70%,

80%, and 95%) of alcohol for 1 min each and finally in 95% alcohol for 5 minutes. The sections were then put in alcoholic solution of eosin for 1-5 mins. Excess eosin was washed with 95% alcohol, until no stains were left. After this, the stains were put in a mix of equal parts of absolute alcohol and xylol for 5 mins.

e. Clearing and Mounting: Clearing agents make the sections transparent and prominent so that, histological features can be studied clearly. Xylol is a good clearing agent for haematoxylin and eosin staining. Stained slides were put in fresh xylol for 5 minutes.

Once the clearing agent was drained, A drop of Canada balsam / DPX was put immediately over the tissue sections following drying of clearing agent and a cover glass was placed gently over it and allowed to dry.

3.4. Statistical analysis

The experiments were done twice and all the data of different sets of experiments were entered in excel sheets. were averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied highly significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p<.01$) and ## implied highly significant ($p<.001$) of the data when compared with the data of nicotine + curcumin treatments.

3.5. Instruments and Apparatus used for Research

Centrifuge

In my study Plastocraft ROTA 3R centrifuge was used for experiment. This machine used for separate the fluid from the particles of different size and densities. It is mainly used in my study to isolate protein, DNA, plasma, serum from animals' peripheral blood. This centrifuge machine maintains the sedimentation principle and throughout the process maintains the required temperature.

Spectrophotometer

In current study UV-3101PC (Shimadzu, Japan) UV-VIS single-beam spectrophotometer was used. Several quantitative estimations of biochemical assays and DNA and various interaction study was done by this UV-Visible spectrophotometer.

LS 50B, Fluorescence spectroscopy was used my study which analyses fluorescence from a sample.

Fourier transform infrared spectroscopy (Perkin Elmer –FTIR-1600, USA) was used in my study for determining the functional group present in the nanocurcumin.

Probe sonicator

In nanotechnology probe sonicator mainly used to break the particle size and made it nano form. In my study for preparing nanocurcumin Hielscher Ultrasonic Processor- UP100H (Germany) was used.

Liophiliser

It is mainly used to extend the shelf life of the product by removing water from the frozen materials. For obtaining the orange powder of the nanocurcumin EYELA-FDU-2000 (Japan) liophiliser was used.

Field Emission Scanning Electron Microscope (FESEM)

FE-SEM, FEI INSPECT F50, (FEI Europe BY, The Netherlands) was used for determining the particle size of the nanocurcumin.

XRD (X-ray diffractometer)

XRD used for determining the crystallographic state of the material. Burker AXS, Inc., Model D8, WI was used for determining the crystallinity of nanocurcumin.

Fluorescent microscope

In current experiment, the fluorescent microscope was used in the comet assay to estimate the percentage of DNA damage within the whole blood cells. In this study Leica Model 300 FX fluorescent microscope was used in this experiment.

Chapter: 4

Results

4. RESULTS

4.1. Characterization of Nanocurcumin

Nanocurcumin was prepared from curcumin by ultra-sonication method. The prepared nano-curcumin appears as yellow coloured powder. The average particle size was measured as 65 ± 5 nm (Figure 4.1.A) by FESEM study. The powder is soluble in water and the solution is reddish yellow in colour with an absorption peak at 350 nm as measured by UV-visible spectrophotometer (Figure 4.1.B). The peaks appeared in The FTIR spectrum were exactly similar with that of curcumin where, peaks at 1,626 cm^{-1} indicate C=C stretching, at 1,507 cm^{-1} and 1,263 cm^{-1} indicate C=O stretching, at 1,153 cm^{-1} and 1,115 cm^{-1} corresponds to C – H stretching. The absorption peaks at 1025 cm^{-1} were due to C-N stretching (Figure 4.1.C). The crystalline nature of prepared nanocurcumin was confirmed by X-ray diffraction (Figure 4.1.D).

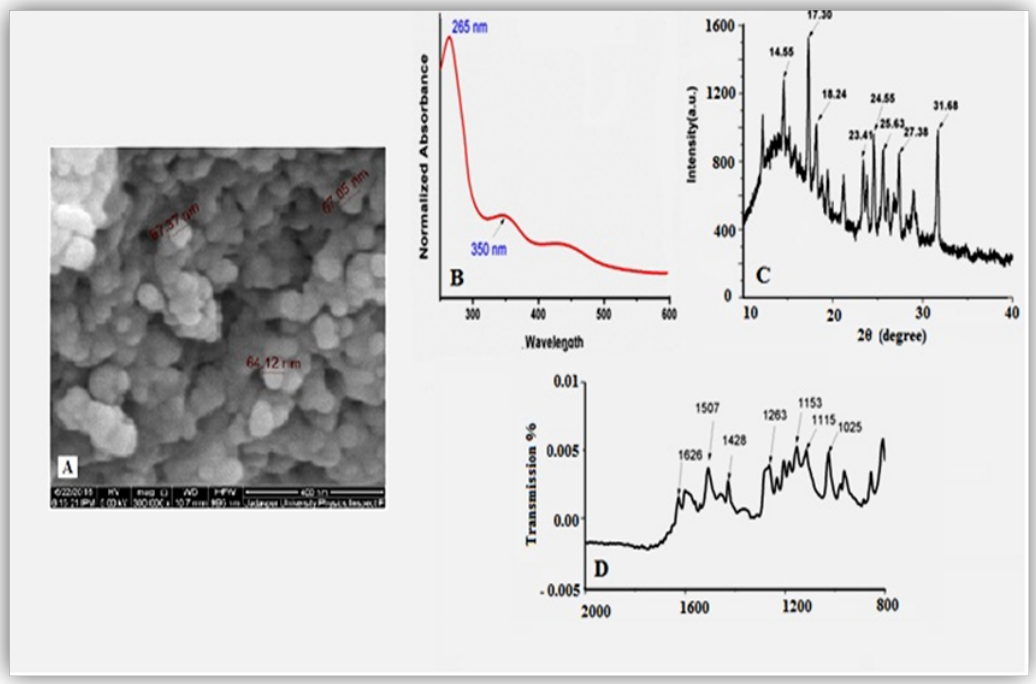

Figure 4.1: (A). Physical appearance and average particle size (65 ± 5 nm) as measured by FESEM. (B). Absorption peak showed at 350 nm by UV-visible spectrophotometer (C). Crystalline properties of prepared nanocurcumin as evidenced by XRD (D). FTIR spectrum of synthesized nanocurcumin

4.2. Ameliorative Effects of nanocurcumin on nicotine induced toxicities in female rat

Nicotine is known to be harmful for our health. Long continuous intake of nicotine shows toxic effects in biochemical, cellular, histo-pathological, immunological and molecular levels. Curcumin shows its potentiality to overcome the toxic effects of nicotine. But its poor pharmacokinetic properties, limits its therapeutic application. In the present study the potentiality of nanocurcumin has been assessed against nicotine toxicity and compared with to that of curcumin.

4.2.1. Effects of nanocurcumin on nicotine induced changes of hepatic enzymes

The activities of all four hepatic enzymes like ACP, ALP, ALT and AST levels were increased significantly (36.4%, 97.9%, 68.0% and 41.4% respectively) among the nicotine treated rats in compared to control group. Both curcumin and nanocurcumin showed significant protective effects against nicotine induced hepatic toxicity in terms of increased levels of those enzymes. But nanocurcumin was found to be more effective in this aspect than that of curcumin. The results of hepatic enzymes level are given in Table 4.1.

4.2.2. Effects of nanocurcumin on nicotine induced changes on renal function parameters

A significant increase in serum urea and creatinine (40.9% and 41.2% respectively) levels were noticed among nicotine exposed animals compared to normal animals (Table 4.2). The administration of curcumin and nano-curcumin along with nicotine showed beneficial effects by declining the levels of urea and creatinine. Nanocurcumin was more effective in terms of reducing serum urea and creatinine (5.8% and 14.3% respectively) than native curcumin (26.5% and 31.1%) among nicotine treated rats. The results of serum urea and creatinine levels are mentioned in Table 4.2.

Table 4.1: Levels of four hepatic enzymes in different study and control groups of female rats under normal dietary condition

Parameter	Groups				
	Control	Nicotine	Nicotine + Curcumin	Nicotine + Nanocurcumin	Nano-curcumin
ACP (m mol/ h/100 ml)	1.43 ± 0.10	1.95 ± 0.15** (36.4↑)	1.56 ± 0.17** (9.1↑)	1.47 ± 0.12** (2.8↑)	1.30 ± 0.11* (9.1↓)
ALP (m mol/ h/100 ml)	4.87 ± 0.60	9.64 ± 1.82** (97.9↑)	7.40 ± 0.98* (51.9↑)	5.55 ± 0.73** (14.0↑)	4.30 ± 0.13* (11.7↓)
AST (IU/L)	14.88 ± 2.10	25.13 ± 5.05** (68.9↑)	20.95 ± 2.49* (40.8↑)	16.97 ± 2.50** (14.0↑)	10.35 ± 0.90** (30.4↓)
ALT (IU/L)	34.58 ± 7.24	48.89 ± 7.79** (41.4↑)	42.29 ± 6.94* (22.3↑)	37.10 ± 2.47** (7.3↑)	36.40 ± 4.84* (7.3↑)

The experimental setup was repeated twice and all data were averaged over n = 12 animals and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the control. Similarly, # implied significant ($p < 0.01$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotine treatment.

Table 4.2: Levels of serum urea and creatinine in different study and control groups of female rats under normal dietary condition

Parameter	Groups				
	Control	Nicotine	Nicotine + Curcumin	Nicotine + Nanocurcumin	Nano-curcumin
Urea (mg/100 ml)	37.16 ± 2.63	52.36 ± 3.60** (40.9↑)	47.00 ± 2.78* (26.5↑)	35.00 ± 2.30** (5.8↓)	32.80 ± 1.19** (11.7↓)
Creatinine (mg/100 ml)	1.19 ± 0.11	1.68 ± 0.14** (41.2↑)	1.56 ± 0.50* (31.1↑)	1.36 ± 0.13* (14.3↑)	0.70 ± 0.15** (41.2↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the control. Similarly, # implied significant ($p < 0.01$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotine treatment.

4.2.3. Effects of nanocurcumin on nicotine induced changes in Lipid profile

A significant increase in triglyceride, cholesterol, VLDL-C and LDL-C (45.8%, 32.9%, 45.5% and 75.4% respectively) and decreased level of HDLC concentration (18.4%) in the serum of the nicotine treated rats were recorded in respect to control arm (Table 4.3). However, curcumin and nanocurcumin showed potential ameliorative effects by decreasing the concentrations of triglyceride, cholesterol, VLDL-C and LDL-C in the serum of the rats. Increased level of HDL-C was recorded in both curcumin and nanocurcumin arms compared to nicotine treated arm. The better role of nanocurcumin than unformulated curcumin in maintaining the level of lipid profile due to nicotine toxicity was observed.

Table 4.3. Levels of lipid profile in different study and control groups of female rats under normal dietary condition

Parameter	Groups				
	Control	**Nicotine**	**Nicotine +Curcumin**	**Nicotine +Nanocurcumin**	**Nano-curcumin**
Triglyceride (mg/dl)	118.6 ± 10.2	172.8 ± 9.4** (45.8↑)	150.9 ± 8.6* (27.3↑)	135.0 ± 5.8* (13.9↑)	99.4 ± 4.8** (16.2↓)
Cholesterol (mg/dl)	104.7 ± 10.7	139.1 ± 11.6** (32.9↑)	104.4 ± 7.1** (0.3↓)	102.9 ± 2.5** (1.7↓)	103.4 ± 9.1* (1.2↓)
HDL (mg/dl)	40.1 ± 1.0	32.7 ± 1.5** (18.4↓)	37.18 ± 1.6** (7.3↓)	38.5 ± 1.6** (4.0 ↓)	47.6 ± 3.6** (18.7↑)
VLDL (mg/dl)	24.0 ± 1.3	34.8 ± 1.4** (45.5↑)	30.7 ± 1.8* (28.3↑)	27.6 ± 1.8* (15.3↑)	19.9 ± 1.2** (17.0↓)
LDL (mg/dl)	41.3 ± 2.5	72.4 ± 4.8** (75.4↑)	57.9 ± 3.2* (40.2↓)	47.8 ± 2.5* (15.7↓)	36.0 ± 5.8** (12.8↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies $P < 0.01$ and ** implies $P < 0.001$ compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.4. Effects of nanocurcumin on nicotine induced changes on MDA

The level of MDA is a parameter to determine the rate of lipid peroxidation by different free radicals in plasma and liver. Nicotine induced peroxidation of lipids by free radicals resulting in increased level of MDA in plasma and liver cells (42.0% and 69.2% respectively). Both curcumin and nanocurcumin showed ability to reduce MDA levels in respect to nicotine treated animals. For reducing MDA level among nicotine treated animals nanocurcumin was more effective than curcumin (Table 4.4).

Table 4.4: Levels of MDA in different study and control groups of female rats under normal dietary condition

Parameter	Groups				
	Control	Nicotine	Nicotine +Curcumin	Nicotine +Nanocurcumin	Nano-curcumin
Plasma (n mol/ ml)	5.64 ± 0.61	8.01 ± 1.31**(42.0↑)	6.90 ± 0.79* (22.3↑)	6.51 ± 0.52* (15.4↑)	5.06 ± 0.50 (10.3↓)
Liver (n mol/mg protein)	14.72 ± 0.36	24.90 ± 2.17**(69.2↑)	20.36 ± 2.96*(38.3↑)	16.83 ± 2.21** (14.3↑)	14.26 ± 0.60* (3.12↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies $P < 0.01$ and ** implies $P < 0.001$ compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.5. Effects of nanocurcumin on nicotine induced changes on antioxidant enzymes

Antioxidants are the enzymes responsible for neutralising the free radicals and thus reduce the oxidative stress. In my present study, it was observed that the level of SOD, CAT, GSH and GPx was declined significantly in nicotine treated group comparing to control group (57.0%, 32.3%, 39.8% and 17.0% respectively). Increasing level of antioxidants by curcumin and nanocurcumin were noticed among nicotine treated animals. This ability was found to be higher in nanocurcumin group than curcumin group (Table 4.5).

Table 4.5. Levels of antioxidant enzymes in different study and control groups of female rats under normal dietary condition

Parameter	Groups				
	Control	Nicotine	Nicotine + Curcumin	Nicotine + Nanocurcumin	Nano-curcumin
SOD (n mol/O2 decomposed/ min/100 mg protein)	9.98 ± 0.40	4.29 ± 0.30** (57.0↓)	6.72 ± 0.14* (32.7↓)	7.61 ± 0.59** (23.7↓)	10.46 ± 1.08* (4.8↑)
CAT (n mol/H2O2 decomposed/ min/100 mg protein)	38.7 ± 7.6	26.2 ± 2.1** (32.3↓)	29.6 ± 1.8* (23.5↓)	31.9 ± 1.3* (17.60↓)	40.4 ± 1.9* (4.4↑)
GSH (µg/mg protein)	34.6 ± 1.0	20.8 ± 0.6** (39.8↓)	28.9 ± 0.5*(16.5↓)	30.2 ± 0.3** (12.7↓)	35.1 ± 0.6* (1.4↑)
GPx (n mol/ min/mg protein)	147.8 ± 2.7	126.4 ± 2.8** (17.0↓)	133.6 ± 2.3*(9.6↓)	136.0 ± 1.8** (8.0↓)	149.0 ± 1.2*(1.0↑)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.6. Effects of nanocurcumin on nicotine induced changes on haemoglobin

The concentration of haemoglobin decreased (26%) in nicotine injected group whereas, native curcumin and nanocurcumin both showed the ability in increasing the haemoglobin concentration in the nicotine treated group (Table 4.6).

Table 4.6: Levels of haemoglobin content in different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine +Curcumin	Nicotine + Nanocurcumin	Curcumin	Nano-curcumin
Hb%	13.3±0.3	9.8±0.6 (26.0↓)	12.2±1.3 (8.1↓)**	12.4±0.2 (6.6↓)**#	12.9±0.2 (3.0↓)	13.2±0.2 (1.0↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.7. Effects of nanocurcumin on nicotine induced changes in total protein in different tissues

The total protein contents of different tissues like- liver, kidney and ovary were decreased significantly in nicotine treated animals (25.5%, 21.5% and 24.3% respectively) (Table 4.7). The protein contents of those tissues were increased more significantly by the administration of animals nanocurcumin ($p < 0.001$) than curcumin ($p < 0.01$).

Table 4.7. Contents of total protein in different tissues of different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine+ Curcumin	Nicotine + Nanocurcumin	Curcumin	Nano-curcumin
Liver (mg/g)	23.9 ± 0.10	17.8 ± 1.0 (25.5↓)**	19.8 ± 1.8 (17.2↓)*	21.2 ± 1.6 (11.5↓)**#	24.1 ± 0.1 (1.0↑)	25.3 ± 0.1 (5.8↑)
Kidney (mg/g)	20.6 ± 3.2	16.2 ± 2.0 (21.5↓)**	17.9 ± 0.3 (13.2↓)*	19.1 ± 1.6 (7.3↓)**##	22.1 ± 0.5 (7.2↑)	22.7 ± 0.5 (10.2↑)
Ovary (mg/g)	20.9 ± 1.1	15.8 ± 1.6 (24.3↓)**	17.8 ± 1.9 (15.0↓) *	18.7 ± 1.8 (10.7↓)*#	21.1 ± 1.4 (1.0↑)	22.1 ± 1.4 (5.7↑)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies $P < 0.01$ and ** implies $P < 0.001$ compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.8. Effects of nanocurcumin on nicotine induced changes in protein carbonyl content in serum

About four times more carbonyl contents were detected in the serum proteins of nicotine induced rats. In contrast, both curcumin or nanocurcumin supplementation showed a reduction of carbonyl content in the serum protein of nicotine-treated animals. The effect of nanocurcumin against protein oxidation is more pronounced than that of curcumin (Table 4.8).

Table 4.8: contents of total protein carbonyl in serum of different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine + Curcumin	Nicotine+ Nano-curcumin	Curcumin	Nano-curcumin
Serum (n mole carbonyl/ mg) protein	0.81± 0.08	3.12± 0.11(285↑)**	1.96±0.18 (141↑)*	1.43±0.7 (76.5↑)**#	0.83± 0.06 (2.46↑)	0.65±0.08 (1.97↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.9. Effects of nanocurcumin on nicotine induced changes in total DNA content and percentage of DNA damage

The whole blood DNA contents was drastically reduced (39.8%) by nicotine as observed in nicotine treated rats (Table 4.9). Nanocurcumin was more effective in increasing the whole blood DNA content among nicotine treated group ($p<0.001$) than native curcumin ($p < 0.01$). Nicotine treatment was responsible for a massive DNA damage (39.97%) of the whole blood cells where as it was only 2.76% in control group. (Table 4.9). This DNA damage due to nicotine was significantly reduced by curcumin (>20%) and more significantly by nanocurcumin (>30%).

Supplementation of curcumin or nanocurcumin alone did not produce any negative effect on DNA content of blood cells rather they showed healthy condition of blood cells (Figure 4.2).

Table 4.9: Contents of total DNA and percentage of DNA damage in different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine +Curcumin	Nicotine +Nano-curcumin	Curcumin	Nano-curcumin
Blood (µg/300µL)	113.95 ± 2.70	68.56 ± 1.52(39.8↓) **	90.77 ± 5.02(20.3↓) **#	107.85 ± 4.42 (5.4↓)*##	114.72 ± 3.33(1.0↑)	116.67 ± 2.51(2.4↑)
% DNA Damaged	2.76 ± 0.30	39.97 ± 0.81	16.99 ± 1.60**	9.20 ± 0.22**##	2.32± 0.54	2.11± 0.48
Tail moment (Arbitrary unit)	70.5 ± 1.6	691.3 ± 10.60	245.5 ± 9.30*	162.00 ± 3.30**##	21.02 ± 3.4	20.6 ± 4.90

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

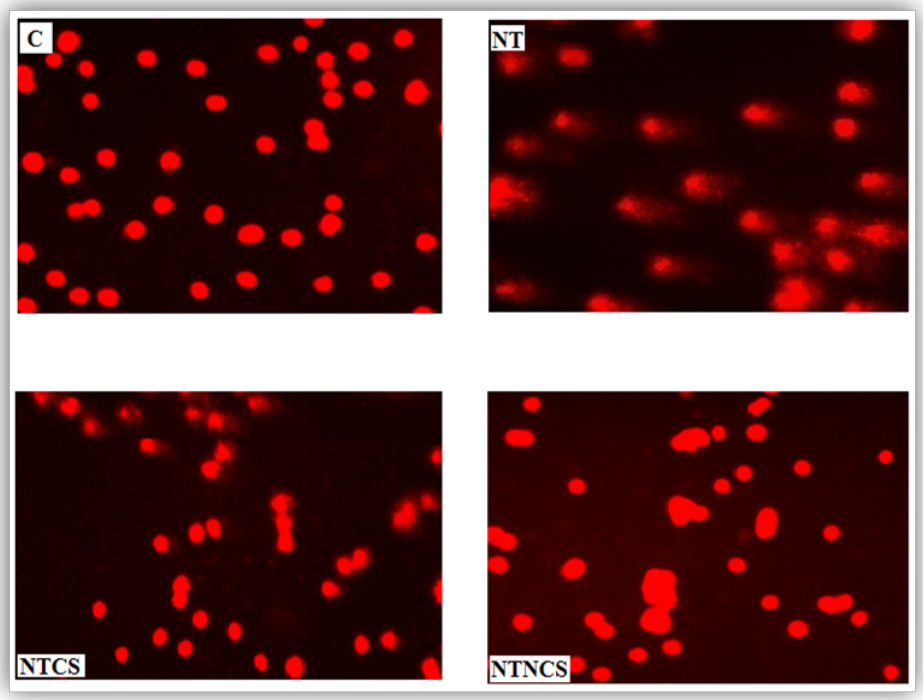

Figure 4.2: Photographs of Comet assay of whole blood of different groups: C= Control, NT=Nicotine, NTCS= Nicotine plus Curcumin and NTNCS= Nicotine plus Nanocurcumin

4.2.10. Molecular Docking study

4.2.10.1. Nicotine Vs. Haemoglobin

Molecular docking study of nicotine vs. haemoglobin showed the formation of complex structures of nicotine-haemoglobin molecule (Figure 4.3A). The best binding energy between nicotine vs. haemoglobin was observed to be around (-) 70.98 kcal among 10 ligand conformations.

4.2.10.2. Nanocurcumin Vs. Haemoglobin

Molecular docking study between nanocurcumin (here curcumin) vs. haemoglobin are given in Figure 4.4A. Out of 10 ligand conformations the best binding energy between curcumin vs. haemoglobin was recorded as (-) 97.41 kcal.

4.2.10.3. Nicotine Vs. DNA

The Slico docking experiment between p53 suppressor DNA and nicotine showed the formation of nicotine - DNA complex molecule (Figure 4.5A). The best binding energy between nicotine-DNA interaction was detected as (-) 69.48 kcal.

4.2.10.4. Nanocurcumin Vs. DNA

Molecular docking study between nanocurcumin (here curcumin) and DNA showed the formation of nanocurcumin – DNA complex molecule (Figure 4.6A). The binding energy between curcumin vs. DNA was about (-) 86.35 kcal.

4.2.10.5. Nicotine Vs. Nanocurcumin

In-silico docking of nicotine vs. nanocurcumin (here curcumin) showed the formation of complex molecule between them with a binding energy of (-) 51.29 kcal (Figure 4.7C).

4.2.10.6. Nicotine Vs. α-LA Protein

In-silico docking studies of nicotine vs. α-LA protein demonstrate clearly the formation of complex structures between nicotine and α-LA protein (Figure. 4.8A). The best binding free energy between the nicotine and α-LA protein was -57.3 Kcal.

4.2.10.7. Nanocurcumin Vs. α-LA Protein

Figure 4.8B showed that the complex structure between the nanocurcumin and α-LA protein and best binding free energy was around - 73.9 kcal.

4.2.10.8. Nicotine Vs. Cyt-c protein

In molecular docking studies of nicotine vs. Cyt-c protein clearly depicted the formation of complex structure between nicotine and Cyt-c protein (Figure 4.8C). The binding free energy between them was - 65.6 kcal.

4.2.10.9. Nanocurcumin Vs. Cyt-C protein

Molecular docking experiment on nanocurcumin vs. Cyt-c protein revealed the complex structure formation between nanocurcumin vs. Cyt-C is more favourable (free energy -74.4 kcal) than nicotine vs. Cytc complex formation. (Figure 4.8D).

4.2.11. UV-Vis study

4.2.11.1. Nicotine Vs. Haemoglobin

Figure 4.3B showed that nicotine interacted with the haemoglobin and was responsible for the suppression of the characteristic's absorbance peak of haemoglobin molecule at 400 nm. Figure 4.3C showed that binding constant between nicotine and haemoglobin molecule was K = 6.5 × 103 M^{-1}.

4.2.11.2. Nanocurcumin Vs. Haemoglobin

UV-Visible spectrophotometer studies of nanocurcumin and haemoglobin showed that there was no change of the characteristic peak of the haemoglobin

molecule (Figure 4.4B). The binding constant between the haemoglobin and nanocurcumin was higher ((binding constant, K = 10.4 × 104 M^{-1}) than the nicotine vs. Haemoglobin (Figure 4.4C). Figure 4.4D gives the information about the protective effect of nanocurcumin against nicotine toxicity by reappeared the characteristic peak of the haemoglobin molecule.

4.2.11.3. Nicotine Vs. DNA

UV-Visible spectral studies of nicotine vs. DNA (Figure 4.5B) showed that nicotine interacted with DNA which resulted suppression of the characteristic's absorbance peak of DNA at 260 nm. This interaction produced multiple absorbance peak of the DNA spectrum. The binding constant between nicotine and DNA was K = 10 × 103 M^{-1}. (Figure 4.5C)

4.2.11.4. Nanocurcumin Vs. DNA

Figure 4.6B showed that nanocurcumin bound with DNA but there was no major change of the characteristic maxima of DNA The binding constant between nanocurcumin and DNA was K = 7.8 × 103 M -1 (Figure 4.6C). Nanocurcumin nullified the destructive effect of nicotine by the reappeared of the characteristic spectrum of the DNA (Figure 4.6D).

4.2.11.5. Nicotine Vs. Nanocurcumin

The UV visible spectral studies showed that the characteristic peak of the nicotine (λ max = 260 nm) was suppressed by the addition of the different concentration of the nanocurcumin solution (10-50μM) to the nicotine solution (500μM) (Figure 4.7A). The binding affinity between nanocurcumin and nicotine was K = 3.5 × 103 M^{-1}(Figure 4.7B).

4.2.11.6. Nicotine –Nanocurcumin- α-LA protein

Figure 4.9A indicates that nicotine causes (500μM) reduction of the characteristic absorption peak of α-LA (λ max = 280 nm). Addition of different concentration of nanocurcumin (10-100 μM) reverses its characteristic spectrum.

4.2.11.7. Nicotine -Nanocurcumin- Cyt-c protein

UV-Visible spectral studies between nicotine and Cyt-c indicate that nicotine bound with this protein and reduced its characteristic absorption peak (λ max = 410 nm). Nanocurcumin showed its positive effect against nicotine toxicity by re-appearing the characteristic peak of the Cyt-c protein (Figure 4.9B).

4.2.12. Fluorescence spectrum study

4.2.12.1. Nicotine –Nanocurcumin- α-LA protein

Figure 4.10A showed that the intrinsic fluorescence emission spectrum of α-LA with emission maxima at λ max = 345 after excitation at 255 nm. Nicotine causes the blue shift of maxima (λ max = 330 nm) of α-LA protein. Addition of different concentration of nanocurcumin (10-100 μM) in the same solution results the increment of the fluorescence intensity of the protein.

4.2.12.2. Nicotine –Nanocurcumin- Cyt-c protein

The Fluorescence spectrum study between the nicotine and Cyt-c protein showed that the blue shift of the maxima of the protein. Gradual addition of the nanocurcumin causes increases the wavelength maxima of the treated protein (Figure 4.10B).

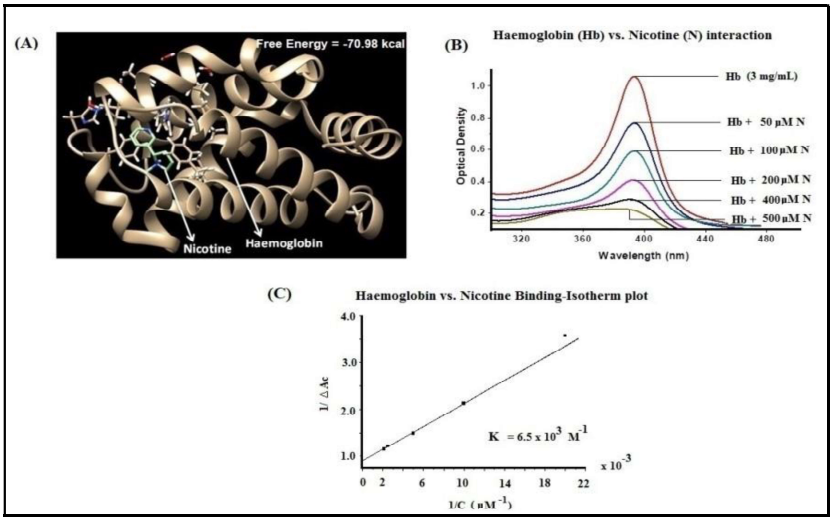

Figure 4.3: Interaction studies between nicotine vs. haemoglobin (Hb). A). Interaction between nicotine vs. Hb as evident by molecular docking. B). Interaction between nicotine vs. Hb as evident by UV-Vis spectra. C). Binding - Isotherm Plot between nicotine vs. Hb.

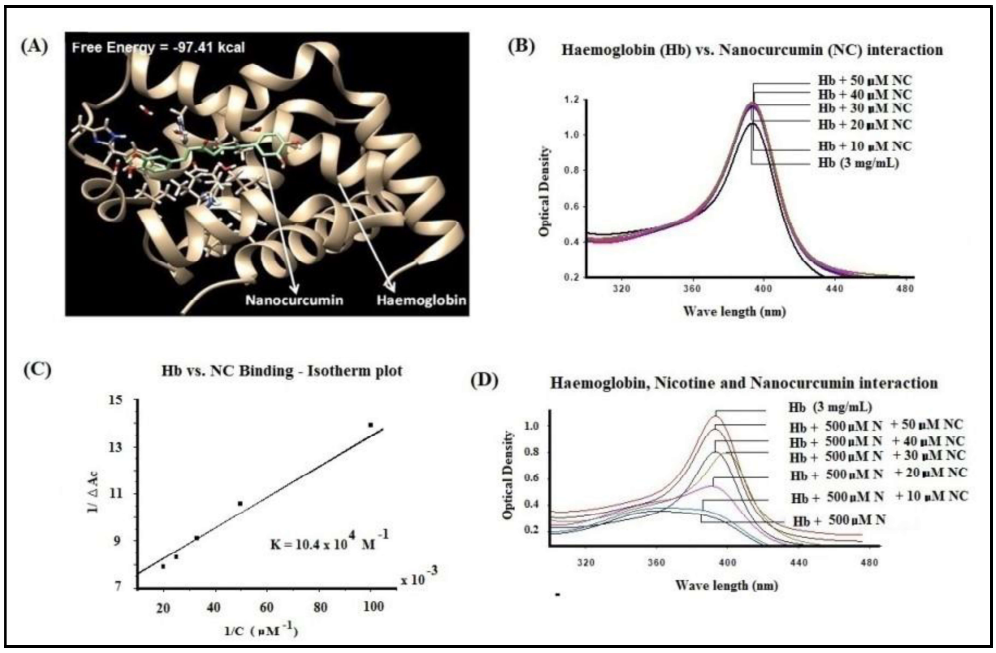

Figure 4.4: Interaction between nanocurcumin vs. haemoglobin (Hb). A). Interaction between nanocurcumin vs. Hb as evident by molecular docking. B). Interaction between nanocurcumin vs. Hb as evident by UV-Vis spectra. C). Binding -Isotherm Plot between nanocurcumin vs. Hb. D). Interaction between nanocurcumin vs. nicotine + Hb as evident by UV-Vis spectra

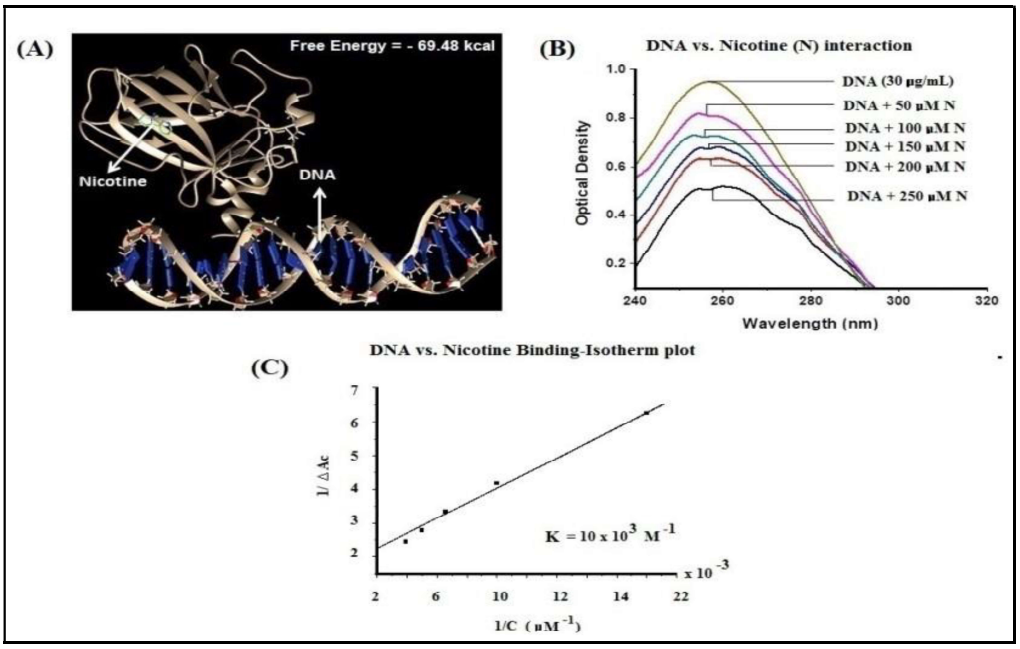

Figure 4.5: Interaction studies between nicotine vs. DNA. A). Interaction between nicotine vs. DNA as evident by molecular docking. B). Interaction between nicotine vs. DNA as evident by UV-Vis spectra. C). Nicotine vs. DNA binding -Isothermal plot

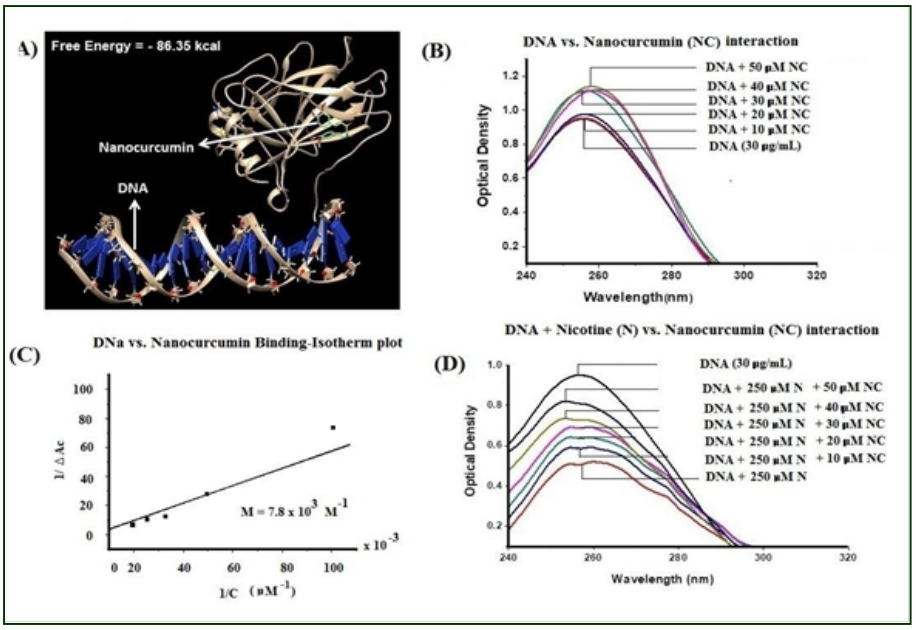

Figure 4.6: Interaction between nanocurcumin vs. DNA. A). Interaction between nanocurcumin vs. DNA as evident by molecular docking. B). Interaction between nanocurcumin vs. DNA as evident by UV-Vis spectra. C). Binding -Isotherm Plot between nanocurcumin vs. DNA. D). Interaction between nanocurcumin vs. nicotine + DNA as evident by UV-Vis spectra.

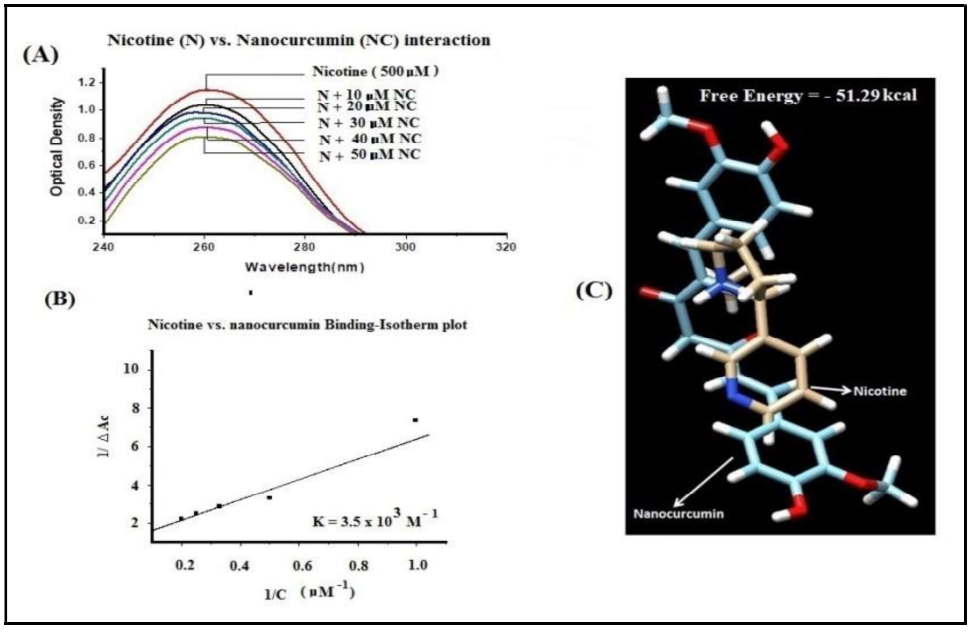

Figure 4.7: Interaction between nicotine vs. Nanocurcumin. A). Interaction between nicotine vs. Nanocurcumin as evident by UV-Vis spectra of. B). Binding -Isotherm Plot between nicotine vs. Nanocurcumin. C). Interaction between nicotine vs. Nanocurcumin as evident by molecular docking

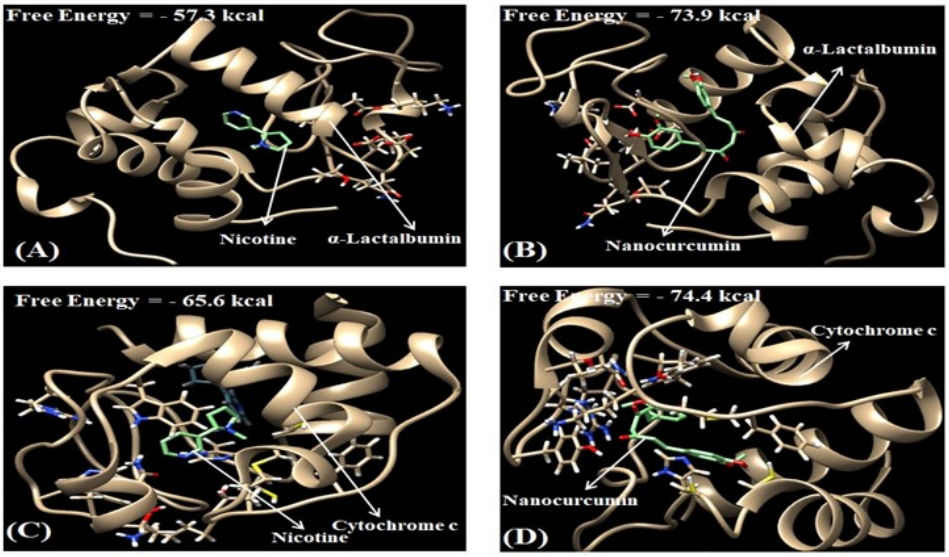

Figure 4.8: Molecular docking study between nicotine/nanocurcumin vs. α-LA/Cyt-c protein. A) Docking interaction between nicotine vs. α-LA; B) Docking interaction between nanocurcumin vs. α-LA; C) Docking interaction between nicotine vs. Cyt-c, and D) Docking interaction between nanocurcumin vs. Cyt-c protein.

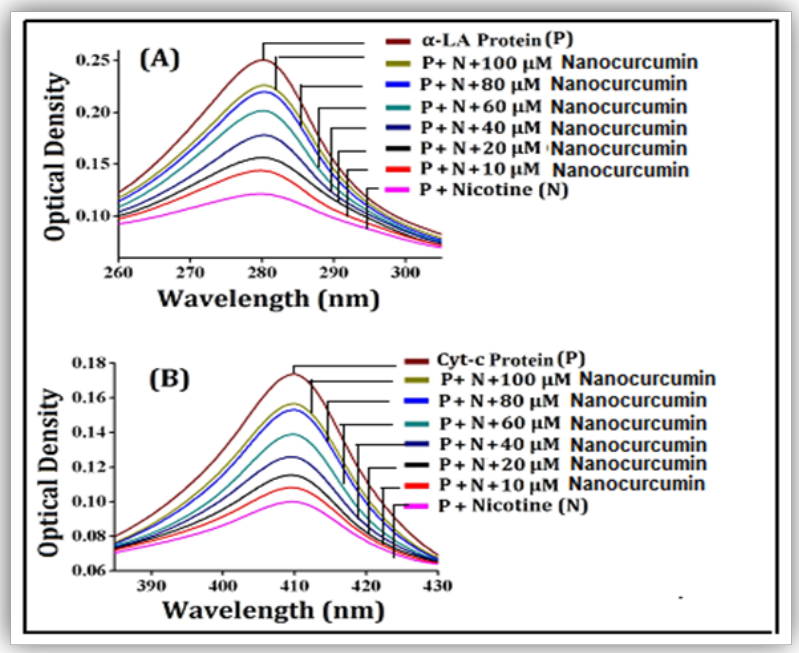

Figure 4.9: A) Shows UV- Visible interaction of nanocurcumin vs. nicotine (500 µM) mediated pure protein of α–LA (50 µg / ml water). B) shows UV-Visible interaction of nanocurcumin vs. nicotine (500 µM) mediated pure protein of Cyt-c (50 µg / ml water).

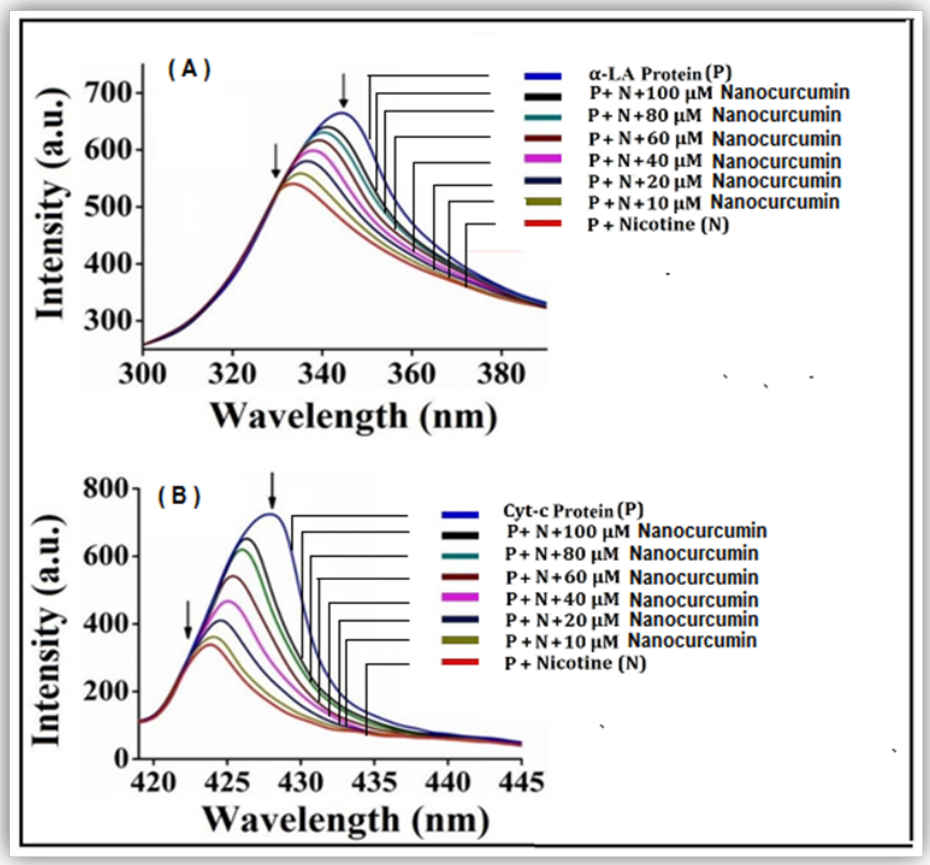

Figure 4.10: A) shows Fluorescence study of nanocurcumin vs. nicotine (500 µM) mediated pure protein of α–LA (50 µg / ml water). B) shows Fluorescence study of nanocurcumin vs. nicotine (500 µM) mediated pure protein of Cyt-c (50 µg / ml water).

4.2.13. Effects of nanocurcumin on nicotine induced changes in cytokine molecules

Cytokines plays an important role in modulating patho-physiological expression of different clinical features regulated by host immune system. Following the treatment of nicotine, the levels of IL-4, IL-6, TNF-α and IFN-γ in the serum were increased significantly group (54.5%, 121.8%, 133.4% and 47.8% respectively) compared to control group (Table 4.10). A significant decline in levels of all four cytokines were observed in nicotine induced rats treated with either curcumin or nanocurcumin. This reduction was highly significant ($p < 0.001$) for nanocurcumin than that of curcumin ($p < 0.01$).

Table 4.10: Levels of cytokines in different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine + Curcumin	Nicotine + Nano-curcumin	Curcumin	Nano-curcumin
IL-4 (pg/mL)	33.6 ± 1.1	51.9 ± 3.5 (54.5↑) **	41.7 ± 2.1 (24.1↑) *	36.5 ± 1.0 (8.6↑)**##	32.1 ± 1.2 (4.5↓)	32.8 ± 1.2 (2.4↓)
IL-6 (pg/mL)	136.8 ± 5.9	303.5 ± 3.5 (121.8↑) **	201.0 ± 1.7 (46.9↑) *	154.1 ± 7.1 (12.6↑) **##	146.4 ± 17.1 (7.0↑)	144.4 ± 17.1 (5.6↑)
TNF-α (pg/mL)	172.8 ± 6.0	403.4 ± 6.5 (133.4↑) **	217.4 ± 3.4 (25.6↑) *	188.5 ± 4.2 (9.1↑) ** ##	150.6 ± 5.6 (12.8↓)	156.4 ± 5.6 (9.5↓)
IFN-γ (pg/mL)	92.0 ± 6.4	136.0 ± 6.0(47.8↑) **	110.2 ± 6.4 (19.8↑) **	97.0 ± 7.7 (5.2↑) *##	89.7 ± 10.3 (3.0↓)	90.3 ± 10.3 (1.8↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies $P < 0.01$ and ** implies $P < 0.001$ compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.14. Effects of nanocurcumin on nicotine induced changes in Apoptosis regulator proteins

A significantly declined level (71.8%) of apoptosis regulator protein, BCL-2 was recorded among nicotine treated animals than the animals of control arm whereas the opposite event was noticed with increased level of BAX (64.7%). Nicotine induced lower level of Bcl-2 was increased effectively ($p < 0.01$) by curcumin and more significantly ($p < 0.001$) by nanocurcumin (Table-4.11). The increased level of Bax due to nicotine decreased significantly ($p < 0.01$) by the treatment of both curcumin and nanocurcumin.

Table 4.11: Levels of apoptosis regulator proteins in different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine +Curcumin	Nicotine +Nanocurcumin	Curcumin	Nano-curcumin
BCL-2 (ng/mL)	**1.99 ± 0.01**	**0.56 ± 0.05** (71.8↓) **	**0.93 ± 0.11** (53.3↓) **	**1.42 ± 0.04** (28.6↓)**##	**2.08± 0.20** (4.5↑)	**2.14± 0.20** (7.5↑)
BAX (ng/mL)	**1.02 ± 0.06**	**1.68 ± 0.21** (64.7↑) **	**1.44 ± 0.02** (39.3↑) *	**1.40 ± 0.02** (37.3↑) **##	**0.98** (3.9↓)	**0.94 ± 0.20** (1.1↓)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.15. Effects of nanocurcumin on nicotine induced changes in steroidogenic female hormones

Nicotine significantly reduced the levels of steroidogenic female hormones; estradiol (23.9%) and progesterone (27.6%). Nicotine induced lower level of these hormones were restored efficiently by curcumin and more effectively with nanocurcumin supplementation (Table 4.12).

Table 4.12: Levels of steroidogenic female hormones in different study and control groups of female rats under normal dietary condition

Parameter	Groups					
	Control	Nicotine	Nicotine+ Curcumin	Nicotine+ Nano-curcumin	Curcumin	Nano-curcumin
Estradiol (pg/mL)	81.1 ± 3.3	61.7 ± 2.4 (23.9↓) **	69.4 ± 2.4 (14.4↓) *	77.8 ± 4.7 (4.1↓)*##	85.0 ± 1.7 (1.0↑)	87.2 ± 1.7 (7.5↑)
Progesterone (ng/mL)	12.41 ± 0.05	8.98 ± 0.02 (27.6↓) **	9.53 ± 0.09(23.2↓)*	11.2 ± 0.22 (9.7↓) *##	12.87 ± 0.80 (5.7↑)	13.68 ± 0.80 (10.2↑)

The experimental setup was repeated twice and all data were averaged over n = 12 animals, and given mean ± S.D. Significance levels were determined using ANOVA, where, * implies P < 0.01 and ** implies P < 0.001 compared to control. The data presented in the parenthesis showed average (%) increase (↑) or decrease (↓) in respect to the control.

4.2.16. Histological study

The histological study of the liver tissue of control group animals is shown in Figure 4.11A. The distorted cell arrangement and the central vein (marked by arrow head) of the nicotine exposed liver are shown in Figure 4.11B. Curcumin and nanocurcumin supplemented liver photographs are presented in Figure 4.11C and Figure 4.11D respectively. The histological studies of the kidney tissues of control rats, nicotine exposure distorted kidney tissue, curcumin and nanocurcumin supplemented kidney tissues are presented in Figures. 4.12A – 4.12D, respectively.

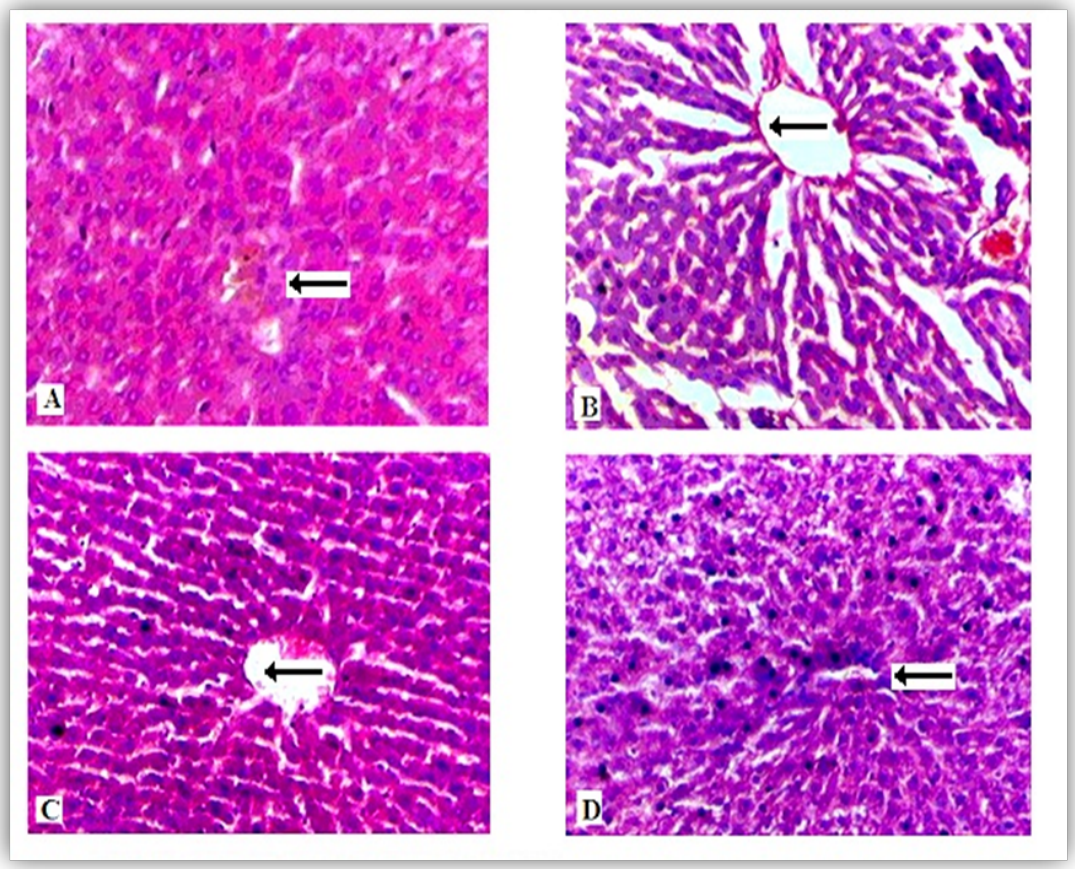

Figure 4.11: Photographs showing the histopathological changes of liver: A). Control group, B). Nicotine treated group, C). Nicotine plus curcumin treated group and D). Nicotine plus nanocurcumin treated group

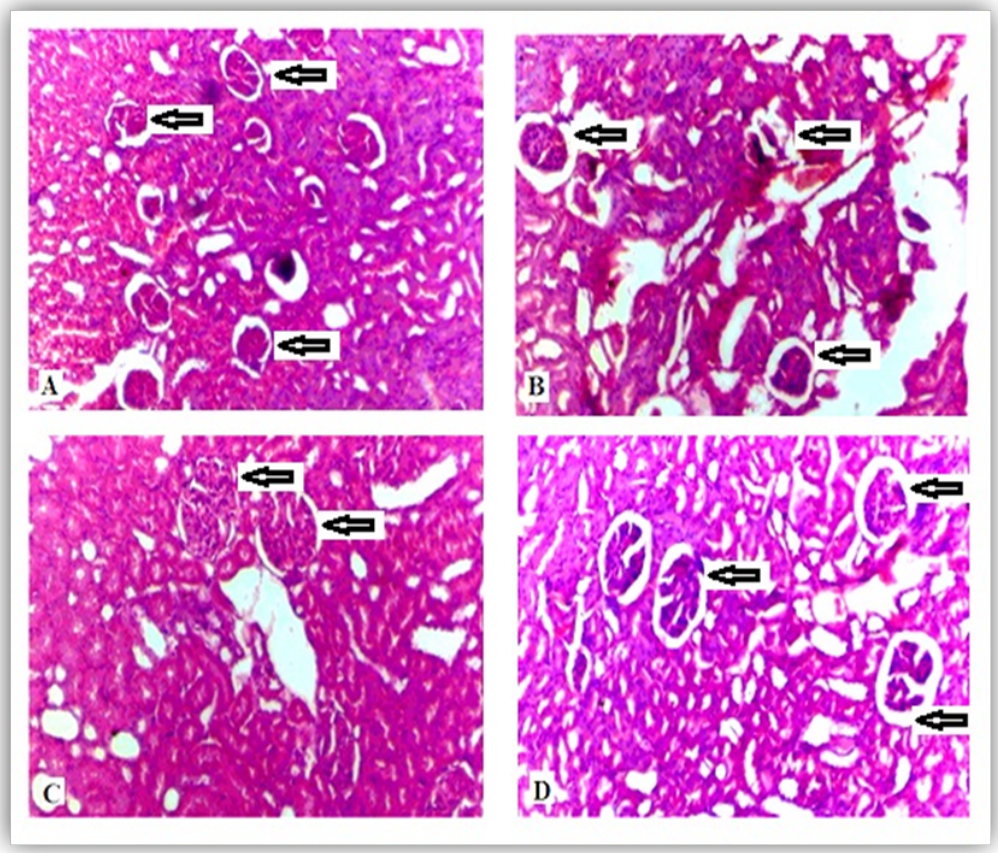

Figure 4.12: Photographs showing the histopathological changes of kidney: A). Control group, B). Nicotine treated group, C). Nicotine plus curcumin treated group and D). Nicotine plus nanocurcumin treated group

Chapter: 5

Discussion

5. DISCUSSION

The ameliorative effect of curcumin against nicotine-induced toxicity is well established in human and animal models in several studies. The pharmacokinetic properties like poor bioavailability, poor absorption, rapid metabolism, chemical instability, and rapid systemic elimination of curcumin are the major barriers for its clinical efficacy in human subjects (Lopresti, 2018). Scientists are trying to overcome these problems by changing its pharmacokinetic properties through translating curcumin to its nano form (Danafar, 2017; Danafar et al., 2017; Nosrati et al., 2018). The advantages of nanoparticles of curcumin enhanced the bioavailability in body fluid and tissues with long half-life and improved drug loaded capacity in tissues (Shaikh et al., 2009; Yadav et al., 2012). The present work was undertaken to study the ameliorative effect of nanocurcumin against nicotine induced toxicity in female rats under normal protein diet condition.

Unlike curcumin, nanocurcumin is freely soluble in water without any surfactants (Arunraj et al., 2014). The solubility testing of synthesized nanocurcumin in water showed a dense reddish coloured solution. The UV-visible spectral study of synthesized nanocurcumin showed the characteristic peak of nanocurcumin seen at 350 nm as evident by (Alam et al., 2012; Ghosh et al., 2011). FTIR spectrum of nanocurcumin showed peaks at 1,626 cm-1 indicating C=C stretching, at 1,507 cm-1 and 1,263 cm-1 indicating C=O stretching, at 1,153 cm-1 and 1,115 cm-1 corresponds to C – H stretching. The absorption peak at 1025 cm-1 was due to C-N stretching. Similar observations were also reported by (Pandit et al., 2015; Yadav et al., 2009; Yen et al., 2010; Sav et al., 2012). The crystalline state of the nanocurcumin was confirmed by the XRD pattern and such results were also obtained by many researchers earlier (Sav et al. 2012; Abd-El-Rahman and Al-Jameel, 2014). The particle size of the prepared nanocurcumin was in the range of 65±5 nm as analysed by FE-SEM. So, the chemical properties of curcumin remained unaltered in its nano form. Nicotine is known to be a toxic agent and creating several harmful effects

in human beings. Curcumin showed advantageous effects against nicotine toxicity (Bandyopadhyay et al., 2008; Banerjee et al., 2012; Chattopadhyay et al., 2015) but such effects of nanocurcumin has not been studied earlier.

Autopsy samples of smokers showed the highest affinity for nicotine is liver, kidney, spleen and lungs (Benowitz, 2009). Nicotine biotransformation takes place predominantly in liver, and responsible for devastating effects. During smoking, nicotine is readily absorbed through the lungs and metabolized in the liver. Nicotine causes damage of the hepatic cell membrane and enhancing the release of hepatocytic cytosomal enzymes and results in their increasing level in serum (Salahshoor et al., 2015). Nicotine induces the oxidative stress resulting in increasing the serum ACP and ALP levels. Oxidative stress causes tissue injury which is directly associated with the generation of reactive oxygen species (ROS) (Sreekala and Indira, 2009). The antioxidant activity of curcumin maintains the structural integrity of the cell membrane of hepatic cells and also reduces the level of hepatic enzymes like ACP, ALP, AST and ALT in serum (Al-Rubaei et al., 2014; Luber et al., 2019; Farzaei et al., 2018). In the present study, it is observed that, such activities of nanocurcumin with a dosage of 4 mg/kg body weight are superior to that of native curcumin (Table 4.1).

The pathogenesis of kidney disease is provoked by nicotine. Nicotine mediated albumin discharge and proteinuria enhanced by nicotine and lead to kidney malfunction (Salahshoor et al., 2019). The filtration capacity of kidney is hampered by nicotine induced oxidative stress. Nicotine was responsible for glomerulas damage and results in the increment of urea and creatinine level in serum (Chaturvedi et al., 2015).

In the present study, increased level of urea and creatinine in serum of nicotine treated animals are recorded like previous studies. (Halimi et al., 2000; Jalili et al., 2016; Begum et al., 2018). It is evident that progressive kidney failure may be related with a gradual decline of renal and non-renal excretion of nicotine, which may increase the rate of nephrotoxicity (Addo et al., 2008). The effects of

heavy metals present in tobacco such as Cadmium, Mercury and Lead might be other reasons for tobacco-induced damages in kidney (Usunobun et al., 2012). Several studies proved that curcumin acts as an anti-inflammatory agent and reduces the possibility of renal damage (Ghosh et al., 2014; Ali et al., 2018; Ghelani et al., 2019).

In my study the mean difference of urea and creatinine are significantly elevated ($P < 0.001$) in nicotine exposed group in compared to controls. Supplementation of nanocurcumin shows a better therapeutic effect for protection of the kidney (Table 4.2).

Cholesterol is an important bio-molecule and plays a crucial role in maintaining the integrity of membrane structure as well as synthesis of the steroid hormones and bile acids. Nicotine stimulates the synthesis of catecholamine which enhanced the lipo-lysis of adipose tissues thus increasing the level of serum cholesterol and triglycerides (Balakrishnan and Menon, 2007). In the present study, it was observed that nicotine significantly increases the triglyceride, cholesterol, LDL-C, VLDL-C in serum and decreased the level of HDL-C. Similar observations were also reported by various researchers (Balakrishnan and Menon, 2007; Chattopadhyay and Chattopadhyay, 2008; Rao and Subash, 2013; Singh, 2016). Nishiyama et al. (2005) reported that proliferator-activated receptor gamma (PPAR-γ) was activated by curcumin by which LDL-C receptor gene expression was suppressed resulting in decreased LDL-C concentration in plasma. Curcumin may reduce the triglyceride level by interacting with multiple targets like peroxisome proliferator-activated receptor alpha (PPAR-α), PPAR-γ, cholesteryl ester transfer protein (CETP), and lipoprotein lipase (Kang and Chen, 2009). The metabolism of lipoproteins is influenced by curcumin (Asai and Miyazawa, 2001). In my study, it was observed that curcumin reduces triglyceride, cholesterol, LDL-C, VLDL- C level in serum and increased HDL-C level in serum which are in agreement with the earlier studies (Qin et al., 2017; Yang et al., 2014). Declined level of cholesterol in curcumin treated group might be due to the enhanced expression of CYP7A1 gene (Kim and Kim, 2010).

Nanocurcumin showed better hypocholesterolaemia effect than curcumin due to its better pharmacokinetic properties. It might be due to either by increased in total cholesterol absorption (Hasimun et al., 2011) or by degradation and quick elimination of total cholesterol (Arafa, 2005).

Various *in vitro* and *in vivo* studies proved that nicotine could induce oxidative stress and hamper peroxidant/antioxidant balance in blood cells, plasma, serum and tissues (Chattopadhyay and Chattopadhyay, 2008; Chattopadhyay et al., 2010). It was also reported that nicotine could reduce the level of antioxidant enzymes (SOD, Catalase, GSH, GPX (Aspera-Werz et al., 2018; Agarwal et al., 2019; Raddam et al., 2017). Free radicals are generated by the oxidative stress that attack the lipid membranes and is responsible for the formation of Malonialdehyde (MDA) resulting in peroxidative tissue damage (Mohammadghasemi et al., 2021; Khademi et al., 2019). Previous studies showed that curcumin decreased the level of MDA by declining the production of free radicals (Alizadeh and Kheirouri, 2019; Lin et al., 2019). However, nanocurcumin showed better amelioration than native curcumin that may be due to its higher bioavailability and more uptakes into the cells. Nanocurcumin showed better defence mechanism due to increased level of antioxidant enzymes, leading to scavenging or neutralizing the free radicals generated by the nicotine stress.

Previous studies showed that nicotine significantly reduced the concentration of haemoglobin in blood in normal condition. Thomas and Lumb (2012) reported that, the binding of oxygen with haemoglobin was affected by carbon monoxide from tobacco smoking, Carbon monoxide have a greater binding affinity (>300 times) with haemoglobin than that of oxygen. Nicotine induced reduction of the RBC count have also been reported by Banerjee et al. (2010) which is may be due to peroxidative damage of RBC membrane resulting decrease in total haemoglobin content of blood. In the present study a significantly lower level of Hb% was recorded among nicotine treated group. From the table 4.6 it is evident that nanocurcumin shows better ameliorative effect on nicotine induced Hb content than that of unformulated curcumin.

Nicotine causes proteolysis and inhibits protein synthesis resulting in loss of muscular mass. Nicotine influences the expression genes responsible for destroying muscle protein content. Nicotine is also responsible for multiple organ dysfunctions like liver, kidney and ovary (Petersen et al., 2007). Present findings showed that nicotine decreases 20 to 25% tissue protein either by decreasing the protein biosynthesis or by increasing the rate of protein catabolism (Table 4.7). Reduction of muscle protein results the structural and functional abnormality of the cells. Curcumin regulates the function of different targets, like apoptotic proteins, cell cycle regulators, growth factors, receptors, protein kinases, and transcription factors (Zhou et al., 2011) that protects our body. Thus, nicotine induced stress in different tissue was blocked by curcumin and nanocurcumin.

Nicotine induction increased oxidative stress that enhances the protein oxidation. It is responsible for the increase of carbonyl content in different tissues resulting dysfunction of immune system, biological enzymes and hormones (Phaniendra et al., 2015). The hydroxyl radical scavenging potentials of curcumin decreased the carbonyl content in different tissue by inhibiting protein oxidation (Alisi et al., 2020). Nanocurcumin, being a more bio-available molecule, showed a better self-protective ability against protein oxidation than that of curcumin (Table 4.8).

Under normal condition the whole DNA content of 300 µL of blood was 109.38 µg; similar results were also recorded previously by Banerjee et al. (2010). The DNA content decreased significantly among the nicotine treated group. The reduction of total DNA contents may be due to nicotine induced oxidative stress. The decreased level of total DNA content of the blood cells was more effectively ($p < 0.001$) corrected by nanocurcumin. That was demonstrated by similar amount of total DNA among nanocurcumin supplemented nicotine induced group to that of control group of animals. The ameliorative effect of nanocurcumin was found to be more prominent than that of native curcumin (Table 4.9).

The average DNA damage in control group was 2.76%. But in nicotine treated group the average DNA damage was found to be significantly higher (40%; $p < 0.001$) than control group. The DNA damage may be due to nicotine induced chromosomal aberration, exchange of sister chromatids and single-strand DNA breakage (Sanner and Grimsru, 2015). Banerjee et al. (2010) reported that nicotine induced oxidative stress increased DNA damage in blood and liver tissues of female albino rats. They stated that curcumin efficiently interacted with both nicotine and as well DNA and reduced the nicotine induced oxidative stress resulting in less DNA damage. The present study showed that nanocurcumin decline the percentage of DNA damage more effectively than that of curcumin against nicotine-induced toxicity.

The molecular docking of nicotine-haemoglobin indicated that, nicotine showed hydrophobic interaction with the amino acid residues like TYR 42, ASN 97, PHE 98 and LEU 101 of haemoglobin thus forming a strong nicotine haemoglobin complex structure. Similarly, nanocurcumin also binds by hydrogen bonds with haemoglobin's active sites at the amino acid residues His 58, SER 102, SER 133. Hydrophobic interactions were also noted with the following amino acid residues of haemoglobin LYS 61, VAL 62, LEU 83, HIS 87, PHE 98 thus forming a nanocurcumin –haemoglobin complex structure. From the molecular docking experiment, it was observed that, the binding free energy between nicotine vs. haemoglobin was higher (-70.98) than that of nanocurcumin vs. haemoglobin (-97.41). It indicates that nanocurcumin can bind with haemoglobin more tightly than nicotine.

The UV spectra study shows that nanocurcumin helps in maintaining high haemoglobin content of erythrocytes under nicotine induced stress condition. It might be due to higher binding affinity of nanocurcumin to haemoglobin ($K = 10.4 \times 104\ M^{-1}$) than nicotine ($K = 6.5 \times 103\ M^{-1}$). Nanocurcumin readily binds with haemoglobin that inhibits the binding of nicotine with haemoglobin. Nanocurcumin also having a binding affinity ($K = 3.5 \times 103\ M^{-1}$) with nicotine that reduces the availability of free nicotine molecules for interaction with the RBC. It justifies the observed higher concentration of haemoglobin

concentration among the nicotine treated animals supplemented with nanocurcumin. The present study showed that nanocurcumin was more effective for maintaining higher concentration haemoglobin with better protective efficacy than curcumin (Banerjee et al., 2010).

Nicotine has an affinity to DNA resulting in formation of nicotine-DNA complex. Nicotine forms hydrogen bond with 241 SER residue of p53 tumour suppressor protein complex with DNA. The stability of nicotine-DNA complex is more strengthen by hydrophobic interactions with amino acid residues like SER 240, ARG 248, ARG 249, ARG 273, VAL 274 etc. of p53 protein associated with DNA. The nanocurcumin-DNA docking showed that, nanocurcumin is nicely accommodated by the active sites of p53 protein associated with DNA through hydrogen bond at the residue GLN 165, ASN 247. Nanocurcumin also formed several hydrophobic interactions with ARG 248, CYS 176, HIS 179 amino acid residues of p53 protein associated with DNA molecule. A binding free energy of - 69.48 kcal between nicotine-DNA was recorded which was higher than that of nanocurcumin vs. DNA (- 86.35 kcal). This observation explains that nanocurcumin can bind with the p53 protein associated DNA molecule more tightly than nicotine. So, the superior stability of nanocurcumin-DNA complex than that of nicotine-DNA complex was observed. Nicotine-nanocurcumin docking showed that, nanocurcumin compete with nicotine while binding with DNA resulting in reducing nicotine induced geno-toxicity in female rats.

A similar binding affinity of nanocurcumin to DNA (K = 7.8 × 103 M-1) as well as with nicotine (K = 10.0 × 103 M-1) was recorded. So nanocurcumin can bind with DNA and prevents nicotine induced DNA damage. The findings of the present study agree with the results of the molecular docking experiment.

From the UV spectra study it was observed that nicotine had a strong affinity to both haemoglobin as well as DNA. For this interaction, the characteristic peak of Hb/DNA is suppressed. Nanocurcumin can strongly bind with Hb and DNA. The availability of free nicotine molecules is reduced for interaction with

Hb/DNA in the presence of nanocurcumin. Thus, nanocurcumin compete with nicotine and results in regaining of structural integrity of Hb/DNA. Such interactions support the results of molecular docking and clarify the ameliorative effect of nanocurcumin in Hb/DNA against nicotine induced rats.

The molecular interactions amongst nicotine, nanocurcumin and cellular proteins like α-LA and Cyt-c protein were well documented by docking experiments. The results showed that nanocurcumin combined with α-LA protein (free energy - 73.9 kcal) to form nanocurcumin - α-LA protein complex. The binding free energy between nicotine and α-LA protein was found to be lower (- 57.3 kcal) than that of nanocurcumin - α-LA protein complex. It favours the formation of nanocurcumin - α-LA protein complex than nicotine - α-LA protein complex.

Due to differences in free binding energy, formation of nanocurcumin-Cyt-c protein (free energy - 74.4 kcal) complex is more favourable than that of nicotine-Cyt-c protein (free energy - 65.6 kcal) complex. This explains that nanocurcumin may resist the nicotine for interaction with the protein molecules thus providing an additional protection against nicotine. This binding ability of nanocurcumin (curcumin) is very strong and makes it as an effective immuno-modulator against nicotine toxicity. The observations of molecular dockings have been supported by the wet-lab interaction studies among nicotine, nanocurcumin and cellular proteins. Nicotine showed an affinity to bind with two important mammalian proteins (α-LA and Cyt-c) (Gergalova et al., 2012). Nanocurcumin showed strong binding ability with nicotine. So, the availability of free nicotine for interaction with both the proteins is low in presence of nanocurcumin. Nanocurcumin thus reduces the nicotine induced damage of α-LA and Cyt-c.

Due to interaction with nicotine the maximum emission wavelength of α-LA protein was shifted from 345 nm towards 330 nm (blue shift). It reflects an increase of absorption energy by the binding of nicotine with the protein and leading to changes in conformational structure of protein. With gradual

addition of nanocurcumin to the nicotine bound protein solution, the intensity as well as the emission maximum wavelength is significantly overlapping the emission maxima of the native protein. Nanocurcumin thus, steadily assists the protein to restore its native state by lowering the energy due to the H-bonding or π-π conjugation. A similar phenomenon is observed in case of Cyt-c emission spectra. The bioavailability of nanocurcumin is greater than that of curcumin due to its higher aqueous solubility. The higher binding ability to nicotine as well as to cellular proteins, it protects the cells from nicotinic effects.

IL-6 plays an essential role in the cytokine network. In the initial stage of inflammation, it is secreted from the local lesion and then goes to the liver by the blood stream. It helps in increasing the secretion of serum amyloid A (SAA), α1-antichymotrypsin, haptoglobin, fibrinogen and C-reactive protein (CRP) and fibrinogen. Long retention of serum amyloid A (SAA) with higher concentration leads to several chronic inflammatory diseases (Castell et al., 1989) High concentration of IL-6 also reduces the iron and zinc level in serum (Tanaka et al., 2014). Elevated expression of IL-6 is also involved with fatigue scores (Wang et al., 2012).

Tumour necrosis factor alpha (TNF-α) is also known as a proinflammatory cytokine. Any alteration (inappropriate or excessive) in TNF-α signalling is involved in pathological complications such as autoimmune diseases and inflammatory disease (Jang et al., 2021). TNF-α is also found to be associated with fatigue condition (Tian et al., 2014). IL-4 is the central cytokine responsible for allergic inflammation (Steinke and Borish, 2001). Interferon gamma (IFNγ) is another pro-inflammatory cytokine responsible for various inflammatory diseases like enhance the chronic lungs disease (Zhu et al., 2019). Previous findings reported that higher level of pro inflammatory cytokines (IL-6, TNF-α) observed in schizophrenia (SZ) and bipolar-I disorder (BD) patient specially who were suffered from childhood trauma (Quidé et al., 2019).

In the present study it was observed that the levels of pro-inflammatory cytokines were elevated among nicotine induced animals. Similar observation was reported previously (Maiti et al., 2015), where nicotine toxicity elevated the secretion of pro-inflammatory cytokines and is responsible for excessive production of ROS. Several studies reported that curcumin, a natural therapeutic agent showed its anti-inflammatory effects by down regulating many pro-inflammatory cytokines and chemokines in a number of cell types and pathologic conditions (Yadav et al., 2015; Gorabi et al., 2021). In my study it was noticed that nanocurcumin showed more beneficial effect in reducing pro inflammatory cytokines level than that of curcumin.

Bcl-2 plays a vital role by influencing the action of cellular existence and hampering the function of pro-apoptotic proteins. Any damage in Bcl-2 gene is involved with different types of cancers and also a probable cause of schizophrenia and autoimmune diseases (Jalili et al., 2017). Hardwick et al. (2013) demonstrated that the damaged Bcl-2 gene prevented the function of anticancer drugs. They also reported that a hetero-dimmer of apoptosis regulator protein Bax with Bcl-2 functioned as an apoptotic activator. In the present study, a decrease in anti-apoptotic protein Bcl-2 and increase in pro-apoptotic protein Bax shows were observed in nicotine stressed rats. So, nanocurcumin showed better immunomodulatory effect against apoptosis than native curcumin.

The present study also shows that, nicotine is responsible for a significant reduction of 17 β-estradiol and progesterone levels among female rats with normal protein diet supplementation. This is in agreement with findings of (Sinha et al., 2012). The decline of 17 β-estradiol levels among nicotine treated group may be due to the anti-estrogenic effect of nicotine. Nicotine is also responsible for declining the levels of progesterone by increasing PGF2α and VEGF-mRNA expressions. Curcumin showed its ability to inhibit the action of nicotine on metabolism of estrogens for its high ability of binding with estradiol. But nanocurcumin was found to be more effective on increasing

17β-estradiol and progesterone levels among nicotine induced animals that might due to its higher bioavailability and binding affinity to nicotine.

The study of histopathology of the liver tissue was done following eosin haematoxylin staining using compound microscope (40X). A regular arrangement of hepatic cell with normal shape of hepatocytes and central vein were observed in liver tissue of the control group animals. The arrangement of hepatic cells was distorted and the central vein and sinusoids space were dilated among nicotine exposed animals under normal diet condition. The normal shape of hepatic cells, diameter of central vein regains their regular arrangement among following curcumin supplementation. Nanocurcumin showed more prominent effects than native curcumin for regaining the normal arrangement of the hepatocytes and the central vein.

The histological studies of the kidney tissues were also revealed the normal arrangement of glomerulus (marked by arrow head), bowman space and proximal /distal tubules of kidney tissue of control rats. Nicotine exposure distorted this arrangement significantly as seen in Figure 4.12B. Curcumin supplementation recovered the normal arrangement glomerulus, bowman space and proximal/distal tubules of kidney tissue to some extent (Figure 4.12C). Supplementation of nanocurcumin restored the normal arrangement glomerulus, bowman space and proximal/distal tubules of kidney tissue effectively (Figure 4.12D).

Chapter: 6

Summary of the thesis

6. SUMMARY OF THE THESIS

- The nanocurcumin synthesized by ultrasonication method in the laboratory was characterised physically. The plasmon resonance (SPR) band pattern analysed by UV-Vis spectrophotometer, confirms the characteristic absorbance peak of 350 nm, which is exactly similar to that of nanocurcumin. The morphological characterization of nanocurcumin by Field Emission Scanning Electron Microscopy showed the average particle size of 65±5 nm which was within the range of nanoparticles (below 100nm). Fourier transform infrared spectroscopy (FTIR) confirmed the functional groups of laboratories synthesized nanocurcumin, were exactly identical to that of curcumin. Crystalline nature of nanocurcumin powder was determined by X-ray diffractometer. Reddish yellow aqueous solution proved its water solubility.

- Different set of animal experiments confirmed its ameliorative effects against nicotine toxicity at biochemical, cellular and molecular levels.

- Nicotine mediated toxicities were well established in control group animals as evidenced by altered hepatic enzymes, Lipid profiles, SODs, free radicals, apoptotic proteins, LFT, RFT, levels of different cytokines and catastrophic changes in molecular levels.

- Nanocurcumin showed better ameliorative effects against nicotine mediated toxicities as compared with native curcumin with significantly lower dosages. Adverse histo-pathological changes at cellular level in vital organs like liver and kidney due to nicotine toxicity were prevented by nanocurcumin more effectively than curcumin.

- The findings of animal experiments about ameliorative effects of nanocurcumin against nicotine induced toxicity were also supported by dry lab molecular docking studies showing its high affinity to

haemoglobin, DNA, various types of proteins. That may reduce the rate of interaction between nicotine and important bio-molecules.

- Better protective efficacy of nanocurcumin than native curcumin might be due to its increased effective surface area, water solubility, high rate of intestinal absorption, cellular distribution and long half-life.
- No adverse side effects were recorded among nanocurcumin treated group indicating its safe usages.
- Similar studies with large number of animals as well as in different cell lines are highly suggested for strengthening the present findings about ameliorative effects of nanoparticles of curcumin against nicotine mediated toxicities.
- Human trial with nanocurcumin against nicotine toxicity among smokers may be undertaken following proper guidelines and permission of Drug controlling authority of India and approval of any registered Human Ethics committee.

REFERENCES

7. REFERENCES

Aali N, Motalleb G. The effect of nicotine on the expressions of the α7 nicotinic receptor gene and Bax and Bcl-2 proteins in the mammary gland epithelial-7 breast cancer cell line and its relationship to drug resistance. Cell Mol Biol Lett. 2015; 20(5):948-64.

Abd El-Rahman SN, Al-Jameel SS. Protection of Curcumin and Curcumin Nanoparticles against Cisplatin Induced Nephrotoxicity in Male Rats. Sch Acad J Biosci. 2014; 2(3):214-223.

Abdul-Razaq SN, Ahmed BM. Effect of cigarette smoking on liver function test and some other related parameters. Zanco J Med Sci. 2013; 17(3):556-562.

Abed AK, Hassen WA, Salman MA. Evaluation of the effect of cigarette smoking on interleukin-4. Int J Psychosol Rehabilitation. 2020; 24(10):2386-2392.

Abu-Taweel GM. Effects of curcumin on the social behavior, blood composition, reproductive hormones in plasma and brain acetylcholinesterase in cadmium intoxicated mice. Saudi J Biol Sci. 2016; 23(2):219-28.

Acar A, Singh D, Srivastava AK. Assessment of the ameliorative effect of curcumin on pendimethalin-induced genetic and biochemical toxicity. Sci Rep. 2022; 12(1):2195.

ACS, 2018. Reports on Nicotine. Available from Https://Www.Acs.Org/Content/Acs/En/Molecule-of-theWeek/Archive /n/ Nicotine.Html

Addo MA, Gbadago JK, Affum HA, Adom T, Ahmed K, Okley GM. Mineral profile of ghanaian dried tobacco leaves and local snuff: A comparative study. J Rad Nucl Chem. 2008; 277:517-524.

Adeyemi DH, Oyeyipo IP, Akanbi KA, Oluwole T. Nicotine alters progesterone and estradiol levels during the first trimester of pregnancy in Wistar rats. JBRA Assist Reprod. 2018; 22(2):78-81.

Agarwal P, Bagewadi A, Keluskar V, Vinuth DP. Superoxide dismutase, glutathione peroxidase, and catalase antioxidant enzymes in chronic tobacco smokers and chewers: A case-control study. Indian J Dent Res. 2019; 30(2):219-225.

Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol. Sci. 2009; 30:85-94.

Ahmadi R, Lotfizadeh M J, Heidari F, Mafi M. Comparing the effects of cigarette and waterpipe smoke on serum levels of LH, FSH, estradiol and progesterone in female rats. Feyz. 2013; 17(3):232-238.

Ahmed MA, Hassan KH, Hassanein KM, Waly H. Role of vitamin C and selenium in attenuation of nicotine induced oxidative stress, P53 and Bcl2 expression in adult rat spleen. Pathophysiol. 2014; 21(3):211-7.

Akel M, Sakr F, Fahs I, Dimassi A, Dabbous M, Ehlinger V, Salameh P, Godeau E. Smoking Behavior among Adolescents: The Lebanese Experience with Cigarette Smoking and Waterpipe Use. Int J Environ Res Public Health. 2022; 19(9):5679.

Akpolat M, Tarlada calı sır Y, Uz Y, Metin M, Kızılay G. Kanser tedavisinde curcuminin yeri. Yeni Tıp Dergisi. 2010; 27:142-147.

Alam S, Panda J, Chauhan V. Novel dipeptide nanoparticles for effective curcumin delivery. Int. J. Nanomed. 2012; 7:4207-4222.

Al-Bishri WM. Hepato therapeutic Efficacy of Native Curcumim and Nano - curcumin: A Novel Therapy Against Hyperthyroidism Induced Liver Oxidative and Inflammatory Damage in Rats . Int. J. Adv. Res. Biol. Sci. 2017; 4(12): 86-97.

Al-Harbi MS, Hamza RZ, Dwari AA Sodium fluoride induced antioxidant defense impairment and renal biomarkers and the ameliorative role of selenium and curcumin in male mice. Asian Pacifi c Journal of Tropical Diseases. 2014; 4: S990-997.

Ali BH, Al-Salam S, Al Suleimani Y, Al Kalbani J, Al Bahlani S, Ashique M, Manoj P, Al Dhahli B, Al Abri N, Naser HT, Yasin J, Nemmar A, Al Za'abi M, Hartmann C, Schupp N. Curcumin Ameliorates Kidney Function and

Oxidative Stress in Experimental Chronic Kidney Disease. Basic Clin Pharmacol Toxicol. 2018; 122(1):65-73.

Ali BH, Karaca T, Al Suleimani Y, Al Za'abi M, Al Kalbani J, Ashique M, Nemmar A. The effect of swimming exercise on adenine-induced kidney disease in rats, and thes influence of curcumin or lisinopril thereon. PLoS One. 2017; 12(4):e0176316.

Alisi IO, Uzairu A, Abechi SE. Molecular design of curcumin analogues with potent antioxidant properties and thermodynamic evaluation of their mechanism of free radical scavenge. Bulletin Natn Res Centre. 2020; 44:137.

Alizadeh M, Kheirouri S. Curcumin reduces malondialdehyde and improves antioxidants in humans with diseased conditions: a comprehensive meta-analysis of randomized controlled trials. Biomed (Taipei). 2019; 9(4):23.

Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974; 20(4):470-5.

Allen AM, Oncken C, Hatsukami D. Women and Smoking: The Effect of Gender on the Epidemiology, Health Effects, and Cessation of Smoking. Curr Addict Rep. 2014; 1(1):53-60.

Allen AM, Yuan NP, Wertheim BC, Krupski L, Bell ML, Nair U. Gender differences in utilization of services and tobacco cessation outcomes at a state quitline. Transl Behav Med. 2019; 9(4):663-668.

Alp H, Aytekin I, Hatipoglu NK, Alp A, Ogun M. Effects of sulforophane and curcumin on oxidative stress created by acute malathion toxicity in rats. Eur Rev Med Pharmacol Sci. 2012; 16(3):144-8.

Al-Rubaei ZM, Mohammad TU, Ali LK. Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study. Pak. J. Biol. Sci. 2014; 17:1237-1241.

Alsalhen KS, Abdalsalam RD. Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. Int Curr Pharm J. 2014; 3(7):291-295.

Alspach E, Lussier DM, Schreiber RD. Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. Cold Spring Harb Perspect Biol. 2018; 11.3:1-22.

Al-tameemi S, Hameed N, Gomes K, Abid H. Cigarette smoking increases plasma levels of IL-6 and TNF-α. Baghdad Journal of Biochemistry and Applied Biological Sciences. 2022; 3(01):60-68.

Amer AS, Mahmoud G. Protective Effects of Vitamin C against Nicotine-Induced Oxidative Damage of Rat Liver and Kidney. J Environ Sci Toxicol Food Technol. 2014; 8(12):50-63.

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007; 4:807-18.

Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB. Curcumin and cancer: An "old-age" disease with an "ageold" solution. Cancer Lett. 2008; 267:133-164.

Ankamwar B. Biomedical Engineering-Technical Applications in Medicine. IntechOpen; London, UK: 2012. Size and Shape Effect on Biomedical Applications of Nanomaterials; pp. 93–114.

Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL4 locus accessibility. Annu Rev Immunol.2006; 24:607-56.

Aoki Y, Ikeda T, Tani N, Shida A, Oritani S, Ishikawa T. Evaluation of the Distribution of Nicotine Intravenous Injection: An Adult Autopsy Case Report with a Review of Literature. Int J Legal Med. 2020; 134(1):243-249.

Arafa HM. Curcumin attenuates diet-induced hypercholesterolemia in rats. Med Sci Monit. 2005; 11(7):BR228-234.

Arany I, Grifoni S, Clark JS, Csongradi E, Maric C, Juncos LA. Chronic nicotine exposure exacerbates acute renal ischemic injury. Am J Physiol Renal Physiol. 2011; 301:F125-33.

Argentin G, Cicchetti R. Genotoxic and antiapoptotic effect of nicotine on human gingival fibroblasts. Toxicol Sci. 2004; 79(1):75-81.

Arunraj T, Rejinold NS, Mangalathillam S, Saroj S, Biswas R, Jayakumar R. Synthesis, characterization and biological activities of curcumin nanospheres. J. Biomed. Nanotechnol. 2014; 10: 238-250.

Asai A, Miyazawa T. Dietary curcuminoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. J Nutr. 2001; 131(11):2932-5.

Ashtary-Larky D, Rezaei Kelishadi M, Bagheri R, Moosavian SP, Wong A, Davoodi SH, Khalili P, Dutheil F, Suzuki K, Asbaghi O. The Effects of Nano-Curcumin Supplementation on Risk Factors for Cardiovascular Disease: A GRADE-Assessed Systematic Review and Meta-Analysis of Clinical Trials. Antioxidants (Basel). 2021; 10(7):1015.

Aspera-Werz RH, Ehnert S, Heid D, Zhu S, Chen T, Braun B, Sreekumar V, Arnscheidt C, Nussler AK. Nicotine and Cotinine Inhibit Catalase and Glutathione Reductase Activity Contributing to the Impaired Osteogenesis of SCP-1 Cells Exposed to Cigarette Smoke. Oxid Med Cell Longev. 2018; 2018:3172480.

Atia MM, Abdel-Tawab HS, Mostafa AM, Mobarak SA. Nanocurcumin and curcumin prevent N, N'-methylenebisacrylamide-induced liver damage and promotion of hepatic cancer cell growth. Sci Rep. 2022; 12(1):8319.

CDC 2010: Centers for Disease Control and Prevention (US). 2010; ISBN-13: 978-0-16-084078-4.

Azab AE, Albasha MO, Elnaif MA. Renal Toxicity Induced by Nicotine in Male Albino Rats and Attenuation by Fenugreek Seeds and Curcumin. J, Biotech. and Bioprocessing. 2021; 4(1):14-21.

Balakrishnan A, Menon VP. Antioxidant properties of hesperidin in nicotine-induced lung toxicity. Fundam Clin Pharmacol. 2007; 21(5):535-46.

Banach M, Serban C, Aronow WS, Rysz J, Dragan S, Lerma EV, Apetrii M, Covic A. Lipid, blood pressure and kidney update 2013. Int Urol Nephrol. 2014; 46(5):947-61.

Bandyopadhyaya G, Sinha S, Chattopadhyay BD, Chakraborty A. Protective role of curcumin against nicotine-induced genotoxicity on rat liver under restricted dietary protein. Eur J Pharmacol. 2008; 588(2-3):151-7.

Banerjee S, Bandyopadhyaya G, Chattopadhyhay K, Chattopadhyhay BD. Amelioration of nicotine-induced damage of blood cells in protein malnourished female rats by curcumin. Int J Pharmacol. 2010; 6(4):609-620.

Banerjee S, Chattopadhyay K, Chhabra JK, Chattopadhyay B. Protein dependent fate of hepatic cells under nicotine induced stress and curcumin ameliorated condition. Eur J Pharmacol. 2012; 684(1-3):132-45.

Banks E, Joshy G, Weber MF et al. Tobacco smoking and all-cause mortality in a large Australian cohort study: findings from a mature epidemic with current low smoking prevalence. BMC Med. 2015; 13:38.

Bansal H, Sandhu VS, Bhandari R, Sharma D. Evaluation of micronuclei in tobacco users: A study in Punjabi population. Contemp Clin Dent. 2012; 3(2):184-7.

Baziar N, Parohan M. The effects of curcumin supplementation on body mass index, body weight, and waist circumference in patients with nonalcoholic fatty liver disease: A systematic review and dose-response meta-analysis of randomized controlled trials. Phytother Res. 2020; 34(3):464-474.

Beauchamp C, Fridovich I. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. Anal Biochem. 1971; 44(1):276-287.

Begum SF, Nagajothi G, Swarnalatha K. Chronic smokeless tobacco consumption contributes to the development of renal diseases in the human male volunteers. J Anal Pharm Res. 2018; 7(6):652-662.

Ben Saad A, Rjeibi I, Alimi H, Ncib S, Bouhamda T, Zouari N. Protective effects of Mentha spicata against nicotine-induced toxicity in liver and erythrocytes of Wistar rats. Appl Physiol Nutr Metab. 2018; 43(1):77-83.

Benowitz NL, Burbank AD. Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. Trends Cardiovasc Med. 2016; 26(6):515-23.

Benowitz NL, Hukkanen J, Jacob P 3rd. Nicotine Chemistry, Metabolism, Kinetics and Biomarkers. Handb Exp Pharmacol. 2009; (192):29-60 (4)

Benowitz NL, Jacob P, 3rd, Fong I, Gupta S. Nicotine Metabolic Profile in Man: Comparison of Cigarette Smoking and Transdermal Nicotine. J Pharmacol Exp Ther. 1994; 268(1):296-303.

Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. Annu Rev Pharmacol Toxicol. 2009; 49:57-71.

Bergström J. Tobacco smoking and chronic destructive periodontal disease. Odontology. 2004; 92(1):1-8.

Bessey DA, Lowery OH, Brocks MJ. In: H.U. Bergmeyer, (Ed), J Biol Chem. Methods of Enzymatic Analysis. Acad Press. NY.1946; 321-329.

Bhabak KP, Mugesh G. Functional mimics of glutathione peroxidase: bioinspired synthetic antioxidants. Acc Chem Res J. 2010; 43:1408-1419.

Bhawana, Basniwal RK, Buttar HP, Jain VK, Jain N. Curcumin Nanoparticles: Preparation, Characterization, and Antimicrobial Study. J Agri Food Chem 2011; 59(5): 2056–61.

Bhoopalan SV, Huang LJ, Weiss MJ. Erythropoietin regulation of red blood cell production: from bench to bedside and back. F1000Res. 2020; 9:F1000 Faculty Rev-1153.

Biswas S, Manna K, Das U, Khan A, Pradhan A, Sengupta A, Bose S, Ghosh S, Dey S. Smokeless tobacco consumption impedes metabolic, cellular, apoptotic and systemic stress pattern: A study on Government employees in Kolkata, India Sci Rep. 2015; 5:18284.

Boulter J, Connolly J, Deneris E, Goldman D, Heinemann S, Patrick J. Functional Expression of Two Neuronal Nicotinic Acetylcholine Receptors from CDNA Clones Identifies a Gene Family. Proc Natl Acad Sci U S A. 1987; 84(21):7763-7.

Brand JS, Chan MF, Dowsett M, Folkerd E, Wareham NJ, Luben RN, van der Schouw YT, Khaw KT. Cigarette smoking and endogenous sex hormones in postmenopausal women. J Clin Endocrinol Metab. 201; 96(10):3184-92.

Braun M, Marsidi LL, Klingelhöfer D, Groneberg DA. Kretek Cigarettes and Particulate Matter Emissions-An Aerosol Spectrometric Study on Typical

Indonesian Brands Flavored With Cloves. Nicotine Tob Res. 2022; 24(5):778-784.

Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S. Effect of Smoking History on [3H] Nicotine Binding in Human Postmortem Brain. J Pharmacol Exp Ther. 1997; 282(1):7-13.

Burke JD, Young HA. IFN- γ : a cytokine at the right time, is in the right place. Semin Immunol. 2019; 43:1-8.

Byron KA, Varigos GA, Wootton AM. IL-4 production is increased in cigarette smokers. Clin Exp Immunol. 1994; 95(2):333-6.

Cannan WJ, Pederson DS. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. J Cell Physiol. 2016; 231(1):3-14.

Cao S, Yang C, Gan Y, Lu Z. The Health Effects of Passive Smoking: An Overview of Systematic Reviews Based on Observational Epidemiological Evidence. PLoS ONE. 2015; 10(10): e0139907.

Carpagnano GE, Kharitonov SA, Foschino-Barbaro MP, Resta O, Gramiccioni E, Barnes PJ. Eur Res J. 2003; 21: 589-593.

Carstens E, Carstens MI. Sensory Effects of Nicotine and Tobacco. Nicotine Tob Res. 2022; 24(3):306-315.

Cases J, Romain C, Marin Pagan C, Chung LH, Rubio-Perez JM, et al. Supplementation with poly-rich extract , perfload , improves physical performance during high-intensity exercise: A Randomized, Double Blind, Gross Over ,Trail. Nutrients. 2017; 9: 421-433.

Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. Febs Lett. 1989; 242(2):237-9.

Catassi A, Servent D, Paleari L, Cesario A, Russo P. Multiple Roles of Nicotine on Cell Proliferation and Inhibition of Apoptosis: Implications on Lung Carcinogenesis. Mutat Res. 2008; 659(3):221-31.

César-Neto JB, Duarte PM, de Oliveira MC, Casati MZ, Tambeli CH, Parada CA, Sallum EA, Nociti FH Jr. Smoking modulates interferon-gamma

expression in the gingival tissue of patients with chronic periodontitis. Eur J Oral Sci. 2006; 114(5):403-8.

Chang CM, Corey CG, Rostron BL, Apelberg BJ. Systematic review of cigar smoking and all cause and smoking related mortality. BMC Public Health. 2015; 15:390.

Changeux JP. Structural Identification of the Nicotinic Receptor Ion Channel. Trends Neurosci. 2018; 41:67-70.

Chatterjee CC, In: Human Physiology. 1994;11th Reprint Ed.

Chatterjee SN, Agarwal S. Liposomes as Membrane Model for Study of Lipid Peroxidation. Free Radic Biol Med. 1988; 4(1):51-72.

Chattopadhyay BD, Tamang A, Kundu D. Protective role of raw-turmeric rhizomes against nicotine-induced complications of Beedi (Indian cigarette) workers. Pharmaceut. Anal. Act. 2015; 6:386.

Chattopadhyay K, Chattopadhyay BD. Effect of nicotine on lipid profile, peroxidation & antioxidant enzymes in female rats with restricted dietary protein. Indian J Med Res. 2008; 127(6):571-6.

Chattopadhyay K, Mondal S, Chattopadhyay B, Ghosh S. Ameliorative effect of sesame lignans on nicotine toxicity in rats. Food Chem Toxicol. 2010; 48(11):3215-20.

Chaturvedi A, Natarajan A, Sharma V, Yaparthy N, Shetty JK, Virupaksha D, et al. Association of age related severity in oxidative stress and blood urea nitrogen levels in patients with dementia: A coastal Karnataka study. Asian J Biomed Pharm Sci. 2015; 5:06.

Chelikhani P, Fita I, Loewen PC. Diversity of structures and properties among catalase. Cell Mol Life Sci. 2004; 61:192-208.

Chen Y, Mai X-M. Smoking and asthma in men and women with normal weight, overweight, and obesity. J Asthma. 2011; 48(5):490-4.

Chen YM, Huang CC, Sung HC, Lee MC, Hsiao CY. Electronic cigarette exposure reduces exercise performance and changes the biochemical profile of female mice. Biosci Biotechnol Biochem. 2019; 83(12):2318-2326.

Chernecky CC, Berger BJ. Laboratory tests and diagnostic procedures. St. Louis MO: Elssvier Saunders. 2013; 16th ed.

Chinnala KM, Konda M. A Review on Choice of Nicotine Buccal Thin Film for Nicotine Replacement Therapy. Wjpmr. 2017; 3(7):96-104.

Chundru VN, Ram MM. Study of oxidant enzymes superoxide dismutase and glutathione peroxidase levels in tobacco chewers and smokers: A pilot study. J Cancer Res Ther. 2013; 9: 210-214.

Cianciulli A, Calvello R, Porro C, Trotta T, Salvatore R, Panaro MA. PI3k/Akt signalling pathway plays a crucial role in the anti-inflammatory effects of curcumin in LPS-activated microglia. Int Immunopharmacol. 2016; 36:282-290.

Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol. 2014; 20(25):8082-91.

Clarke E, Thompson K, Weaver S, Thompson J, O'Connell G. Snus: a compelling harm reduction alternative to cigarettes. Harm Reduct J. 2019; 16:62 .

Cohen G, Dembiec D, Marcus J. Measurement of Catalase Activity in Tissue Extract. Anal Biochem. 1970; 34(1):30-38.

Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J. Autoimmun. 2009; 33:197-207.

Cordelli E, Bignami M, Pacchierotti F. Comet assay: a versatile but complex tool in genotoxicity testing. Toxicol Res (Camb). 2021; 10(1):68-78.

Corona-Rivera A, Urbina-Cano P, Bobadilla-Morales L, Vargas-Lares Jde J, Ramirez-Herrera MA, Mendoza-Magaua ML, Troyo-Sanroman R, Diaz-Esquivel P, Corona-Rivera JR. Protective in vivo effect of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. J Appl Genet. 2007; 48(4):389-96.

Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T, Ballivet M. A Neuronal Nicotinic Acetylcholine Receptor Subunit (Alpha 7) Is Developmentally Regulated and Forms a Homo-Oligomeric Channel Blocked by Alpha-BTX. Neuron. 1990; 5(6):847-56.

D'Souza MS, Markou A. Neuronal Mechanisms Underlying Development of Nicotine Dependence: Implications for Novel Smoking-Cessation Treatments. Addict Sci Clin Pract. 2011; 6(1):4-16.

d'Adesky ND, de Rivero Vaccari JP, Bhattacharya P, Schatz M, Perez-Pinzon MA, Bramlett HM, Raval AP. Nicotine Alters Estrogen Receptor-Beta-Regulated Inflammasome Activity and Exacerbates Ischemic Brain Damage in Female Rats. Int J Mol Sci. 2018; 19(5):1330.

Dai W, Wang H, Fang J, Zhu Y, Zhou J, Wang X, Zhou Y, Zhou M. Curcumin provides neuroprotection in model of traumatic brain injury via the Nrf2-ARE signalling pathway. Brain Res Bull. 2018; 140:65-71.

Dalberto D, Nicolau CC, Garcia ALH, Nordin AP, Grivicich I, Silva JD. Cytotoxic and genotoxic evaluation of cotinine using human neuroblastoma cells (SH-SY5Y). Genet Mol Biol. 2020; 43(2):e20190123

Danafar H, Sharafi A, Askarlou S, Manjili HK. Preparation and Characterization of PEGylated Iron Oxide-Gold Nanoparticles for Delivery of Sulforaphane and Curcumin. Drug Res (Stuttg). 2017; 67(12):698-704.

Danafar H. Study of the composition of polycaprolactone/poly (ethylene glycol)/polycaprolactone copolymer and drug-to-polymer ratio on drug loading efficiency of curcumin to nanoparticles. Jundishapur J Nat Pharm Prod. 2017; 12(1): e34179.

Dasgupta P, Rizwani W, Pillai S, Kinkade R, Kovacs M, Rastogi S, Banerjee S, Carless M, Kim E, Coppola D, Haura E, Chellappan S. Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. Int J Cancer. 2009; 124(1):36-45.

Dastani M, Rahimi HR, Askari VR, Jaafari MR, Jarahi L, Yadollahi A, Rahimi VB. Three months of combination therapy with nano-curcumin reduces the inflammation and lipoprotein (a) in type 2 diabetic patients with mild to moderate coronary artery disease: Evidence of a randomized, double-blinded, placebo-controlled clinical trial. Biofactors. 2022 Jun 8.

Dei Cas M, Ghidoni R. Dietary Curcumin: Correlation between Bioavailability and Health Potential. Nutrients. 2019; 11(9):2147.

Delijewski M, Wrześniok D, Otręba M, Beberok A, Rok J, Buszman E. Nicotine impact on melanogenesis and antioxidant defense system in HEMn-DP melanocytes. Mol Cell Biochem. 2014; 395(1-2):109-16.

DeMayo FJ, Zhao B, Takamoto N, Tsai SY. Mechanisms of action of estrogen and progesterone. Ann N Y Acad Sci. 2002; 955:48-59.

Den Hartogh DJ, Gabriel A, Tsiani E. Antidiabetic Properties of Curcumin II: Evidence from In Vivo Studies. Nutrients. 2019; 12(1):58.

Den Hartogh DJ, Gabriel A, Tsiani E. Antidiabetic Properties of Curcumin I: Evidence from In Vitro Studies. Nutrients. 2020; 12(1):118.

Dende C, Meena J, Nagarajan P, Nagaraj VA, Panda AK, Padmanaban G. Nanocurcumin is superior to native curcumin in preventing degenerative changes in Experimental Cerebral Malaria. Sci Rep. 2017; 7(1):10062.

Devassy JG, Nwachukwu ID, Jones PJ. Curcumin and cancer: barriers to obtaining a health claim. Nutr Rev. 2015; 73(3):155-65.

Durazzo,T.C.; Mattsson, N.; Weiner, M.W.; Alzheimer's Disease Neuroimaging Initiative. Smoking and increased Alzheimer's disease risk: A review of potential mechanisms. Alzheimers Dement. 2014; 10(3):S122-S245.

Edwards RL, Luis PB, Varuzza PV, Joseph AI, Presley SH, Chaturvedi R, Schneider C. The anti-inflammatory activity of curcumin is mediated by its oxidative metabolites. J Biol Chem. 2017; 292(52):21243-21252.

El-Desoky GE, Wabaidur SM, AlOthman ZA, Habila MA. Evaluation of Nano-curcumin effects against Tartrazine-induced abnormalities in liver and kidney histology and other biochemical parameters. Food Sci Nutr. 2022; 10(5):1344-1356

El-Desoky GE, Wabaidur SM, AlOthman ZA, Habila MA. Regulatory Role of Nano-Curcumin against Tartrazine-Induced Oxidative Stress, Apoptosis-Related Genes Expression, and Genotoxicity in Rats. Molecules. 2020; 25(24):5801.

Elisia I, Lam V, Cho B, Hay M, Li MY, Yeung M, Bu L, Jia W, Norton N, Lam S, Krystal G. The effect of smoking on chronic inflammation, immune function and blood cell composition. Sci Rep. 2020; 10:19480.

El-Zayadi AR. Heavy smoking and liver. World J Gastroenterol. 2006; 12(38):6098-101.

Fadus MC, Lau C, Bikhchandani J, Lynch HT. Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med. 2016; 7(3):339-346.

Fant RV, Henningfield JE, Shiffman S, Strahs KR, Reitberg DP. A Pharmacokinetic Crossover Study to Compare the Absorption Characteristics of Three Transdermal Nicotine Patches. Pharmacol Biochem Behav. 2000; 67(3):479-482.

Farhadi M, Bakhshandeh M, Shafiei B, Mahmoudzadeh A, Hosseinimehr S J. The Radioprotective Effects of Nano-Curcumin Against Genotoxicity Induced by Iodine-131 in Patients with Differentiated Thyroid Carcinoma (DTC) by Micronucleus Assay. Int J Cancer Manag. 2018; 11(6):e14193.

Farzaei MH, Zobeiri M, Parvizi F, El-Senduny FF, Marmouzi I, Coy-Barrera E, Naseri R, Nabavi SM, Rahimi R, Abdollahi M. Curcumin in Liver Diseases: A Systematic Review of the Cellular Mechanisms of Oxidative Stress and Clinical Perspective. Nutrients. 2018; 10(7):855.

Flora G, Gupta D, Tiwari A. Preventive efficacy of bulk and nanocurcumin against lead-induced oxidative stress in mice. Biol Trace Elem Res. 2013; 152(1):31-40.

Friedman RB, Young DS. Effects of Disease on Clinical Laboratory Tests. AACC. 2000; 5th Ed.

Galaris D, Barbouti A, Pantopoulos K. Iron homeostasis and oxidative stress: An intimate relationship. Biochim Biophys Acta Mol Cell Res. 2019; 1866(12):118535.

Gallucci G, Tartarone A, Lerose R, Lalinga AV, Capobianco AM. Cardiovascular risk of smoking and benefits of smoking cessation. J Thorac Dis. 2020; 12(7):3866–76.

Gandhi KK, Foalds J, Steinberg MB. Lower Quit rates among African American and Latino menthol cigarette smokers at a tobacco treatment clinic. Int J Clin Pract. 2009; 63:360- 367.

Gatoo MA, Naseem S, Arfat MY, Dar AM, Qasim K, Zubair S. Physicochemical properties of nanomaterials: implication in associated toxic manifestations. Biomed Res Int. 2014; 2014:498420.

GBD 2019 Tobacco Collaborators. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. Lancet. 2021; 397:2337-2360.

Gergalova G, Lykhmus O, Kalashnyk O, Koval L, Chernyshov V, Kryukova E, Tsetlin V, Komisarenko S, Skok M. Mitochondria express α7 nicotinic acetylcholine receptors to regulate Ca2+ accumulation and cytochrome c release: study on isolated mitochondria. PLoS One. 2012; 7(2):e31361.

Ghelani H, Razmovski-Naumovski V, Chang D, Nammi S. Chronic treatment of curcumin improves hepatic lipid metabolism and alleviates the renal damage in adenine-induced chronic kidney disease in Sprague-Dawley rats. BMC Nephrol. 2019; 20(1):431.

Gheni N, Nanakali NMQ. Protective effect of aqueous extract of neem (azadirachta indica) leaves against nicotine induced oxidative stress in a model of hyperlipidemia.biochem. Cell Arch. 2020; 20(1)1407-1413.

Ghosh M, Singh AT, Xu W, Sulchek T, Gordon LI, Ryan RO. Curcumin nanodisks: formulation and characterization. Nanomedicine. 2011; 7(2):162-7.

Ghosh SS, Gehr TW, Ghosh S. Curcumin and chronic kidney disease (CKD): major mode of action through stimulating endogenous intestinal alkaline phosphatase. Molecules. 2014; 19(12):20139-56.

Gipson CD, Fowler CD. Nicotinic Receptors Underlying Nicotine Dependence: Evidence from Transgenic Mouse Models. Curr Top Behav Neurosci. 2020; 45:101-121.

Global Burden of Disease [Database].Washington, DC: Institute of Health Metrics; 2019. IHME, Accessed 17 July 2021.

Goodwin RD, Pagura J, Spiwak R, Lemeshow AR, Sareen J. Predictors of persistent nicotine dependence among adults in the United States. Drug Alcohol Depend. 2011; 118(2-3):127-33.

Gopal KS, Padma M. Evaluation of cytogenic damage in the form of micronuclei in oral exfoliated buccal cells in tobacco users. Indian J Dent Res. 2018; 29(6):773-780.

Gorabi AM, Razi B, Aslani S, Abbasifard M, Imani D, Sathyapalan T, Sahebkar A. Effect of curcumin on proinflammatory cytokines: A meta-analysis of randomized controlled trials. Cytokine. 2021; 143:155541.

Gourlay SG, Benowitz NL. Arteriovenous Differences in Plasma Concentration of Nicotine and Catecholamines and Related Cardiovascular Effects after Smoking, Nicotine Nasal Spray, and Intravenous Nicotine. Clin Pharmacol Ther. 1997; 62(4):453-463.

Gram IT, Park SY, Wilkens LR, Haiman CA, Le Marchand L. Smoking-related risks of colorectal cancer by anatomical subsite and sex. Am J Epidemiol. 2020; 189(6):543–553.

Grundy MS. National Cholesterol Education Program. Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA. 1993; 269(23):3015-3023.

Guo L, Urban JF, Zhu J, Paul WE. Elevating calcium in Th2 cells activates multiple pathways to induce IL-4 transcription and mRNA stabilization. J Immunol. 2008; 181:3984-93.

Gupta B, Gupta R, Sharma KK, Gupta A, Mahanta TG, Deedwania PC. Low Prevalence of AHA-Defined Ideal Cardiovascular Health Factors: A Study of Urban Indian Men and Women. Glob Heart. 2017; 12(3):219-225.

Gupta RC. Nonrandom Binding of the Carcinogen N-Hydroxy-2 Acetylaminofluorene to Repetitive Sequence of Rat Liver DNA in Vivo. Biochemistry. 1984; 81:6943-6947.

Haga Y, Kanda T, Sasaki R, Nakamura M, Nakamoto S, Yokosuka O. Nonalcoholic fatty liver disease and hepatic cirrhosis: Comparison with

viral hepatitis-associated steatosis. World J Gastroenterol. 2015; 21(46):12989-95.

Halimi JM, Giraudeau B, Vol S, Cacès E, Nivet H, Lebranchu Y, Tichet J. Effects of current smoking and smoking discontinuation on renal function and proteinuria in the general population. Kidney Int. 2000; 58(3):1285-92.

Hamouda AK, Bautista MR, Akinola LS, Alkhlaif Y, Jackson A, Carper M, Toma WB, Garai S, Chen YC, Thakur GA, Fowler CD, Damaj MI. Potentiation of (α4)2(β2)3, but not (α4)3(β2)2, nicotinic acetylcholine receptors reduces nicotine self-administration and withdrawal symptoms. Neuropharmacol. 2021; 190:108568.

Hanna DH, Saad GR. Nanocurcumin: preparation, characterization and cytotoxic effects towards human laryngeal cancer cells. RSC Advances. 2020; 10(35):20724-20737.

Hardwick JM, Soane L. Multiple functions of BCL-2 family proteins. Cold Spring Harb Perspect Biol. 2013; 5(2):a008722.

Hartel C, Adam N, Strunk T, Temming P, Muller-Steinhardt M, Schultz C. Cytokine responses correlate differentially with age in infancy and early childhood. Clin. Exp. Immunol. 2005; 142:446-453.

Hasan H, Arhouma T, Khanfar M, Azzam M. Study the Relationship between the Nicotine and Lipid Profile with Some Hematology Parameters in Serum of Smoker and Non-Smoker Blood Samples. J Biosci Med. 2022; 10:20-36.

Hashish EA, Elgaml SA. Hepatoprotective and Nephroprotective Effect of Curcumin Against Copper Toxicity in Rats. Indian J Clin Biochem. 2016; 31(3):270-7.

Hasimun P, Sukandar EY, Adnyana IK, Tjahjono DH. Synergistic effect of curcuminoid and s-methyl cysteine in regulation of cholesterol homeostasis. Int J Pharmacol. 2011; 7:268-272.

Hassan AN, Le Foll B. Survival Probabilities and Predictors of Major Depressive Episode Incidence Among Individuals With Various Types of Substance Use Disorders. J Clin Psychiatry. 2021; 82(5):2013637.

Hassanzadeh K, Buccarello L, Dragotto J, Mohammadi A, Corbo M, Feligioni M. Obstacles against the Marketing of Curcumin as a Drug. Int J Mol Sci. 2020; 21(18):6619.

Hawk PB, Oser BL, Summerson WH, Oser BL Ltd.Practical Physiological Chemistry,. Mc Graw Hill Book Comp, New York, London. 1954;13th Ed.

He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: how are they linked? Molecules. 2015; 20(5):9183-213.

Hecht SS, Carmella SG, Chen M, Dor Koch JF, Miller AT, Murphy SE, Jensen JA, Zimmerman CL, Hatsukami DK. Quantitation of Urinary Metabolites of a Tobacco-Specific Lung Carcinogen after Smoking Cessation. Cancer Res. 1999; 59(3):590-596.

Hecht SS, Hochalter JB, Villalta PW, Murphy SE. 2′-Hydroxylation of Nicotine by Cytochrome P450 2A6 and Human Liver Microsomes: Formation of a Lung Carcinogen Precursor. Proc Natl Acad Sci U S A. 2000; 97(23):12493-12497.

Hegde VH, Ambili. Comparison of Nicotine Concentration and PH of Commercially Available Smokeless Tobacco Products. J Oral Res Rev. 2017; (9):21-24.

Henningfield JE, Keenan RM. Nicotine Delivery Kinetics and Abuse Liability. J Consult Clin Psychol. 1993; 61(5):743-750.

Hirayama T. Non-smoking wives of heavy smokers have a higher risk of lung cancer: a study from Japan. Br Med J (Clin Res Ed). 1981; 282(6259):183-5.

Hoshyar N, Gray S, Han H, Bao G. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. Nanomed (Lond). 2016; 11(6):673-92.

Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet. 2011; 378(9799):1297-305.

Igaki T, Miura M. Role of Bcl-2 family members in invertebrates. Biochim Biophys Acta. 2004; 1644(2-3):73-81.

Iranloye BO, Bolarinwa AF. Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. Niger J Physiol Sci. 2009; 24(1):7-12.

Ishida M, Ishida T, Tashiro S, Uchida H, Sakai C, Hironobe N, Miura K, Hashimoto Y, Arihiro K, Chayama K, Kihara Y, Yoshizumi M. Smoking cessation reverses DNA double-strand breaks in human mononuclear cells. PLoS One. 2014; 9(8):e103993.

Ishida J, Ohtsu H, Tachibana Y, Nakanishi Y, Bastow KF, Nagai M, Wang HK, Itokawa H, Lee KH. Antitumor agents. part 214: synthesis and evaluation of curcumin analogues as cytotoxic agents. Bioorg Med Chem. 2002; 10(11): 3481–3487.

Jäger R, Lowery RP, Calvanese AV, Joy JM, Purpura M, Wilson JM. Comparative absorption of curcumin formulations. Nutr J. 2014; 13:11.

Jagetia GC. Antioxidant activity of curcumin protects against the radiation-induced micronuclei formation in cultured human peripheral blood lymphocytes exposed to various doses of γ-Radiation. Int J Radiat Biol. 2021; 97(4):485-493.

Jain G, Jaimes EA. Nicotine signalling and progression of chronic kidney disease in smokers. Biochem Pharmacol. 2013; 86(8):1215-23.

Jalali M, Mahmoodi M, Mosallanezhad Z, Jalali R, Imanieh MH, Moosavian SP. The effects of curcumin supplementation on liver function, metabolic profile and body composition in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. Complement Ther Med. 2020; 48:102283.

Jalili C, Ahmadi S, Roshankhah S, Salahshoor M. Effect of Genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. Int J Reprod BioMed. 2016; 14:95-102.

Jalili C, Salahshoor MR, Moradi MT, Ahookhash M, Taghadosi M, Sohrabi M. Expression Changes of Apoptotic Genes in Tissues from Mice Exposed to Nicotine. Asian Pac J Cancer Prev. 2017; 18(1):239-244.

Jamil A, Rashid A, Naveed AK, Asim M. Effect of smoking on interleukin-6 and correlation between IL-6 and serum amyloid A-low density lipoprotein in smokers. J Postgrad Med Inst. 2017; 31(4):336-8.

Jamwal R. Bioavailable curcumin formulations: A review of pharmacokinetic studies in healthy volunteers. J Integr Med. 2018; 16(6):367-374.

Jandikova H, Duskova M, Starka L. The Influence of Smoking and Cessation on the Human Reproductive Hormonal Balance. Physiol Res. 2017; 66(3):S323 - S331.

Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, Lee SR, Yang SH. The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics. Int J Mol Sci. 2021; 22(5):2719.

Jantawong C, Priprem A, Intuyod K, Pairojkul C, Pinlaor P, Waraasawapati S, Mongkon I, Chamgramol Y, Pinlaor S. Curcumin-loaded nanocomplexes: Acute and chronic toxicity studies in mice and hamsters. Toxicol Rep. 2021; 8:1346-1357.

Jarhahzadeh M, Alavinejad P, Farsi F, Husain D, Rezazadeh A. The effect of turmeric on lipid profile, malondialdehyde, liver echogenicity and enzymes among patients with nonalcoholic fatty liver disease: a randomized double blind clinical trial. Diabetol Metab Syndr. 2021; 13(1):112.

Jena SS, Kabi S, Panda BN, Kameswari BC, Payal, Behera IC, Tripathy SK, Mahanta S. Smokeless Tobacco and Stroke - A Clinico-epidemiological Follow-up Study in A Tertiary Care Hospital. J Clin Diagn Res. 2016; 10(10):OC40-OC43.

Jensen KP, Valentine G, Gueorguieva R, Sofuoglu M. Differential Effects of Nicotine Delivery Rate on Subjective Drug Effects, Urges to Smoke, Heart Rate and Blood Pressure in Tobacco Smokers .Psychopharmacol. 2020; 237:1359-1369.

Jha P, Peto R. Global effects of smoking, of quitting, and of taxing tobacco. N Engl J Med. 2014; 370(1):60-8.

Jha P. The hazards of smoking and the benefits of cessation: a critical summation of the epidemiological evidence in high-income countries. Elife. 2020; 9:e49979.

Jha P. 21st- century hazards of smoking and benefits of cessation in the United States. N. Engl. J. Med. 2013; 368:341-350.

Jie Z, Chao M, Jun A, Wei S, LiFeng M. Effect of Curcumin on Diabetic Kidney Disease: A Systematic Review and Meta-Analysis of Randomized, Double-Blind, Placebo-Controlled Clinical Trials. Evid Based Complement Alternat Med. 2021; 2021:6109406.

Jones ME, Schoemaker MJ, Wright LB, Ashworth A, Swerdlow AJ. Smoking and risk of breast cancer in the Generations Study cohort. Breast Cancer Res. 2017; 19(1):118.

Jung YS, Yoon SJ. Burden of Cancer Due to Cigarette Smoking and Alcohol Consumption in Korea. Int J Environ Res Public Health. 2022; 19(6):3493.

Junttila IS. Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. Front Immunol. 2018; 9:888.

Kalpana C, Rajasekharan KN, Menon VP. Modulatory effects of curcumin and curcumin analog on circulatory lipid profiles during nicotine-induced toxicity in Wistar rats. J Med Food. 2005; 8(2):246-50.

Kang Q, Chen A. Curcumin suppresses expression of low-density lipoprotein (LDL) receptor, leading to the inhibition of LDL-induced activation of hepatic stellate cells. Br J Pharmacol. 2009; 157:1354-1367.

Karthikeyan A, Senthil N, Min T. Nanocurcumin: A Promising Candidate for Therapeutic Applications. Front Pharmacol. 2020; 11:487.

Kawahara T, Ito H, Yao M, Uemura H. Impact of smoking habit on overactive bladder symptoms and incontinence in women. Int J Urol. 2020; 27(12):1078-86.

Khademi F, Totonchi H, Mohammadi N, Zare R, Zal F. Nicotine-Induced Oxidative Stress in Human Primary Endometrial Cells. Int.J.Toxicol. 2019; 38(3):202-208.

Khademi F, Totonchi H, Mohammadi N, Zare R, Zal F. Nicotine-Induced Oxidative Stress in Human Primary Endometrial Cells. The Neuroscientist. 2019; 38(3):432-439.

Khaitan T, Shukla AK, Gupta P, Naik SR, Verma P, Kumar S. Liver and thyroid profile in educating smokeless tobacco users and its role in oral health promotion. J Educ Health Promot. 2019; 29(8):224.

Khalid A, Qureshi HJ, Nazir S, Lone KP. Effect of Second Hand Smoke Exposure on Serum Estradiol and Progesterone Levels in women having First Trimester Spontaneous Abortions. Pak J Med Health Sci. 2017; 11(1):163-167.

Kheiripour N, Plarak A, Heshmati A, Asl SS, Mehri F, Ebadollahi-Natanzi A, Ranjbar A, Hosseini A. Evaluation of the hepatoprotective effects of curcumin and nanocurcumin against paraquat-induced liver injury in rats: Modulation of oxidative stress and Nrf2 pathway. J Biochem Mol Toxicol. 2021; 35(5):e22739.

Kido R, Sato I, Tsuda S. Detection of in Vivo DNA Damage Induced by Ethanol in Multiple Organs of Pregnant Mice Using the Alkaline Single Cell Gel Electrophoresis (Comet) Assay. J Vet Med Sci. 2006; 68(1):41-47.

Kim CS, Choi JS, Joo SY, Bae EH, Ma SK, Lee J, Kim SW. Nicotine-Induced Apoptosis in Human Renal Proximal Tubular Epithelial Cells. PLoS One. 2016; 11(3):e0152591.

Kim M, Kim Y. Hypocholesterolemic effects of curcumin via up-regulation of cholesterol 7a-hydroxylase in rats fed a high fat diet. Nutr Res Pract. 2010; 4(3):191-5.

Kind PRN, King EJ. Estimation of Plasma Phosphatase by Determination of Hydrolysed Phenol with Amino-Antipyrine. J Clin Path. 1954; 7(4):322-326.

Kizildag S, Hosgorler F, Güvendi G, Koc TB, Kandis S, Argon A, Ates M, Uysal N. Nicotine lowers TNF-α, IL-1b secretion and leukocyte accumulation via nAChR in rat stomach, Toxin Reviews. 2021; 40(1):17-24.

Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr. 2017; 57(13):2889-2895.

Kong G, Cavallo DA, Goldberg A, LaVallee H, Krishnan-Sarin S. Blunt Use among Adolescents and Young Adults: Informing Cigar Regulations. Tob Regul Sci. 2018; 4(5):50-60.

Lai Y, Yang H, Han W, Guo T, Lv H, Li J, Qian JM. Cigarette smoking associated with chronic pancreatitis: a case control study in China. Tob. Induced Dis. 2017; 15:38.

Laldinsangi C. Toxic effects of smokeless tobacco on female reproductive health: A review. Curr Res Toxicol. 2022; 3:100066.

Lampe V, Milobedeska J. Studien über Curcumin. Ber Dtsch Chem Ges. 1913; 46:2235-40.

Lan Jiabo. Overview of Application of Nanomaterials in Medical Domain. Magnetomotive Photoacoustic in Biomedical Applications, Contrast Med Molecule Imag. 2022; 2022,Article ID -3507383:5 pages.

Le Foll B, Piper ME, Fowler CD, Tonstad S, Bierut L, Lu L, Jha P, Hall WD. Tobacco and nicotine use. Nat Rev Dis Primers. 2022; 24:8(1):19.

Lee G, Jung K-H, Shin D, Lee C, Kim W, Lee S, Kim J, Bae H. Cigarette Smoking Triggers Colitis by IFN-γ+ CD4+ T Cells. Front. Immunol. 2017; 8:1344.

Lee S, Kang S, Joo YS, Lee C, Nam KH, Yun HR, Park JT, Chang TI, Yoo TH, Kim SW, Oh KH, Kim YH, Park SK, Kang SW, Choi KH, Ahn C, Han SH. Smoking, Smoking Cessation, and Progression of Chronic Kidney Disease: Results From KNOW-CKD Study. Nicotine Tob Res. 2021; 23(1):92-98.

Levander OA, DeLoach DP, Morris VC, Moser PB. Platelet glutathione peroxidase activity as an index of selenium status in rats. J Nutr. 1983; 113(1):55-63.

Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 1994; 233: 346 - 357.

Li LF, Chan RL, Lu L, Shen J, Zhang L, Wu WK, Wang L, Hu T, Li MX, Cho CH. Cigarette smoking and gastrointestinal diseases: the causal relationship

and underlying molecular mechanisms (review). Int J Mol Med. 2014; 34(2):372-80.

Liang Z, Wu R, Xie W, Zhu M, Xie C, Li X, Zhu J, Zhu W, Wu J, Geng S, Xu W, Zhong C, Han H. Curcumin reverses tobacco smoke induced epithelial mesenchymal transition by suppressing the MAPK pathway in the lungs of mice. Mol Med Rep. 2018; 17(1):2019-2025.

Lilkova E, Petkov P, Ilieva N, Krachmarova E, Nacheva G, Litov L. Molecular modeling of the effects of glycosylation on the structure and dynamics of human interferon-gamma. J Mol Model. 2019; 25(127):1-13.

Lin X, Bai D, Wei Z, Zhang Y, Huang Y, Deng H, Huang X. Curcumin attenuates oxidative stress in RAW264.7 cells by increasing the activity of antioxidant enzymes and activating the Nrf2-Keap1 pathway. PLoS One. 2019; 14(5):e0216711.

Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN, Kamat AA, Spannuth WA, Gershenson DM, Lutgendorf SK, Aggarwal BB, Sood AK. Curcumin inhibits tumour growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res. 2007; 13(11):3423-30.

Lindell G, Lunell E, Graffner H. Transdermally Administered Nicotine Accumulates in Gastric Juice. Eur J Clin Pharmacol. 1996; 51(3-4):315-318.

Lopresti AL. The Problem of Curcumin and Its Bioavailability: Could Its Gastrointestinal Influence Contribute to Its Overall Health-Enhancing Effects? Adv Nutr. 2018; 9(1):41-50.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin¬ phenol reagent. J Biol Chem. 1951; 193(1):265-275.

Luber RP, Rentsch C, Lontos S, Pope JD, Aung AK, Schneider HG, Kemp W, Roberts SK, Majeed A. Turmeric Induced Liver Injury: A Report of Two Cases. Case Reports Hepatol. 2019; 2019:6741213.

Lunell E, Molander L, Ekberg K, Wahren J. Site of Nicotine Absorption from a Vapour Inhaler–Comparison with Cigarette Smoking. Eur J Clin Pharmacol. 2000; 55(10):737-741.

Machova Urdzikova L, Karova K, Ruzicka J, Kloudova A, Shannon C, Dubisova J, Murali R, Kubinova S, Sykova E, Jhanwar-Uniyal M, Jendelova P. The Anti-Inflammatory Compound Curcumin Enhances Locomotor and Sensory Recovery after Spinal Cord Injury in Rats by Immunomodulation. Int J Mol Sci. 2015; 17(1):49.

Maier CM, Chan PH. Role of superoxide dismutase in oxidative damage and neurodegenerative disorder. Neuroscientist. 2002; 8:323-334.

Maimela NR, Liu S, Zhang Y. Fates of CD8+ T cells in Tumor Microenvironment. Comput Struct Biotechnol J. 2018; 17:1-13.

Maiti M, Chattopadhyay K, Verma M, Chattopadhyay B. Curcumin protects against nicotine-induced stress during protein malnutrition in female rat through immunomodulation with cellular amelioration. Mol Biol Rep. 2015; 42(12):1623-37.

Malenica M, Prnjavorac B, Bego T, Dujic T, Semiz S, Skrbo S, Gusic A, Hadzic A, Causevic A. Effect of Cigarette Smoking on Haematological Parameters in Healthy Population. Med Arch. 2017; 71(2):132-136.

Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, Faure P. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron. 2006; 50(6):911-21.

Manikandan S, Mabrouk KE, Ballamuruganan A. Synthesis of Nanocurcumin and Evaluation of its Properties for Biomedical Applications. Trends Biomater Artif Organs. 2022; 36(S1):67-69.

Marchiani A, Rozzo C, Fadda A, Delogu G, Ruzza P. Curcumin and curcumin-like molecules: from spice to drugs. Curr Med Chem. 2014; 21(2):204-22.

Marcy TW, Merrill WW. Cigarette smoking and respiratory tract infection. Clin Chest Med. 1987; 8(3):381-91.

Martínez-Castillo M, Villegas-Sepúlveda N, Meraz-Rios MA, Hernández-Zavala A, Berumen J, Coleman MA, Orozco L, Cordova EJ. Curcumin differentially affects cell cycle and cell death in acute and chronic myeloid leukemia cells. Oncol Lett. 2018; 15(5):6777-6783.

Marzouk HS, Awaad AS, Abo-Eleneen RE, El-Bakry AM .Effects of experimentally induced nicotine on the liver of neonatal albino rat. Adv Anim Vet Sci. 2022; 10(1):151-159.

Masella R, Di Benedotto R, Vari R, Filesi C, Ginovannini C. Novel mechanism s of natural antioxidant compounds in biological system: Involvement of glutathione and glutathione reductase enzymes. J Nutr Biochem. 2005; 16:577-586.

Mbulo L, Palipudi KM, Smith T, Yin S, Munish VG, Sinha DN, Gupta PC, Swasticharan L. Patterns and related factors of bidi smoking in India. Tob Prev Cessat. 2020; 6:28.

McKay BE, Placzek AN, Dani JA. Regulation of Synaptic Transmission and Plasticity by Neuronal Nicotinic Acetylcholine Receptors. Biochem Pharmacol. 2007; 74:1120-1133.

Mendonça LM, Machado Cda S, Teixeira CC, Freitas LA, Bianchi ML, Antunes LM. Comparative study of curcumin and curcumin formulated in a solid dispersion: Evaluation of their antigenotoxic effects. Genet Mol Biol. 2015; 38(4):490-8.

Mendoza JL, Escalante NK, Jude KM, Sotolongo Bellon J, Su L, Horton TM, Tsutsumi N, Berardinelli SJ, Haltiwanger RS, Piehler J, Engleman EG, Garcia KC. Structure of the IFNγ receptor complex guides design of biased agonists. Nature. 2019; 567(7746):56-60.

Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol. 2007; 595:105-25.

Menshawy MM, Sharaf WM, Esmail RS, Farrag AR. Histopathological and histochemical effects of nicotine on the liver and kidney of adult male rats. J Arab Soc Med Res. 2019; 14:7-13.

Merghani TH, Saeed A, Alawad A. Changes in plasma IL4, TNFá and CRP in response to regular passive smoking at home among healthy school children in Khartoum, Sudan. Afr Health Sci. 2012; 12(1):41-7.

Metzler M, Pfeiffer E, Schulz SI, Dempe JS. Curcumin uptake and metabolism. Biofactors. 2013; 39:14-20.

Milano F, Mari L, van de Luijtgaarden W, Parikh K, Calpe S, Krishnadath KK. Nano-curcumin inhibits proliferation of esophageal adenocarcinoma cells and enhances the T cell mediated immune response. Front Oncol. 2013; 3:137.

Mishra A, Chaturvedi P, Datta S, Sinukumar S, Joshi P, Garg A. Harmful effects of nicotine. Indian J Med Paediatr Oncol. 2015; 36(1):24-31.

Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, Yazdi M, Bonyadi F, Mohajeri D, Mazani M, Marzban H, Łos MJ, Ghavami S. Pre-administration of turmeric prevents methotrexate-induced liver toxicity and oxidative stress. BMC Complement Altern Med. 2015; 15:246.

Mohajeri M, Rezaee M, Sahebkar A. Cadmium-induced toxicity is rescued by curcumin: A review. Biofactors. 2017; 43(5):645-661.

Mohammadghasemi F, Khanaki K, Moravati H, Faghani M. The amelioration of nicotine-induced reproductive impairment in male mouse by Sambucus ebulus L. fruit extract. Anat Cell Biol. 2021; 54(2):232-240.

Mohammed AM, Hussen DF, Rashad H, Hasheesh A. The Micronuclei Scoring as a Biomarker for Early Detection of Genotoxic Effect of Cigarette Smoking. Asian Pac J Cancer Prev. 2020; 21(1):87-92.

Mohammed ES, El-Beih NM, El-Hussieny EA, El-Ahwany E, Hassan M, Zoheiry M. Effects of free and nanoparticulate curcumin on chemically induced liver carcinoma in an animal model. Arch Med Sci. 2020; 17(1):218-227.

Mohanapriya S, Maheswaran T, Ganapathy N, Yoithapprabhunath TR, Dineshshankar J, Ilayaraja V, Vinodhini RS, Devi R. Evaluation of DNA damage in tobacco associated human buccal cells using comet assay. Med Pharm Rep. 2021; 94(2):214-219.

Moktar A, Ravoori S, Vadhanam MV, Gairola CG, Gupta RC. Cigarette smoke-induced DNA damage and repair detected by the comet assay in HPV-transformed cervical cells. Int J Oncol. 2009; 35(6):1297-304.

Moss GA, Bondar RL, Buzzelli DM. Kinetic Enzymatic Method for Determining Serum Creatinine. Clin Chem. 1975; 21:1422-1426.

Mueck AO, Seeger H. Smoking, estradiol metabolism and hormone replacement therapy. Curr Med Chem Cardiovasc Hematol Agents. 2005; 3(1):45-54.

Murphy SE. Biochemistry of Nicotine Metabolism and Its Relevance to Lung Cancer. J Biol Chem. 2021; 296:100722.

Narake SS, Gupta PC. Nasal use of snuff. Indian J Cancer. 2014; 51(1):S88.

Nath MC, Rahman AKMS, Nath MC, Dutta A, Khan ZH, Ghosh E, Akhter S, Islam MS, Sultana S, Begum R, Rahman MM. The Effect of Cigarette Smoking on Fasting Lipid Profile: A Single Center Study. Fortune J Health Sci. 2022; 5(2):363-373.

National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General (Centers for Disease Control and Prevention). 2014.

Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. J Med Chem. 2017; 60(5):1620-1637.

Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K, Kitahara M. Curcuminoids and sesquiterpenoids in turmeric (Curcuma Longa L.) suppress an increase in blood glucose level in type 2 diabetic KK-ay mice. J Agric Food Chem. 2005; 53:959-963.

No DS, Algburi A, Huynh P, Moret A, Ringard M, Comito N, Drider D, Takhistov P, Chikindas ML. Antimicrobial efficacy of curcumin nanoparticles against Listeria monocytogenes is mediated by surface charge. J Food Saf. 2017; 37(4):12353.

Nosrati H, Sefidi N, Sharafi A, Danafar H, Kheiri Manjili H. Bovine Serum Albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug. Bioorg Chem. 2018; 76:501-509.

Nouri-shirazi M, guinet E. Evidence for the immunosuppressive role of nicotine on humandendritic cell functions. Immunol. 2003; 109:365-373.

(501) Schraufstatter E, Bernt H. Antibacterial action of curcumin and related compounds. Nature. 1949; 164:456.

Nuryunarsih D, Lewis S, Langley T. Health Risks of Kretek Cigarettes: A Systematic Review. Nicotine Tob Res. 2021; 23(8):1274-1282.

Oboni JB, Marques-Vidal P, Bastardot F, Vollenweider P, Waeber G. Impact of smoking on fertility and age of menopause: a populationbased assessment. BMJ Open. 2016; 6(11):e012015-e012015.

Ohori H, Yamakoshi H, Tomizawa M, Shibuya M, Kakudo Y, Takahashi A, Takahashi S, Kato S, Suzuki T, Ishioka C, Iwabuchi Y, Shibata H. Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medicinal treatment of cancer. Mol Cancer Ther. 2006; 5(10):2563-71.

Oliveira AJM, Deventer VEH, Bachmann ML, et al. Evaluation of Four Different Equations for Calculating LDL-C with Eight Different Direct HDL-C Assays. Clin Chim Acta. 2013; 23 (423):135-140.

Olsson P, Skogstrand K, Nilsson A, Turesson C, Jacobsson LTH, Theander E, Houen G, Mandl T. Smoking, disease characteristics and serum cytokine levels in patients with primary Sjögren's syndrome. Rheumatol Int. 2018; 38(8):1503-1510.

Oyeyipo IP, Raji Y, Bolarinwa AF. Nicotine alters serum antioxidant profile in male albino rats. N Am J Med Sci. 2014; 6(4):168-71.

Palipudi KM, Gupta PC, Sinha DN, Andes LJ, Asma S, McAfee T; GATS Collaborative Group. Social determinants of health and tobacco use in thirteen low and middle income countries: evidence from Global Adult Tobacco Survey. PLoS One. 2012; 7(3):e33466.

Panahi Y, Alishiri GH, Parvin S, Sahebkar A. Mitigation of Systemic Oxidative Stress by Curcuminoids in Osteoarthritis: Results of a Randomized Controlled Trial. J Diet Suppl. 2016; 13(2):209-20.

Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A, Sahebkar A. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. Phytother Res. 2014a; 28(11):1625-31.

Panahi Y, Saadat A, Beiraghdar F, Sahebkar A. Adjuvant therapy with bioavailability-boosted curcuminoids suppresses systemic inflammation and improves quality of life in patients with solid tumors: a randomized double-blind placebo-controlled trial. Phytother Res. 2014b; 28(10):1461-7.

Pandit RS, Gaikwad SC, Agarkar GA, Gade AK, Rai M. Curcumin nanoparticles: physico-chemical fabrication and its in vitro efficacy against human pathogens. 3 Biotech. 2015; 5(6):991-997.

Pang RD, Liautaud MM, Kirkpatrick MG, Huh J, Monterosso J, Leventhal AM. Ovarian Hormones and Transdermal Nicotine Administration Independently and Synergistically Suppress Tobacco Withdrawal Symptoms and Smoking Reinstatement in the Human Laboratory. Neuropsychopharmacol. 2018; 43(4):828-837.

Patil TN, Srinivasan M. Hypocholesteremic effect of curcumin in induced hypercholesteremic rats. Indian J Exp Biol. 1971; 9:167-9.

Pau CT, Keefe CC, Welt CK. Cigarette smoking, nicotine levels and increased risk for metabolic syndrome in women with polycystic ovary syndrome. Gynecol Endocrinol. 2013; 29(6):551-5.

Perez-Paramo YX, Chen G, Ashmore JH, Watson CJW, Nasrin S, Adams-Haduch J, Wang R, Gao YT, Koh WP, Yuan JM, Lazarus P. Nicotine-N'-Oxidation by Flavin Monooxygenase Enzymes. Cancer Epidemiol Biomarkers Prev. 2019; 28(2):311-320.

Perkins KA, Karelitz JL, Giedgowd GE, Conklin CA. Negative mood effects on craving to smoke in women versus men. Addict Behav. 2013; 38(2):1527-1531.

Peters EN, Schauer GL, Rosenberry ZR, Pickworth WB. Does marijuana "blunt" smoking contribute to nicotine exposure?: Preliminary product testing of nicotine content in wrappers of cigars commonly used for blunt smoking. Drug and Alcohol Depend. 2016; 168:119-122.

Petersen AM, Magkos F, Atherton P, Selby A, Smith K, Rennie MJ, Pedersen BK, Mittendorfer B. Smoking impairs muscle protein synthesis and increases

the expression of myostatin and MAFbx in muscle. Am J Physiol Endocrinol Metab. 2007; 293(3):E843-8.

Petrescu F, Voican SC, Silosi I. Tumor necrosis factor-alpha serum levels in healthy smokers and nonsmokers. Int J Chron Obstruct Pulmon Dis. 2010; 5:217-22.

Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Ind J Clin Biochem. 2015; 30(1):11-26.

Picciotto MR, Addy NA, Mineur YS, Brunzell DH. It Is Not "Either/or": Activation and Desensitization of Nicotinic Acetylcholine Receptors Both Contribute to Behaviors Related to Nicotine Addiction and Mood. Prog Neurobiol. 2008; 84:329-342.

Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signalling shapes nervous system function and behavior. Neuron. 2012; 76(1):116-29.

Pirie K, Peto R, Reeves GK, Green J, Beral V; Million Women Study Collaborators. The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK. Lancet. 2013; 381(9861):133-41.

Pivari F, Mingione A, Brasacchio C, Soldati L. Curcumin and Type 2 Diabetes Mellitus: Prevention and Treatment. Nutrients. 2019; 11(8):1837.

Pölkki M, Rantala MJ. Smoking affects womens' sex hormone-regulated body form. Am J Public Health. 2009; 99(8):1350.

Poojary KK, Nayak G, Vasani A, Kumari S, Dcunha R, Kunhiraman JP, Gopalan, D, Rao RR, Mutalik S, Kalthur SG, Murari MS, Raghu SV, Adiga SK, Kalthur G. Curcumin nanocrystals attenuate cyclophosphamide-induced testicular toxicity in mice. Toxicology Appl Pharmacol. 2021; 433:115772.

Prasad R, Kumar M, Trivedi SP. Antigenotoxic effect of turmeric powder extract curcumin against chromium trioxide induced genotoxicity in fish Channa punctatus. J Entomol Zool Stud. 2017; 5(1):89-94.

Prasad S, Tyagi AK. Curcumin and its analogues: a potential natural compound against HIV infection and AIDS. Food Funct. 2015; 6(11):3412-9.

Priyadarsini KI, Maity DK, Naik GH, Kumar MS, Unnikrishnan MK, Satav JG, Mohan H. Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radic Biol Med. 2003; 35(5):475-84.

Priyadarsini KI. The Chemistry of Curcumin: From Extraction to Therapeutic Agent. Molecules. 2014; 19(12):20091-20112.

Qasim H, Alarabi AB, Alzoubi KH, Karim ZA, Alshbool FZ, Khasawneh FT. The effects of hookah/waterpipe smoking on general health and the cardiovascular system. Environ Health Prev Med. 2019; 24(1):58.

Qin S, Huang L, Gong J, Shen S, Huang J, Ren H, Hu H. Efficacy and safety of turmeric and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: a meta-analysis of randomized controlled trials. Nutr J. 2017; 16(1):68.

Quidé Y, Bortolasci CC, Spolding B, Kidnapillai S, Watkeys OJ, Cohen-Woods S, Berk M, Carr VJ, Walder K, Green MJ. Association between childhood trauma exposure and pro-inflammatory cytokines in schizophrenia and bipolar-I disorder. Psychol Med. 2019; 49(16):2736-2744.

Rabinoff M, Caskey N, Rissling A, Park C. Pharmacological and chemical effects of cigarette additives. Am J Public Health. 2007; 97(11):1981-91.

Rachmawati H, Soraya IS, Kurniati NF, Rahma A. In Vitro Study on Antihypertensive and Antihypercholesterolemic Effects of a Curcumin Nanoemulsion. Sci Pharm. 2016; 84(1):131-40.

Raddam QN, Moafaq MZ, Mostafa AA, Nahla KA. Smoking Effects on Blood Antioxidants Level: Lactate Dehydrogenase, Catalase, Superoxide Dismutase and Glutathione Peroxidase in University Students. J Clin Exp Pathol. 2017; 7: 331.

Rafiee S, Bagherniya M, Askari G, Sathyapalan T, Jamialahmadi T, Sahebkar A. The Effect of Curcumin in Improving Lipid Profile in Patients with

Cardiovascular Risk Factors: A Systematic Review of Clinical Trials. Adv Exp Med Biol. 2021; 1291:165-177.

Rahimi HR, Nedaeinia R, Sepehri Shamloo A, Nikdoust S, Kazemi Oskuee R. Novel delivery system for natural products: Nano-curcumin formulations. Avicenna J Phytomed. 2016; 6(4):383-98.

Rahimi S, Khosravi A, Aazami S, et al. Effect of smoking on cyanide, IL-2 and IFN-γlevels in saliva of smokers and nonsmokers. Polish Ann Med 2018; 25:203-206.

Rajalakshmi N, Dhivya S. A Review on the preparation methods of Curcumin Nanoparticles. PharmaTutor. 2018; 6(9):6-10.

Ramalingam A, Santhanathas T, Shaukat Ali S, Zainalabidin S. Resveratrol supplementation protects against nicotine-induced kidney injury. Int J Environ Res Public Health. 2019; 16:13.

Rao Ch S, Subash Y E. The effect of chronic tobacco smoking and chewing on the lipid profile. J Clin Diagn Res. 2013; 7(1):31-4.

Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and Oxidative Stress in Chronic Kidney Disease-Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. Int J Mol Sci. 2019; 21(1):263.

Ravindran J, Prasad S, Aggarwal BB. Curcumin and cancer cells: how many ways can curry kill tumour cells selectively? AAPS J. 2009; 11(3):495-510.

Ray A, Rana S, Banerjee D, Mitra A, Datta R, Naskar S, Sarkar S. Improved bioavailability of targeted Curcumin delivery efficiently regressed cardiac hypertrophy by modulating apoptotic load within cardiac microenvironment. Toxicol Appl Pharmacol. 2016; 290:54-65.

Raymond WD, Hamdorf M, Furfaro M, Eilertsen GO, Nossent JC. Smoking associates with increased BAFF and decreased interferon-γ levels in patients with systemic lupus erythematosus. Lupus Sci Med. 2021; 8(1):e000537.

Reitman S, Frankel S. A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. Amer J Clin Pathol. 1957; 28(1):56-63.

Rose JE, Behm FM, Westman EC, Coleman RE. Arterial Nicotine Kinetics during Cigarette Smoking and Intravenous Nicotine Administration: Implications for Addiction. Drug Alcohol Depend. 1999; 56(2):99-107.

Rostami AA, Campbell JL, Pithawalla YB, Pourhashem H, Muhammad-Kah RS, Sarkar MA, Liu J, McKinney WJ, Gentry R, Gogova M. Author Correction: A comprehensive physiologically based pharmacokinetic (PBPK) model for nicotine in humans from using nicotine-containing products with different routes of exposure. Sci Rep. 2022; 12(1):2436.

Ruan X, Mueck AO. Impact of smoking on estrogenic efficacy. Climacteric. 2015; 18(1):38-46.

Rutledge SM, Asgharpour A. Smoking and Liver Disease. Gastroenterol Hepatol (N Y). 2020; 16(12):617-625.

Sadoughi S. Investigation the Effect of Curcumin on the Hormones of Pituitary-Ovarian Axis in Alloxan-induced Diabetic Rats. J Ardabil Univ Med Sci. 2016; 16(4) :441-451.

Sahebkar A, Serbanc MC, Ursoniuc S, Banach, M. Effect of curcuminoids on oxidative stress: A systematic review and meta-analysis of randomized controlled trials. J. Funct. Foods. 2015; 18:898–909.

Salahshoor M, Mohamadian S, Kakabaraei S, Roshankhah S, Jalili C. Curcumin improves liver damage in male mice exposed to nicotine. J Tradit Complement Med. 2015; 6(2):176-83.

Salahshoor MR, Roshankhah S, Motavalian V, Jalili C. Effect of Harmine on Nicotine-Induced Kidney Dysfunction in Male Mice. Int J Prev Med. 2019; 10:97.

Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. J Neurosci. 2004; 24(45):10035-9.

Salas R, Sturm R, Boulter J, De Biasi M. Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. J Neurosci. 2009; 29(10):3014-8.

Salehi B, Del Prado-Audelo ML, Cortés H, Leyva-Gómez G, Stojanović-Radić Z, Singh YD, Patra JK, Das G, Martins N, Martorell M, Sharifi-Rad M, Cho WC, Sharifi-Rad J. Therapeutic Applications of Curcumin Nanomedicine Formulations in Cardiovascular Diseases. J Clin Med. 2020; 9(3):746.

Sandhiutami NMD, Arozal W, Louisa M, Rahmat D, Mandy T. Comparative Effect of Curcumin and Nanocurcumin on Nephroprotection at Cisplatin-induced Rats. J Pharm Bioallied Sci. 2019; 11(4):S567-S573.

Sanner T, Grimsru TK. Nicotine: Carcinogenicity and Effects on Response to Cancer Treatment – A Review. Front Oncol 2015; 5:196.

Sarawi WS, Alhusaini AM, Fadda LM, Alomar HA, Albaker AB, Alghibiwi HK, Aljrboa AS, Alotaibi AM, Hasan IH, Mahmoud AM. Nano-Curcumin Prevents Copper Reproductive Toxicity by Attenuating Oxidative Stress and Inflammation and Improving Nrf2/HO-1 Signaling and Pituitary-Gonadal Axis in Male Rats. Toxics. 2022; 10(7):356.

Alghriany AAI, Omar HEM, Mahmoud AM, Atia MM. Assessment of the Toxicity of Aluminum Oxide and Its Nanoparticles in the Bone Marrow and Liver of Male Mice: Ameliorative Efficacy of Curcumin Nanoparticles. ACS Omega. 2022; 7(16):13841-13852.

Sav A, Khetrapal N, Amin P. Preparation of curcumin solid dispersions using hydrophilic polymers by different techniques and in vitro characterization. Asian J. Pharm. Sci. 2012; 7(4): 271-279.

Savige JA, Paspaliaris B, Silvestrini R, Davies D, Nikoloutsopoulos T, Sturgess A, Neil J, Pollock W, Dunster K, Hendle M. A review of immunofluorescent patterns associated with antineutrophil cytoplasmic antibodies (ANCA) and their differentiation from other antibodies. J Clin Pathol. 1998; 51(8):568-75.

Selvam C, Jachak SM, Thilagavathi R, Chakraborti AK. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as

antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg Med Chem Lett. 2005; 15(7):1793-7.

Setthacheewakul S, Mahattanadul S, Phadoongsombut N, Pichayakorn W, Wiwattanapatapee R. Development and evaluation of self-microemulsifying liquid and pellet formulations of curcumin, and absorption studies in rats. Eur J Pharm Biopharm. 2010; 76(3):475-85.

Setyawati I, Anggraeni R. Effectiveness of Sweet Orange Peel Extract (Citrus sinensis) on The Improvement of Liver Functions of Animal Trials Induced by Cigarette Smoke. J Young Pharm. 2018; 10(2):132-135.

Sezer Z, Yilmaz TE, Gungor ZB, Kalay F, Guzel E. Effects of vitamin E onnicotine-induced lipid peroxidation in rat granulosa cells: Folliculogenesis Reprod Biol. 2020; 20(1):63-74.

Shah MZUH, Shrivastava VK. Turmeric extract alleviates endocrine-metabolic disturbances in letrozole-induced PCOS by increasing adiponectin circulation: A comparison with Metformin. Metabol Open. 2021; 13:100160.

Shaik FB, Nagajothi G, Swarnalatha K, Kumar CV, Dhania KN, Kumar CS, Maddu N. Possible Association of Smokeless Tobacco Dependent Impairment in the Erythrocytes and Platelets Membranes of Human Male Volunteers: An Observation. Asian Pac J Cancer Prev. 2019; 20(7):2167-2176.

Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MN. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci. 2009; 37(3-4):223-30.

Shao XR, Wei XQ, Song X, Hao LY, Cai XX, Zhang ZR, Peng Q, Lin YF. Independent effect of polymeric nanoparticle zeta potential/surface charge, on their cytotoxicity and affinity to cells. Cell Prolif. 2015; 48(4):465-74.

Sharifi-Rad J, Rayess YE, Rizk AA, Sadaka C, Zgheib R, Zam W, Sestito S, Rapposelli S, Neffe-Skocińska K, Zielińska D, Salehi B, Setzer WN, Dosoky NS, Taheri Y, El Beyrouthy M, Martorell M, Ostrander EA, Suleria HAR,

Cho WC, Maroyi A, Martins N. Turmeric and Its Major Compound Curcumin on Health: Bioactive Effects and Safety Profiles for Food, Pharmaceutical, Biotechnological and Medicinal Applications. Front Pharmacol. 2020; 11:01021.

Sharma OP. Antioxidant activity of curcumin and related compounds. Biochem Pharmacol. 1976; 25:1811–2.

Shein M, Jeschke G. Comparison of Free Radical Levels in the Aerosol from Conventional Cigarettes, Electronic Cigarettes, and Heat-Not-Burn Tobacco Products. Chem Res Toxicol. 2019; 32(6):1289-1298.

Singh D. Effect of Cigarette Smoking on Serum Lipid Profile in Male Population of Udaipur. Biochem Anal Biochem. 2016; 5:3.

Singh DP, Gulati D, Singh P. Smoking and its association with serum lipid levels. Int J Med Res Rev. 2016; 4(11):2064-2070.

Singh KP, Lawyer G, Muthumalage T, Maremanda KP, Khan NA, McDonough SR, Ye D, McIntosh S, Rahman I. Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries. ERJ Open Res. 2019; 5(4):00182-2019.

Singh NP, Mccoy MT, Tice RR, et al. A Simple Technique for Quantification of Low Levels of DNA Damage in Individual Cells. Exp Cell Res. 1988; 175:184-191.

Singh R, Singh K. Histopasthological changes in the rat liver and kidney with references to nicotine toxicity. J drug deliv ther. 2019; 9(3):436-42.

Sinha AK, Misra GC, Patel DK. Effect of cigarette smoking on lipid profile in the young. J Assoc Physicians India. 1995; 43(3):185-8.

Sinha S, Maiti M, Chattopadhyay K, Chattopadhyay BD. Potential amelioration of curcumin against nicotine-induced toxicity of protein malnourished female rats. J Pharmacol Toxicol. 2012; 7:166-180.

Sinnhuber RO, Yu TC, Yu TC. Characterization of the Red Pigment Formed in the 2-Thiobarbituric Acid Determination of Oxidative Rancidity. Food Res. 1958; 23:626-634.

Smart DJ, Helbling FR, Verardo M, McHugh D, Vanscheeuwijck P. Mode-of-action analysis of the effects induced by nicotine in the in vitro micronucleus assay. Environ Mol Mutagen. 2019; 60(9):778-791.

Sobkowiak R, Musidlak J, Lesicki A. In vitro genoprotective and genotoxic effect of nicotine on human leukocytes evaluated by the comet assay. Drug Chem Toxicol. 2014; 37(3):322-8.

Sohn SI, Priya A, Balasubramaniam B, Muthuramalingam P, Sivasankar C, Selvaraj A, Valliammai A, Jothi R, Pandian S. Biomedical Applications and Bioavailability of Curcumin-An Updated Overview. Pharmaceutics. 2021; 13(12):2102.

Sokolowska M, Quesniaux VFJ, Akdis CA, Chung KF, Ryffel B, Togbe D. Acute Respiratory Barrier Disruption by Ozone Exposure in Mice. Front. Immunol. 2019; 10:2169.

Söylemez E, Kayaaltı Z, Aliyev V, Söylemezoğlu T. Effect of cigarette smoking on DNA damage according to nine comet assay parameters in female and male groups. Ankara Üniversitesi Tip Fakültesi Mecmuasi. 2012; 64(2):81-85.

Sreekala S, Indira M. Effects of exogenous selenium on nicotine-induced oxidative stress in rats. Biol Trace Elem Res. 2009; 130(1):62-71.

Srimal RC, Dhawan BN. Pharmacology of diferuloyl methane (curcumin), a nonsteroidal anti-inflammatory agent. J Pharm Pharmacol. 1973; 25:447–52.

Srinivasan M. Effect of curcumin on blood sugar as seen in a diabetic subject. Indian J Med Sci. 1972; 26:269-70.

Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res. 2001; 2(2):66-70.

Suchanda S. Calculation of VLDL-Cholesterol from Triglycerides and Total Cholesterol Levels. Biomedicine. 2008; 28:219-221.

Sudheer AR, Muthukumaran S, Kalpana C, Srinivasan M, Menon VP. Protective effect of ferulic acid on nicotine-induced DNA damage and cellular

changes in cultured rat peripheral blood lymphocytes: a comparison with N-acetylcysteine. Toxicol In Vitro. 2007; 21(4):576-85.

Sun A, Shoji M, Lu YJ, Liotta DC, Snyder JP. Synthesis of EF24-tripeptide chloromethyl ketone: a novel curcumin-related anticancer drug delivery system. J Med Chem. 2006; 49(11):3153-8.

Suter MA, Aagaard KM. The Impact of Tobacco Chemicals and Nicotine on Placental Development. Prenat Diagn. 2020; 40(9):1193-1200.

Syed M, Shangloo P. Effect of nicotine smoke on liver and kidneys of adult male albino rats: an experimental study. Int J Med Biomed Studies. 2020; 4(9).

Szkup M, Jurczak A, Karakiewicz B, Kotwas A, Kopeć J, Grochans E. Influence of cigarette smoking on hormone and lipid metabolism in women in late reproductive stage. Clin Interv Aging. 2018; 13:109-115.

Tabanelli R, Brogi S, Calderone V. Improving Curcumin Bioavailability: Current Strategies and Future Perspectives. Pharmaceutics. 2021; 13(10):1715.

Tan X, Vrana K, Ding ZM. Cotinine: Pharmacologically Active Metabolite of Nicotine and Neural Mechanisms for Its Actions. Front Behav Neurosci. 2021; 15:758252.

Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014; 6(10):a016295.

Tanner JA, Novalen M, Jatlow P, Huestis MA, Murphy SE, Kaprio J, Kankaanpää A, Galanti L, Stefan C, George TP, Benowitz NL, Lerman C, Tyndale RF. Nicotine Metabolite Ratio (3-Hydroxycotinine/Cotinine) in Plasma and Urine by Different Analytical Methods and Laboratories: Implications for Clinical Implementation. Cancer Epidemiol Biomarkers Prev. 2015; 24(8):1239-46.

Tao X, Li H, XING Y, LIU F, HU Y, Tao H, Mu M; Pang G, Zhang R. Nicotine Protects Dendritic Cells from Apoptosis and Support DCs-dependent CD4+ T-cell Priming in vitro. Indian J Pharm Sci. 2019; 81(6): 1000-1010.

Tau GZ, Cowan SN, Weisburg J, Braunstein NS, Rothman PB. Regulation of IFN-γ signalling is essential for the cytotoxic activity of CD8+ T cells. J Immunol. 2001; 167(10):5574-82.

The WHO, 2020.The WoArld Health Organaisations Reports on Tobacco. Available from Https://Www.Who.Int/News-Room/Fact-Sheets/Detail/Tobacco(2)

Thirumalai T, Therasa S, Viviyan Elumalai EK, David E. J Pharm Sci Res. 2010; 2(9):579-582.

Thomas C, Lumb A. Physiology of haemoglobin, Continuing Education in Anaesthesia and Critical Care Pain. 2012; 12(5):251-256.

Tian T, Wang M, Ma D. TNF-α, a good or bad factor in hematological diseases? Stem Cell Investig. 2014; 1:12.

Tomàs J, Garcia N, Lanuza MA, Santafé MM, Tomàs M, Nadal L, Hurtado E, Simó A, Cilleros V. Presynaptic Membrane Receptors Modulate ACh Release, Axonal Competition and Synapse Elimination during Neuromuscular Junction Development. Front Mol Neurosci. 2017; 16(10):132.

Tsochatzis E, Papatheodoridis GV, Manolakopoulos S, Tiniakos DG, Manesis EK, Archimandritis AJ. Smoking is associated with steatosis and severe fibrosis in chronic hepatitis C but not B. Scand J Gastroenterol. 2009; 44(6):752-9.

Tsujimoto Y. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? Genes Cells. 1998; 3(11):697-707.

Ubeyitogullari A, Ciftci ON. A novel and green nanoparticle formation approach to forming low-crystallinity curcumin nanoparticles to improve curcumin's bioaccessibility. Sci Rep. 2019; 9(1):19112.

Ugur S, Ulu R, Dogukan A, Gurel A, Yigit IP, Gozel N, Aygen B, Ilhan N. The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. Ren Fail. 2015; 37(2):332-6.

Uhl M, Helma C, Knasmuller S. Evaluation of the Single Cell Gel Electrophoresis Assay with Human Hepatoma (Hep G2) Cells. Mutat Res. 2000; 468(2):213-225.

Ung TT, Nguyen TT, Lian S, Li S, Xia Y, Kim NH, Jung YD. Nicotine stimulates IL-6 expression by activating the AP-1 and STAT-3 pathways in human endothelial EA.hy926 cells. J Cell Biochem. 2019; 120(4):5531-5541.

Usunobun U, Adegbeg J, Ademuyiwa O, Okugbo TF, Evuen U, Osibemhe M, Okolie NP. Nnitrosodimethylamine (NDMA), Liver Function Enzymes, Renal Function Parameters and Oxidative Stress Parameters: A Review. British J Pharm Toxicol. 2012; 3:165-176.

Valizadeh H, Abdolmohammadi-Vahid S, Danshina S, Ziya Gencer M, Ammari A, Sadeghi A, Roshangar L, Aslani S, Esmaeilzadeh A, Ghaebi M, Valizadeh S, Ahmadi M. Nano-curcumin therapy, a promising method in modulating inflammatory cytokines in COVID-19 patients. Int Immunopharmacol. 2020; 89(Pt B):107088.

Verma RK, Kumari P, Maurya RK, Kumar V, Verma RB, Singh RK. Medicinal properties of turmeric (Curcuma longa L.): A review. Int J Chem Stud. 2018; 6:1354-1357.

Vu T, Jin L, Datta PK. Effect of Cigarette Smoking on Epithelial to Mesenchymal Transition (EMT) in Lung Cancer. J Clin Med. 2016; 5(4):44.

Wadgave U, Nagesh L. Nicotine Replacement Therapy: An Overview. Int J Health Sci (Qassim). 2016; 10(3):425-35.

Walker HK, Hall WD, Hurst JW. BUN and Creatinine In: Clinical Methods: The History, Physical, and Laboratory Examinations. 1990; 3rd Ed.

Wang C, Wang S, Chang T, Yao W, Chou P. Smoking and Alanine Aminotransferase Levels in Hepatitis C Virus Infection:Implications for Prevention of Hepatitis C Virus Progression. Arch Intern Med. 2002; 162(7):811-815.

Wang H, Chen H, Fu Y, Liu M, Zhang J, Han S, Tian Y, Hou H, Hu Q. Effects of Smoking on Inflammatory-Related Cytokine Levels in Human Serum. Molecules. 2022; 27(12):3715.

Wang XS, Williams LA, Krishnan S, Liao Z, Liu P, Mao L, Shi Q, Mobley GM, Woodruff JF, Cleeland CS. Serum sTNF-R1, IL-6, and the development of fatigue in patients with gastrointestinal cancer undergoing chemoradiation therapy. Brain Behav Immun. 2012; 26(5):699-705.

Wannamethee SG, Shaper AG. Cigarette smoking and serum liver enzymes: the role of alcohol and inflammation. Ann Clin Biochem. 2010; 47(4):321-6.

Warnakulasuriya S, Straif K. Carcinogenicity of smokeless tobacco: Evidence from studies in humans & experimental animals. Indian J Med Res. 2018; 148(6):681-686.

Warner KE. How to think–not feel–about tobacco harm reduction. Nicotine Tob. Res. 2019; 21:1299-1309.

Weinberger AH, Gbedemah M, Wall MM, Hasin DS, Zvolensky MJ, Goodwin RD. Cigarette use is increasing among people with illicit substance use disorders in the United States, 2002-14: emerging disparities in vulnerable populations. Addiction. 2018; 113(4):719-728.

Weizenecker R, Deal WB. Tobacco Coppers Sickness. J Florida Med Assoc. 1970; 57(12):13-14.

Werner M, Gabrielson DG, Eastman G. Ultra-Micro Determination of Serum Triglyceride by Bioluminescent Assay. Clin Chem. 1981; 27(2):268-271.

Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol Cancer. 2011; 10:12.

Wills L, Kenny PJ. Addiction-related neuroadaptations following chronic nicotine exposure. J Neurochem. 2021; 157(5):1652-1673.

Wittenberg RE, Wolfman SL, De Biasi M, Dani JA. Nicotinic Acetylcholine Receptors and Nicotine Addiction: A Brief Introduction. Neuropharmacol. 2020; 177:108256.

Wooltorton JR, Pidoplichko V I, Broide RS, Dani JA. Differential Desensitization and Distribution of Nicotinic Acetylcholine Receptor Subtypes in Midbrain Dopamine Areas J Neurosci. 2003; 23:3176-3185.

World Health Organisation 2011. WHO report on the global tobacco epidemic Avenue Appia, CH-1211 Geneva 27, Switzerland. www. Who.int/tobacco.

Wu S, Zhou Y, Liu S, Zhang H, Luo H, Zuo X, Li T. Regulatory effect of nicotine on the differentiation of Th1, Th2 and Th17 lymphocyte subsets in patients with rheumatoid arthritis. Eur J Pharmacol. 2018; 831:38-45.

Yacoub R, Habib H, Lahdo A, Al Ali R, Varjabedian L, Atalla G, Kassis Akl N, Aldakheel S, Alahdab S, Albitar S. Association between smoking and chronic kidney disease: a case control study. BMC Public Health. 2010; 10:731.

Yadav A, Flora S, Kushwaha P. Nanocurcumin Prevents Oxidative Stress Induced following Arsenic and Fluoride Co-exposure in Rats. Defence Life Science Journal. 2016; 1(1):69-77.

Yadav A, Lomash V, Samim M, Flora SJ. Curcumin encapsulated in chitosan nanoparticles: a novel strategy for the treatment of arsenic toxicity. Chem Biol Interact. 2012; 199(1):49-61.

Yadav R, Jee B, Awasthi SK. Curcumin Suppresses the Production of Pro-inflammatory Cytokine Interleukin-18 in Lipopolysaccharide Stimulated Murine Macrophage-Like Cells. Indian J Clin Biochem. 2015; 30(1):109-12.

Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. 2009; 10(3):752-62.

Yang S, Jensen MK, Rimm EB, Willett W, Wu T. Erythrocyte superoxide dismutase , glutathione peroxidase and catalase activity and risk of coronary heart disease in generally healthy women. Am J Epidemiol. 2014; 180: 901-908.

Yang YS, Su YF, Yang HW, Lee YH, Chou JI, Ueng KC. Lipid-lowering effects of curcumin in patients with metabolic syndrome: a randomized, double-blind, placebo-controlled trial. Phytother Res. 2014; 28(12):1770-7.

Yavarpour-Bali H, Ghasemi-Kasman M, Pirzadeh M. Curcumin-loaded nanoparticles: a novel therapeutic strategy in treatment of central nervous system disorders. Int J Nanomedicine. 2019; 14:4449-4460.

Yen F, Wu F, Tzeng C, Lin L, Ling C. Curcumin nanoparticles improves the physicochemical properties of Curcumin and effectively enhances its antioxidant and antihepatoma activities. J. Agri. Food Chem. 2010; 58,7376-7382.

Yershova K, Yuan JM, Wang R, Valentin L, Watson C, Gao YT, Hecht SS, Stepanov I. Tobacco-specific N-nitrosamines and polycyclic aromatic hydrocarbons in cigarettes smoked by the participants of the Shanghai Cohort Study. Int J Cancer. 2016; 139(6):1261-9.

Zaidi MR, Merlino G. The two faces of interferon-g in cancer. Clin Cancer Res. 2011;17(19):1-7.

Zeng H, Kong X, Zhang H, Chen Y, Cai S, Luo H, Chen P. Inhibiting DNA methylation alleviates cigarette smoke extract-induced dysregulation of Bcl-2 and endothelial apoptosis. Tob Induc Dis. 2020;18:51.

Zeng H, Shi Z, Kong X, Chen Y, Zhang H, Peng H, Luo H, Chen P. Involvement of B-cell CLL/lymphoma 2 promoter methylation in cigarette smoke extract-induced emphysema. Exp Biol Med (Maywood). 2016; 241(8):808-16.

Zeng Q, Shen LJ, Li S, Chen L, Guo X, Qian CN, Wu PH. The effects of hemoglobin levels and their interactions with cigarette smoking on survival in nasopharyngeal carcinoma patients. Cancer Med. 2016; 5(5):816-26.

Zhang W, Lin H, Zou M, Yuan Q, Huang Z, Pan X, Zhang W. Nicotine in Inflammatory Diseases: Anti-Inflammatory and Pro-Inflammatory Effects. Front Immunol. 2022; 13:826889.

Zhao L, Gu Q, Xiang L, Dong X, Li H, Ni J, Wan L, Cai G, Chen G. Curcumin inhibits apoptosis by modulating Bax/Bcl-2 expression and alleviates oxidative stress in testes of streptozotocin-induced diabetic rats. Ther Clin Risk Manag. 2017; 13:1099-1105.

Zhao Y, Ma X, Wang J, He X, Hu Y, Zhang P, Wang R, Li R, Gong M, Luo S, Xiao X. Curcumin protects against CCl4-induced liver fibrosis in rats by inhibiting HIF-1α through an ERK-dependent pathway. Molecules. 2014; 19(11):18767-80.

Zheng B, McClements DJ. Formulation of More Efficacious Curcumin Delivery Systems Using Colloid Science: Enhanced Solubility, Stability, and Bioavailability. Molecules. 2020; 25(12):2791.

Zheng CM, Lee YH, Chiu IJ, Chiu YJ, Sung LC, Hsu YH, Chiu HW. Nicotine Causes Nephrotoxicity through the Induction of NLRP6 Inflammasome and Alpha7 Nicotinic Acetylcholine Receptor. Toxics. 2020; 8(4):92.

Zhou H, Beevers CS, Huang S. The targets of curcumin. Curr Drug Targets. 2011; 12(3):332-47.

Zhu J. T helper 2 (Th2) cell differentiation, type 2 innate lymphoid cell (ILC2) development and regulation of interleukin-4 (IL-4) and IL-13 production. Cytokine. 2015; 75:14-24.

Zhu Y, Song D, Song Y, Wang X. Interferon gamma induces inflammatory responses through the interaction of CEACAM1 and PI3K in airway epithelial cells. J Transl Med. 2019; 17(1):147.

Zingg JM, Hasan ST, Meydani M. Molecular mechanisms of hypolipidemic effects of curcumin. Biofactors. 2013; 39(1):101-21.

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RESEARCH ARTICLE

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Potential amelioration of nicotine-induced toxicity by nanocurcumin

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Abstract

Curcumin, a nontoxic bioactive agent of turmeric significantly reduces nicotine-induced toxicity both at cellular and genetic levels. The clinical implication of native curcumin is hindered in the target cells due to its low aqueous solubility, poor bioavailability and poor pharmacokinetics. The problem was tried to overcome by preparing nanocurcumin with a view to improve its aqueous solubility and better therapeutic efficacy against nicotine-induced toxicity. The prepared nanocurcumin was characterized by Ultraviolet-visible spectroscopy; Field emission scanning electron microscopy (FE-SEM); X-ray diffraction (XRD); and Fourier transform infrared spectroscopy (FTIR). Female albino rats of Wistar strain were daily exposed to effective dose of nicotine (2.5 mg/kg, injected subcutaneously) and supplemented with effective dose of curcumin (80 mg/kg body weight orally) or nanocurcumin (4 mg/kg body weight orally) for 21 days. The preventive efficacies of curcumin and nanocurcumin were evaluated against the changes in liver function enzymes, kidney function parameters, lipid profiles, lipid-peroxidation, anti-oxidant status, and tissues damages etc. Results revealed that nanocurcumin more effectively ameliorated the nicotine-induced toxicities at much lower concentration due to its higher aqueous solubility and more bioavailability. The nanocurcumin can be used as a potential therapeutic agent for better efficacy against nicotine-induced toxicities than native curcumin.

KEYWORDS
curcumin, nanoparticles, nicotine, toxicity

1 | INTRODUCTION

Smoking has enormous negative health consequences worldwide, and the use of tobacco is still rising globally (Macky & Eriksen, 2002). Tobacco addiction is mainly due to its major alkaloid, i.e., nicotine, which has been associated with many disorders. An average tobacco rod contains 10–14 mg of nicotine (Kozolowski et al., 1998), and on average about 1 to 1.5 mg of nicotine is absorbed systemically during smoking a cigarette (Benowitz & Jacob, 1984). Digard, Proctor, Kulasekaran, Malmqvist, and Richter (2013) have presented some good pharmacokinetic data for nicotine absorption. They have generated new information on comparative nicotine absorption from a cigarette, loose snus, and pouched snus typical of products sold in Scandinavia and observed that absorption kinetics of nicotine are dependent on quantity of tobacco by weight and total nicotine content rather than product form. Nicotine induces oxidative stress both in vivo and in vitro that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma, and tissues (Maiti, Chattopadhyay, Verma, & Chattopadhyay, 2015). Oxidative stress generates free radicals that attack on membrane lipids resulting in the formation of malondialdehyde (MDA), which causes increased peroxidative tissue damage (Chattopadhyay & Chattopadhyay, 2008). Animal studies have shown significantly higher

levels of MDA, conjugated dienes, hydro peroxides, and free fatty acids in the serum of rats induced by cigarette smoke (Chattopadhyay & Chattopadhyay, 2008; Sinha, Maiti, Chattopadhyay, & Chattopadhyay, 2012). Previous studies have demonstrated that acute exposure to tobacco smoke induces marked changes in the immune system of the host both at molecular and cellular levels (Flouris & Oikonomou, 2010; Flouris et al., 2013; Maiti et al., 2015). It also aggravates Th1/Th2 cytokine imbalance, alters transcription factor, lipid peroxidation in the liver and other tissues and affects on the activities of antioxidant enzymes in rats (Maiti et al., 2015; Sinha et al., 2012).

Banerjee, Chattopadhyay, Chhabra, and Chattopadhyay (2012) have shown that acute nicotine exposure aggravates DNA damage in the hepatic cells, promotes hepatic cell death through upregulating pro-apoptotic proteins and signaling molecules in the protein malnourished individuals. Conversely, long-term smoking is associated with increased risk for many noncommunicable diseases (Ambrose & Barua, 2004; Domagala-Kulawik, 2008) where smokers have a dose dependent heightened basal inflammatory profile (Fröhlich et al., 2003; Tracy et al., 1997). However, it is unknown if such consequences exist among the smokers who present with a relatively shorter smoking history.

Curcumin, a dietary spice present in turmeric (*Curcuma longa*) has been known since ancient times to posses therapeutic properties like antihepatoxic, antioxidant, anti-inflammatory, antidiabetic, antitumour cancer, hepato-protective, and anti-HIV activities (Bandyopadhyaya, Sinha, Chattopadhyay, & Chakraborty, 2008; Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995). Curcumin significantly ameliorates the toxicological effects and regulates the imbalance between cell survival and cell death caused by nicotine (Banerjee et al., 2012). It appears as a very active agent and a potential blocker against the nicotine-induced stress (Chattopadhyay, Tamang, & Kundu, 2015; Maiti et al., 2015) and inhibits the lipid peroxidation, lowers the biochemical marker enzymes and enhances the antioxidant status (Bandyopadhyaya et al., 2008; Kalpana & Menon, 2004). The protective and antioxidant effects of curcumin are superior to those of vitamins E and A (Ao, Chu, & Wang, 2014). Curcumin encapsulation formulations have been made in the recent past but their effectiveness has been limited due to their larger particle sizes. The size, shape, and surface properties play an important role in determining the cellular uptake of nanoparticles in mammalian cells (Chen, Hung, Liau, & Huang, 2009; Chithrani, Ghazani, & Chan, 2006; Nan, Bai, Son, Lee, & Ghandehari, 2008).

Due to its fast metabolic turnover in the liver and intestinal wall, the concentration of curcumin is found to be very low in human blood resulting limited distribution in the tissues following oral dosing (Carroll et al., 2011; Ringman et al., 2012; Vareed et al., 2008). Maximum plasma curcumin concentrations in humans, even upon intake of doses as high as 10 or 12 g curcumin, remain in the low nanomolar range (<160 nmol/L) (Vareed et al., 2008). Despite its promising pharmacological activities, its low bioavailability still remains a major hurdle as usage of curcumin. Over a period of time, a lot of efforts have been tried to overcome the limitation of bioavailability of curcumin such as the inhibition of curcumin metabolism with adjuvant and novel solid and liquid oral delivery systems (Cuomo et al., 2011; Kanai et al., 2012; Sasaki et al., 2011; Schiborr et al., 2014). This study was designed for preparation and characterization of uniform nanocurcumin to observe its protective role against nicotine-induced toxicity of female populations particularly who are habituated with smoking and used various tobacco products.

2 | MATERIALS AND METHODS

Nicotine hydrogen tartrate and curcumin were purchased from Sigma Chemicals Company, St. Louis. All other analytical grade chemicals were supplied by Spectrochem Pvt. Ltd. India and Merck India.

2.1 | Preparation of nanocurcumin

Nanocurcumin was prepared by slightly modifying the method of Basniwal, Buttar, Jain, and Jain (2011). For synthesis of nanocurcumin, a solution was prepared by dissolving 0.018 M curcumin in 30 mL dichloromethane (DCM). Thereafter, it was mixed drop wise (flow rate 0.2 mL/min) in warm (50°C) Milli-Q ultrapure water for 10 min under ultrasonic-pulse sonication conditions with constant stirring at 600 rpm [Hielscher Ultrasonic Processor- UP100H, Germany]. The colored solution containing nanocurcumin was separated by centrifugation. It was autoclaved and then freeze-dried at −80°C. After that the sample was lyophilized (EYELA-FDU- 2000, Japan) to obtain a dry orange powder of nanocurcumin.

2.2 | Characterization of nanocurcumin

The plasmon resonance (SPR) band pattern of the newly synthesized nanocurcumin was optically analyzed by UV-Vis spectrophotometer (UV-3101PC, Shimadzu, Japan). Morphological investigation was done by Field Emission Scanning Electron Microscopy (FE-SEM, FEI INSPECT F50, The Netherlands). The as prepared nanocurcumin was characterized by Fourier transform infrared spectroscopy (FTIR) (Perkin- Elmer FTIR-1600, USA) by mixing dried powder of nanoparticles with KBr. Spectra were taken of 4 cm^{-1}. The data of FTIR revealed the information about the functional groups which were present in the nanocurcumin.

The crystalline structure or phase of nanocurcumin powder was determined by X-ray diffractometer (Bruker AXS, Inc., Model D8, WI). The experiment was performed in symmetrical reflection mode with a Cobalt (Co) line as the source of radiation. The crystalline state of the samples was estimated by a Standard runs using 40 kV voltage, 40 mA current with a scanning rate of 0.02° min^{-1} over a range of 2Θ (5 to 40°).

2.3 | Animals and treatments

A total of 30 female albino rats of Wistar strain (*Rattus norvegicus*), 60–75 days old, weighing 140–150 g were procured from the Animal housing facility and maintained according to the guidelines of the Institutional Animal Ethics Committee of the Jadavpur University, Kolkata, India (Constituted as per the "Gazette of India" notification part II sec.3 (ii) 17 of the Ministry of Environment and forestry, Government of India, dated 8th September 1998 for the "prevention to cruelty to

animal 1968"). The animals were acclimatized under standard condition of temperature and humidity with 12 hr light and dark cycles. One week before the commencement of treatments, animals were fed the standard pellet diet (Hindustan Liver Ltd., India). The animals were divided into 5 groups, each containing 6 animals as follows-

2.3.1 | Control group

These rats were fed with normal protein diet containing 18% casein, 70% carbohydrate, 7% fat, 4% salt mixture, and 1% vitamin mixture (Hawk, Oser, & Summerson, 1954).

2.3.2 | Nicotine group

These rats were fed with normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) for 21 days.

2.3.3 | Nicotine and curcumin group

These rats were fed with normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of curcumin (80 mg/kg body weight) orally for 21 days.

2.3.4 | Nicotine and nanocurcumin group

These rats were fed with normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of nanocurcumin (4 mg mg/kg body weight) orally for 21 days.

2.3.5 | Nanocurcumin group

These rats were fed with normal protein diet and supplemented with effective dose of nonocurcumin (4 mg/kg body weight) orally for 21 days.

Nicotine hydrogen tartrate salt was dissolved in normal saline water (2.5 g/mL) and required volume of drug solution was taken in an eppendorf tube to which distilled water was added to make the final volume of 0.2 mL and injected subcutaneously to the animals. The doses of nicotine were selected on the basis of our previous studies (Mandal, Dasgupta, & Chattopadhyay, 2004). The dose of curcumin was selected from the study of Kalpana and Menon (2004). The effective dose of nanocurcumin was determined prior to this study by supplementing the animals with different concentrations of nanocurcumin (2 – 8 mg/kg body weight) and monitoring the different biochemical parameters. Both curcumin and nanocurcumin were administered orally 1 hr after the administration of nicotine. The animal under control groups received subcutaneous injection of 0.2 mL physiological saline only. After 21 days of treatment, animals were kept under fasting condition for 12 hr and sacrificed on the next day after mild anesthesia. Blood samples were collected from the heart immediately after sacrifice and stored in both with or without anticoagulant (heparin) containing containers. Serum and plasma were separated by centrifugation and stored at −20°C for further analysis. Liver and kidney were dissected out and stored in vacuum desiccators at −20°C to prevent auto-oxidation and future studies.

2.4 | Biochemical assays

Acid phosphatase activity (ACP) in the serum was assayed according to the method illustrated by Bergmeyer and Bernt (1963) by using para nitrophenyl-phosphate as the substrate. The alkaline phosphatase (ALP), alanine-transaminase (ALT), and aspartase-transaminase (AST) activities in the serum were measured by using the standard kit supplied by ARKRAY Healthcare Pvt. Ltd., Surat, India. The lipid components such as TC (Allain, Poon, Chan, Richmond, & Fu, 1974), HDL-C (Warnick, Nguyen, & Albers, 1985), and triglyceride (Werner, Gabrielson, & Eastman, 1981) in the serum were estimated by using standard kits supplied by Ranbaxy Diagnostic Ltd., Mumbai, India. VLDL-C and LDL-C were calculated from the values of triglyceride, TC and HDL-C by using the Friedwald and Fredicksons formula (Friedewald, Levy, & Fredrickson, 1972). Plasma and liver lipid peroxidations were measured by the assay of thiobarbituric acid reactive substances (TBARS) according to the standard method (Chatterjee & Agarwal, 1988). The amount of MDA both in the plasma and the liver tissues was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Superoxide dismutase (SOD) and catalase (CAT) activities in the liver were determined from rats of all the groups (Beauchamp & Fridovich, 1971; Cohen, Dembiec, & Marcus, 1970). The glutathione-reductase (GSH) and glutathione-peroxidase enzymes activities of the liver tissue were determined by the methods described by Griffith (1980) and Paglia and Valentine (1967), respectively. Protein concentration of the tissue was determined by using the method of Lowry, Rosebrough, Farr, and Randall (1951). The entire biochemical assays were repeated at least two times to get a statistical significance.

2.5 | Histological examination

Tissues from the liver and kidney were cleaned properly and fixed by using the Bouin's fluid. After fixation, the tissues were washed several times by different graded alcohol (50, 70, 80, and 95%) to remove the excess fluid and then embedded in the paraffin. The embedded tissues were sliced by using the rotary microtome. The paraffin sections were washed in xylol before staining with eosin and hematoxylin stains.

2.6 | Statistical analysis

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments.

3 | RESULTS

The morphological characterization of nanocurcumin was observed by FESEM where the average particle size was seen as 65 ± 5 nm (Figure 1a). The characteristic absorbance peak of the prepared nanocurcumin

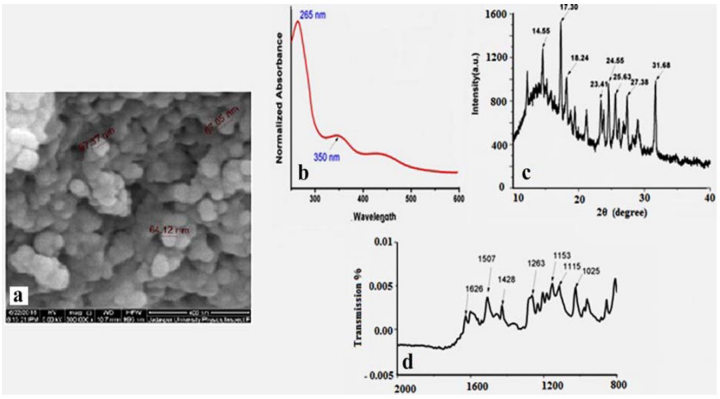

FIGURE 1 Characterization of Cur-NPs. (a) SEM picture of as prepared Cur-NPs. (b) Absorption spectrum of Cur-NPs. (c) XRD spectrum of Cur-NPs. (d) FTIR spectrum of Cur-NPs [Color figure can be viewed at wileyonlinelibrary.com]

was observed at 350 nm by UV-visible spectrophotometer (Figure 1b). The characteristic peaks in the FTIR spectrum further confirmed the formation of nanocurcumin as seen in Figure 1c. X-ray diffraction (XRD) analyzes showed that the as prepared nanocurcumin was crystalline nature (Figure 1d).

The data from Table 1 indicated that exposure to nicotine produced significant changes in the serum ACP, ALP, ALT, and AST levels. The activities of the all four hepatic enzymes of rats were increased due to nicotine treatment. The effective dose of nanocurcumin was determined as 4 mg/kg body weight (Supporting Information Tables). Both curcumin and nanocurcumin reduced the activities of those enzymes. The ameliorative effect of nanocurcumin was more prominent than normal curcumin as seen from the Table 1. It was also observed that there were significant increase in urea and creatinine levels in the nicotine exposed animals compared to the control group animals (Table 2). The administration of curcumin and nanocurcumin showed beneficial effects by decreasing the urea and creatinine levels of serum. Nicotine induced significant changes in the cholesterol, triglyceride, LDL, and VLDL concentrations and decreased HDL concentration in the serum of the nicotine treated animals (Table 3). However, administration of curcumin and nanocurcumin showed potential ameliorative effects by decreasing the concentrations of the cholesterol, triglyceride, LDL and VLDL levels in the serum of the rats. The results of lipid peroxidation both in the plasma and liver tissue of rats are shown in Table 4. The degenerative effects of antioxidant enzymes caused by nicotine and ameliorative effects caused by curcumin and nanocurcumin are shown in Table 5.

The histological study of the liver tissue of control group animals is shown in Figure 2a. The distorted cell arrangement and the central vein (marked by arrow head) of the nicotine exposed liver are shown in Figure 2b. Curcumin and nanocurcumin supplemented rat liver photographs are presented in Figure 2c,d, respectively. The histological studies of kidney tissues of the control, nicotine treated, curcumin and nanocurcumin supplemented groups are presented in Figure 3a–d, respectively.

4 | DISCUSSION

Several studies in animal models demonstrated the efficacy of curcumin against nicotine-induced toxicity. The clinical efficacy of curcumin in human subjects is limited due to its undesirable pharmacokinetic properties although; some clinical trials have unequivocally demonstrated the safety and efficacy of turmeric in human subjects (Gupta et al., 2013). Scientists are trying to improve the pharmacokinetic properties of curcumin for translating its wide range of activities into clinical benefits, e.g., formulation and applications of nanocurcumin (Danafar,

TABLE 1 Effect of nanocurcumin on hepatic enzymes

Enzymes	Control	Nicotine	Groups Nicotine + Curcumin	Nicotine + Nanocurcumin	Nanocurcumin
ACP (m mol/h/100 mL)	1.43 ± 0.10	1.95 ± 0.15 (36.4↑)	1.56 ± 0.17** (9.1↑)	1.47 ± 0.12**# (2.8↑)	1.30 ± 0.11* (9.1↓)
ALP (m mol/h/100 mL)	4.87 ± 0.60	9.64 ± 1.82 (97.9↑)	7.40 ± 0.98* (51.9↑)	5.55 ± 0.73**## (14.0↑)	4.30 ± 0.13* (11.7↓)
AST (IU/L)	14.88 ± 2.10	25.13 ± 5.05 (68.9↑)	20.95 ± 2.49* (40.8↑)	16.97± 2.50**## (14.0↑)	10.35 ± 0.90** (30.4↓)
ALT (IU/L)	34.58 ± 7.24	48.89 ± 7.79 (41.4↑)	42.29 ± 6.94* (22.3↑)	37.10 ± 2.47**# (7.3↑)	36.40 ± 4.84* (5.3↑)

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

TABLE 2 Effect of nanocurcumin on renal function parameters

Parameter	Control	Nicotine	Groups Nicotine + Curcumin	Nicotine + Nanocurcumin	Nanocurcumin
Urea (mg/100 mL)	37.16 ± 2.63	52.36 ± 3.60 (40.9↑)	47.00 ± 2.78* (26.5↑)	35.00± 2.30**## (5.8↓)	32.80 ± 1.19** (11.7↓)
Creatinine (mg/100 mL)	1.19 ± 0.11	1.68 ± 0.14 (41.2↑)	1.56 ± 0.50* (31.1↑)	1.36 ± 0.13**## (14.3↑)	0.70 ± 0.15** (41.2↓)

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

2017; Danafar, Sharafi, Askarlou, & Manjili, 2017; Kheiri Manjili et al., 2017; Nosrati, Sefidi, Sharafi, Danafar, & Manjili, 2018). Nanoparticles are a novel drug delivery mode on account of its high bioavailability in aqueous media, controlled drug release property, and higher physical stability and drug loading (Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009; Yadav, Lomash, Samim, & Flora, 2012). Here, we report for the first time the effectiveness of orally administered nanocurcumin against the nicotine-induced toxicity in rats.

Nanocurcumin is soluble in water (Basniwal, Buttar, Jain, & Jain, 2011), while curcumin powder is insoluble in water (Bhawana et al., 2011). Solubilized nanocurcumin forms a dense reddish color solution which reveals the confirmation of nanocurcumin synthesis. The characteristic peak of nanocurcumin (at 350 nm) as seen in the UV-visible spectrum corroborates with the result of Alam, Panda, and Chauhan (2012) and Ghosh et al. (2011) and similarly confirms the synthesis of nanocurcumin. In FTIR analysis, the characteristic peaks appeared correspond to different functional groups (Figure 1c). Among these, the absorption peak at 1,626 cm^{-1} can be assigned for C = C stretching, peaks at 1,507 cm^{-1} and 1,263 cm^{-1} correspond to C = O and at 1,428 cm^{-1} corresponds to C = H. The absorption peaks at 1,153 cm^{-1} and 1,115 cm^{-1} are due to C – H stretching. The absorption peak at 1,025 cm^{-1} may be due to C – N stretching. These finding are in accordance with the results obtained by many researchers earlier (Sav, Khetrapal, & Amin, 2012; Yadav, Suresh, Devi, & Yadav, 2009; Yen, Wu, Tzeng, Lin, & Lin, 2010). The XRD pattern confirms the crystalline structure of nanocurcumin which corresponds with the results obtained by Sav et al. (2012) and Yen et al. (2010). The particle size of the prepared nanocurcumin was in the range of 65 ± 5 nm as analyzed by FE-SEM.

Analysis on human autopsy samples from smokers shows that the highest affinity for nicotine is in the liver, kidney, spleen, and lung (Benowitz, 2009). The activities of ACP and ALP are to free attached phosphate groups from the other molecules during digestion. Nicotine increases the concentrations of ACP and ALP in the serum due to the increased oxidative stress. It indicates the tissue injury which is directly involved in the generation of reactive oxygen species (ROS) as explained by Sreekala and Indira (2009). In curcumin treated group, both ACP and ALP levels are decreased due to improved antioxidant status within the tissues. Supplementation of nanocurcumin almost normalizes the ACP and ALP activities in the serum indicating the more prominent effect due its better bioavailability (Table 1).

Increased activities of the AST and ALT enzymes by nicotine administration indicate liver tissue damage that is occurred due to loss of functional integrity of the cells membranes (Balakrishnan & Menon, 2007). When the liver tissue is injured or damaged, the liver cells spill those enzymes in the blood, raising the enzyme levels in the blood, and signaling the liver disease. Curcumin decreases the levels of the liver enzymes by preserving the structural integrity of the cell membrane (Al-Jassabi & Azirun, 2010; Salahshoor, Mohamadian, Kakabaraei, Roshankhah, & Jalili, 2016). Nanocurcumin shows its better efficacy to restore the functions of those two enzymes in the nicotine stressed condition (Table 1).

Urea and creatinine are being the two important indicators for kidney disorder. The data presented in the Table 2 shows that the mean difference of urea and creatinine are significantly elevated ($p < .001$) in the serum of nicotine exposed group as compared to the control group. Earlier studies reported that a progressive kidney failure can be associated with a gradual decrease of renal and nonrenal elimination of

TABLE 3 Effect of nanocurcumin on lipid profile

Parameter	Control	Nicotine	Groups Nicotine + Curcumin	Nicotine + Nanocurcumin	Nanocurcumin
Triglyceride (mg/dL)	118.6 ± 10.2	172.8 ± 9.4 (45.8↑)	150.9 ± 8.6* (27.3↑)	135.0 ± 5.9**## (13.9↑)	99.4 ± 4.8** (16.2↓)
Cholesterol (mg/dL)	104.7 ± 10.7	139.1 ± 11.6 (32.9↑)	104.4 ± 7.1** (0.3↓)	102.9 ± 2.5**# (1.7↓)	103.4 ± 9.1** (1.2↓)
HDL (mg/dL)	40.1 ± 1.0	32.7 ± 1.5 (18.4↓)	37.18 ± 1.6* (7.3↓)	38.5 ± 1.6*# (4.0 ↓)	47.6 ± 3.6** (18.7↑)
VLDL (mg/dL)	24.0 ± 1.3	34.8 ± 1.4 (45.5↑)	30.7 ± 1.8* (28.3↑)	27.6 ± 1.8**# (15.3↑)	19.9 ± 1.2** (17.0↓)
LDL (mg/dL)	41.3 ± 2.5	72.4 ± 4.8 (75.4↑)	57.9 ± 3.2* (40.2↓)	47.8 ± 2.5**## (15.7↓)	36.0 ± 5.8** (12.8↓)

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

TABLE 4 Effect of nanocurcumin on lipid peroxidation

MDA level	Control	Nicotine	Groups Nicotine + Curcumin	Nicotine + Nanocurcumin	Nanocurcumin
Plasma (nmol/mL)	5.64 ± 0.61	8.01 ± 1.31 (42.0↑)	6.90 ± 0.79* (22.3↑)	6.51 ± 0.52*# (15.4↑)	5.06 ± 0.50** (10.3↓)
Liver (nmol/mg protein)	14.72 ± 0.36	24.90 ± 2.17 (69.2↑)	20.36 ± 2.96* (38.3↑)	16.83 ± 2.21**## (14.3↑)	14.26 ± 0.60** (3.12↓)

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

nicotine, and this increases the rate of nephrotoxicity (Addo et al., 2008). Also, the effects of heavy metals in tobacco like Cadmium (Cd), Mercury (Hg), and Lead (Pb), might be the another possible factors for tobacco-induced renal damage (Usunobun et al., 2012). Supplementation of nanocurcumin may be a better therapeutic agent ($p < .001$) compared to curcumin ($p < .01$) to protect the kidney as indicated in our results (Table 2).

Cholesterol is an extremely important biological molecule that has an important role to maintain the integrity of membrane structure as well as being a precursor that helps to synthesize the steroid hormones and bile acids. Nicotine stimulates catecholamine synthesis which lipolyses adipose tissue resulting an increment in the serum cholesterol level as well as of triglyceride level (Balakrishnan & Menon, 2007). Our findings show significantly ($p < .001$) increased serum cholesterol and triglyceride levels of the nicotine treated rats (Table 3) which are in agreement with the earlier studies (Balakrishnan & Menon, 2007; Chattopadhyay & Chattopadhyay, 2008). In curcumin treated group, the level of cholesterol is decreased probably due to the increase in the CYP7A1 gene expression which is a rate limiting enzyme in the biosynthesis of bile acid from cholesterol as suggested by Kim and Kim (2010). Nanocurcumin shows obviously better hypocholesterolemic effect than curcumin either by increasing total cholesterol absorption as suggested by Hasimun, Sukandar, Adnyana, and Tjahjono (2011) or by degrading and eliminating the total cholesterol (Arafa, 2005). Triglyceride lowering effect by nanocurcumin is probably due to multiple inductions of fatty acid catabolism as reported by Asai and Miyazawa (2001). It is already reported that nicotine increases lipid peroxidation for which Malondialdehyde (MDA) levels are increased in the plasma and liver tissues (Chattopadhyay & Chattopadhyay, 2008; Chattopadhyay, Mondal, Chattopadhyay, & Ghosh, 2010). We have also similarly observed that curcumin down regulates ROS levels in the serum and protects the hepatic cell membranes of nicotine-induced rats from lipid peroxidation (Table 4). However, better amelioration (% change) observed with nanocurcumin than free curcumin that may be related to higher bioavailability and more uptake of nanocurcumin into the cells. Nanocurcumin similarly increases the antioxidant enzymes levels and thereby increases the defense mechanism by scavenging or neutralizing the free radicals generated due to nicotine more fruitfully compared to native curcumin as shown in Table 5.

Histological study of the liver tissue was accomplished by using compound microscope (10×). The regular arrangement of the hepatic cell and normal shape of the central vein (marked by arrow head) of the liver tissue were observed in case of control group animals (Figure 2a). The hepatic cell arrangement was distorted and the central vein and the sinusoids space were dilated by the nicotine exposure under the normal diet condition as seen in Figure 2b. Curcumin supplementation helped the hepatic cell and the central vein to regain their regular arrangement and normal shape (Figure 2c). Nanocurcumin supplementation showed more prominent effects than curcumin supplementation for regaining the regular arrangement and normal shape of the hepatic cell and the central vein (Figure 2d).

Histological studies of kidney tissues were also revealed the normal arrangement of the glomerulus (marked by arrow head), the bowman space and the proximal/distal tubules of kidney tissue of control rats (Figure 3a). Nicotine exposure distorted those arrangements significantly as seen in Figure 3b. Curcumin supplementation recovered the

TABLE 5 Effect of nanocurcumin on antioxidant enzymes

Enzymes	Control	Nicotine	Groups Nicotine + Curcumin	Nicotine + Nanocurcumin	Nanocurcumin
SOD (nmol/O_2 decomposed/ min/100 mg protein)	9.98 ± 0.40	4.29 ± 0.30 (57.0↓)	6.72 ± 0.14* (32.7↓)	7.61 ± 0.59**# (23.7↓)	10.46 ± 1.08** (4.8↑)
CAT (nmol/H_2O_2 decomposed/ min/mg protein)	38.7 ± 7.6	26.2 ± 2.1 (32.3↓)	29.6 ± 1.8* (23.5↓)	31.9 ± 1.3*# (17.60↓)	40.4 ± 1.9** (4.4↑)
GSH (μg/mg protein)	34.6 ± 1.0	20.8 ± 0.6 (39.8↓)	28.9 ± 0.5* (16.5↓)	30.2 ± 0.3**# (12.7↓)	35.1 ± 0.6** (1.4↑)
GPx (nmol/min/mg protein)	147.8 ± 2.7	126.4 ± 2.8 (17.0↓)	133.6 ± 2.3* (9.6↓)	136.0 ± 1.8**# (8.0↓)	149.0 ± 1.2** (1.0↑)

The experimental setup was repeated twice and all data were averaged over $n = 12$ animals, and given mean ± S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

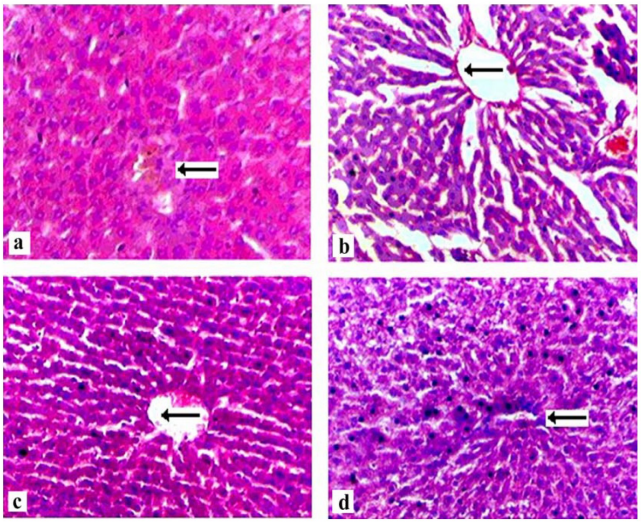

FIGURE 2 Histological sections of rat liver (arrowhead indicates the central vein). (a) Control rat. (b) Nicotine (2.5 mg/kg body weight) treated rat. (c) Nicotine and curcumin (80 mg/kg body weight) treated rat. (d) Nicotine and nanocurcumin (4 mg/kg body weight) treated rat [Color figure can be viewed at wileyonlinelibrary.com]

normal arrangement of the glomerulus, the bowman space and the proximal/distal tubules of kidney tissue to some extent (Figure 3c). Supplementation of nanocurcumin restored the normal arrangement of the glomerulus, the bowman space and the proximal/distal tubules of kidney tissue more effectively (Figure 3d).

One possible problem with nanoparticles is its undesirable toxicity arising from the type of nanomaterial used. Inorganic nanomaterial can as well be toxic to the human body whereas, protein based nanomaterials can be used as potential therapeutic nanocarriers since they naturally occur, and generally exhibit less cytotoxicity (Tabatabaei Rezaei, Nabid, Niknejad, & Entezami, 2012; Zhao et al., 2013). Khosropanah et al. (2016), while studying the antiproliferative effects of curcumin and nanocurcumin in MDA-MB231 breast cancer cell line have shown that although there is a burst release of nanocurcumin that limits for targeted tumor delivery, nanocurcumin still exhibits major advantages over solvent solubilized curcumin. According to them, the nanoformulation does not result in lung accumulation and thus increases overall systemic curcumin exposure. Nanocurcumin also inhibits proliferation of esophageal adenocarcinoma cells and enhances the T cell mediated immune response (Milano et al., 2013). Our study also suggests that

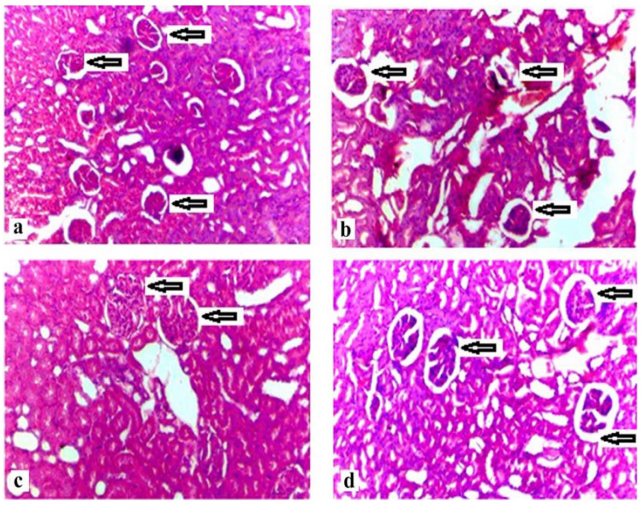

FIGURE 3 Histological sections of rat kidney (arrowhead indicates the glomerulus). (a) Control rat. (b) Nicotine (2.5 mg/kg body weight) treated rat. (c) Nicotine and curcumin (80 mg/kg body weight) treated rat. (d) Nicotine and nanocurcumin (4 mg/kg body weight) treated rat [Color figure can be viewed at wileyonlinelibrary.com]

nanocurcumin more effectively ameliorates nicotine-induced toxicities through normalizing the function of the hepato-functional enzymes, kidney function parameters, lipid profiles, anti-oxidant status, and maintaining the structural integrity of different tissues under nicotine stress condition. It can be inferred that nanocurcumin is a safer, more effective and better therapeutic agent than curcumin against the nicotine-induced complications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest of any kind related to this work.

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REFERENCES

Addo, M. A., Gbadago, J. K., Affum, H. A., Adom, T., Ahmed, K., & Okley, G. M. (2008). Mineral profile of Ghanaian dried tobacco leaves and local snuff: A comparative study. *Journal of Radioanalytical and Nuclear Chemistry*, *277*(3), 517–524.

Alam, S., Panda, J., & Chauhan, V. (2012). Novel dipeptide nanoparticles for effective curcumin delivery. *International Journal of Nanomedicine*, *7*, 4207–4222.

Al-Jassabi, S., & Azirun, M. S. (2010). Phenytoin-induced hepatic 8-hydroxy-deoxyguanosine in DNA of Balb/C mice and its reduction by curcumin. *American-Eurasian Journal of Toxicolocial Sciences*, *2*, 129–133.

Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, *20*(4), 470–475.

Ambrose, J. A., & Barua, R. S. (2004). The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology*, *43*(10), 1731–1737.

Ao, G. Z., Chu, X. J., Ji, Y. Y., & Wang, J. W. (2014). Antioxidant properties and PC12 cell protective effects of a novel curcumin analogue (2E, 6E) - 2, 6-bis (3, 5-dimethoxybenzylidene) cyclohexane (MCH). *International Journal of Molecular Sciences*, *15*(3), 3970–3988.

Arafa, H. M. (2005). Curcumin attenuates diet induced hypercholesterolemia in rats. *Medical Science Monitor*, *11*, 228–234.

Asai, A., & Miyazawa, T. (2001). Dietary curcuminodoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. *The Journal of Nutrition*, *131*(11), 2932–2935.

Balakrishnan, A., & Menon, V. P. (2007). Antioxidant properties of hesperidin in nicotine-induced lung toxicity. *Fundamental & Clinical Pharmacology*, *21*(5), 535–544.

Bandyopadhyaya, G., Sinha, S., Chattopadhyay, B. D., & Chakraborty, A. (2008). Protective role of curcumin against nicotine induced genotoxicity on rat liver under restricted dietary protein. *European Journal of Pharmacology*, *588*(2–3), 151–157.

Banerjee, S., Chattopadhyay, K., Chhabra, J. K., & Chattopadhyay, B. (2012). Protein dependent fate of hepatic cells under nicotine induced stress and curcumin ameliorated condition. *European Journal of Pharmacology*, *684*(1–3), 132–145.

Basniwal, R. K., Buttar, H. S., Jain, V. K., & Jain, N. (2011). Curcumin nanoparticles: Preparation, characterization, and antimicrobial study. *Journal of Agricultural and Food Chemistry*, *59*(5), 2056–2061.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, *44*(1), 276–287.

Benowitz, N. L. (2009). Pharmacology of nicotine: Addiction, smoking-induced disease and therapeutics. *Annual Review of Pharmacology and Toxicology*, *49*, 57–71.

Benowitz, N. L., & Jacob, P. (1984). Daily intake of nicotine during cigarette smoking. *Clinical Pharmacology and Therapeutics*, *35*(4), 499–504.

Bergmeyer, H. U., & Bernt, E. (1963). Glutamate-pyruvate transaminase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis*. New York: Academic Press Inc.

Bhawana, Basniwal, R. K., Buttar, H. S., Jain, V. K., & Jain, N. (2011). Curcumin nanoparticles: Preparation, characterization, and antimicrobial study. *Journal of Agricultural and Food Chemistry*, *59*(5), 2056–2061.

Carroll, R. E., Benya, R. V., Turgeon, D. K., Vareed, S., Neuman, M., Rodriguez, L., … Brenner, D. E. (2011). Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. *Cancer Prevention Research (Philadelphia, PA)*, *4*(3), 354–364.

Chatterjee, S. N., & Agarwal, S. (1988). Liposome as membrane model for study of lipid peroxidation. *Free Radical Biology & Medicine*, *4*(1), 51–72.

Chattopadhyay, B. D., Tamang, A., & Kundu, D. (2015). Protective role of raw-turmeric rhizomes against nicotine-induced complications of Beedi (Indian cigarette) workers. *Pharmaceutica Analytica Acta*, *6*, 386.

Chattopadhyay, K., & Chattopadhyay, B. D. (2008). Effect of nicotine on lipid profile, per oxidation and antioxidant enzymes in female rats with restricted dietary protein. *The Indian Journal of Medical Research*, *127*, 571–576.

Chattopadhyay, K., Mondal, S., Chattopadhyay, B., & Ghosh, S. (2010). Ameliorative effect of sesame lignans on nicotine toxicity in rats. *Food and Chemical Toxicology*, *48*(11), 3215–3220.

Chen, Y. S., Hung, Y. C., Liau, I., & Huang, G. S. (2009). Assessment of the in vivo toxicity of gold nanoparticles. *Nanoscale Research Letters*, *4*(8), 858–864.

Chithrani, B. D., Ghazani, A. A., & Chan, W. C. W. (2006). Determining the size and shape dependence of gold nanoparticles uptake into mammalian cells. *Nano Letters*, *6*(4), 662–668.

Cohen, G., Dembiec, D., & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, *34*, 30–38.

Cuomo, J., Appendino, G., Dern, A. S., Schneider, E., McKinnon, T. P., Brown, M. J., … Dixon, B. M. (2011). Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *Journal of Natural Products*, *74*(4), 664–669.

Danafar, H. (2017). Study of the composition of polycaprolactone/poly (ethylene glycol)/polycaprolactone copolymer and drug-to-polymer ratio on drug loading efficiency of curcumin to nanoparticles. *Jundishapur Journal of Natural Pharmaceutical Products*, *12*(1), e34179.

Danafar, H., Sharafi, A., Askarlou, S., & Manjili, H. K. (2017). Preparation and characterization of PEGylated iron oxide-gold nanoparticles for delivery of sulforaphane and curcumin. *Drug Research*, *67*(12), 698–704.

Digard, H., Proctor, C., Kulasekaran, A., Malmqvist, U., & Richter, A. (2013). Determination of nicotine absorption from multiple tobacco products and nicotine gum. *Nicotine & Tobacco Research*, *15*(1), 255–261.

Domagala-Kulawik, J. (2008). Effects of cigarette smoke on the lung and systemic immunity. *Journal of Physiology and Pharmacology*, *59*(6), 19–34.

Flouris, A. D., & Oikonomou, D. N. (2010). Electronic cigarettes: Miracle or menace? *BMJ (Clinical Research Ed.)*, *340*, c311.

Flouris, A. D., Chorti, M. S., Poulianiti, K. P., Jamurtas, A. Z., Kostikas, K., Tzatzarakis, M. N., … Koutedakis, Y. (2013). Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. *Inhalation Toxicology*, *25*(2), 91–101.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*, 499–502.

Fröhlich, M., Sund, M., Löwel, H., Imhof, A., Hoffmeister, A., & Koenig, W. (2003). Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). *European Heart Journal*, *24*(14), 1365–1372.

Ghosh, M., Singh, A., Xu, W., Sulchek, T., Gordon, L., & Ryan, R. (2011). Curcumin nanodisks: Formulation and characterization. *Nanomedicine*, *7*(2), 162–167.

Griffith, O. W. (1980). Determination of glutathione and glutathione sulfide using glutathione reductase and 2-vinyl pyridine. *Analytical Biochemistry*, *106*(1), 207–212.

Gupta, S. C., Sung, B., Kim, J. H., Prasad, S., Li, S., & Aggarwal, B. B. (2013). Multitargeting by turmeric, the golden spice: From kitchen to clinic. *Molecular Nutrition & Food Research*, *57*(9), 1510–1528.

Hasimun, P., Sukandar, E. Y., Adnyana, I. K., & Tjahjono, D. H. (2011). Synergistic effect of curcuminoid and s-methyl cysteine in regulation of cholesterol homeostasis. *International Journal of Pharmacology*, *7*(2), 268–272.

Hawk, P. B., Oser, B. L., & Summerson, W. H. (1954). *Practical physiological chemistry* (13th ed.). New York: Mc Graw Hill Book Comp. Ltd.

Kalpana, C., & Menon, V. P. (2004). Modulatory effects of curcumin on lipid peroxidation and antioxidant status during nicotine induced-toxicity. *Polish Journal of Pharmacology*, *56*, 581–586.

Kanai, M., Imaizumi, A., Otsuka, Y., Sasaki, H., Sasaki, H., Hashiguchi, M., … Chiba, T. (2012). Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. *Cancer Chemotherapy and Pharmacology*, *69*, 65–70.

Kheiri Manjili, H., Ghasemi, P., Malvandi, H., Mousavi, M. S., Attari, E., & Danafar, H. (2017). Pharmacokinetics and in vivo delivery of curcumin by copolymeric mPEG-PCL micelles. *European Journal of Pharmaceutics and Biopharmaceutics*, *116*, 17–30.

Khosropanah, M. H., Dinarvand, A., Nezhadhosseini, A., Haghighi, A., Hashemi, S., Nirouzad, F., … Dehghani, H. (2016). Analysis of the antiproliferative effects of curcumin and nanocurcumin in MDA-MB231 as a breast cancer cell line. *Iranian Journal of Pharmaceutical Research*, *15*(1), 231–239.

Kim, M., & Kim, Y. (2010). Hypocholesterolemic effects of curcumin via up-regulation of cholesterol 7a-hydroxylase in rats fed a high fat diet. *Nutrition Research and Practice*, *4*(3), 191–195.

Kozolowski, L. T., Mehta, N. Y., Sweeney, C. T., Schwartz, S. S., Vogler, G. P., Jarvis, M. J., & West, R. J. (1998). Filter ventilation and nicotine content of tobacco in cigarettes from Canada, the United Kingdom and the United states. *Tobacco Control*, *7*, 369–375.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275.

Macky, J., & Eriksen, M. (2002). *The Tobacco atlas*. Geneva: World Health Organization.

Maiti, M., Chattopadhyay, K., Verma, M., & Chattopadhyay, B. D. (2015). Curcumin protects against nicotine-induced stress during protein malnutrition in female rat through immunomodulation with cellular amelioration. *Molecular Biology Reports*, *42*(12), 1623–1637.

Mandal, K., Dasgupta, S., & Chattopadhyay, B. D. (2004). Nicotine induced alterations on reproductive organs of female rats under protein deficient condition. *Indian Journal of Experimental Biology*, *42*, 330–332.

Milano, F., Mari, L., van de Luijtgaarden, W., Parikh, K., Calpe, S., & Krishnadath, K. K. (2013). Nano-curcumin inhibits proliferation of esophageal adenocarcinoma cells and enhances the T cell mediated immune response. *Frontiers in Oncology*, *3*, 137.

Nan, A., Bai, X., Son, S. J., Lee, S. B., & Ghandehari, H. (2008). Cellular uptake and cytotoxicity of silica nanotubes. *Nano Letters*, *8*(8), 2150–2154.

Nosrati, H., Sefidi, N., Sharafi, A., Danafar, H., & Manjili, H. K. (2018). Bovine Serum Albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug. *Bioorganic Chemistry*, *76*, 501–509.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, *70*, 158–169.

Ringman, J. M., Frautschy, S. A., Teng, E., Begum, A. N., Bardens, J., Beigi, M., … Cole, G. M. (2012). Oral curcumin for Alzheimer's disease: Tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. *Alzheimer's Research & Therapy*, *4*(5), 43. https://doi.org/10.1186/alzrt146.

Ruby, A. J., Kuttan, G., Babu, K. D., Rajasekharan, K. N., & Kuttan, R. (1995). Antitumour and antioxidant activity of natural curcuminoids. *Cancer Letters*, *94*(1), 79–83.

Salahshoor, M., Mohamadian, S., Kakabaraei, S., Roshankhah, S., & Jalili, C. (2016). Curcumin improves liver damage in male mice exposed to nicotine. *Journal of Traditional and Complementary Medicine*, *6*(2), 176–183.

Sasaki, H., Sunagawa, Y., Takahashi, K., Imaizumi, A., Fukuda, H., Hashimoto, T., … Morimoto, T. (2011). Innovative preparation of curcumin for improved oral bioavailability. *Biological & Pharmaceutical Bulletin*, *34*(5), 660–665.

Sav, A., Khetrapal, N., & Amin, P. (2012). Preparation of curcumin solid dispersions using hydrophilic polymers by different techniques and *in vitro* characterization. *Asian Journal of Pharmaceutics*, *7*(4), 271–279.

Schiborr, C., Kocher, A., Behnam, D., Jandasek, J., Toelstede, S., & Frank, J. (2014). The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. *Molecular Nutrition & Food Research*, *0*, 1–12.

Shaikh, J., Ankola, D. D., Beniwal, V., Singh, D., & Kumar, M. N. V. R. (2009). Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *European Journal of Pharmaceutical Sciences*, *37*(3–4), 223–230.

Sinha, S., Maiti, M., Chattopadhyay, K., & Chattopadhyay, B. D. (2012). Potential amelioration of curcumin against nicotine-induced toxicity of protein malnourished female rats. *Journal of Pharmacological and Toxicological Methods*, *7*, 166–180.

Sreekala, S., & Indira, M. (2009). Effects of exogenous selenium on nicotine-induced oxidative stress in rats. *Biological Trace Element Research*, *130*(1), 62–71.

Tabatabaei Rezaei, S. J., Nabid, M. R., Niknejad, H., & Entezami, A. A. (2012). Multifunctional and thermoresponsive unimolecular micelles for tumor-targeted delivery and site-specifically release of anticancer drugs. *Polymer*, *53*, 3485–3497.

Tracy, R. P., Psaty, B. M., Macy, E., Bovill, E. G., Cushman, M., Cornell, E. S., & Kuller, L. H. (1997). Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *17*(10), 2167–2176.

Usunobun, U., Adegbeg, J., Ademuyiwa, O., Okugbo, T. F., Evuen, U., Osibemhe, M., & Okolie, N. P. (2012). Nnitrosodimethylamine (NDMA), liver function enzymes, renal function parameters and oxidative stress parameters: A review. *British Journal of Pharmacology and Toxicology*, *3*, 165–176.

Vareed, S. K., Kakarala, M., Ruffin, M. T., Crowell, J. A., Normolle, D. P., Djuric, Z., & Brenner, D. E. (2008). Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiology, Biomarkers & Prevention*, *17*(6), 1411–1417.

Warnick, G. R., Nguyen, T., & Albers, A. A. (1985). Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. *Clinical Chemistry*, *31*, 217–222.

Werner, M., Gabrielson, D. G., & Eastman, J. (1981). Ultramicro determination of serum triglycerides by biolumnineescent assay. *Clinical Chem*, *27*, 268–271.

Yadav, A., Lomash, V., Samim, M., & Flora, S. J. (2012). Curcumin encapsulated in chitosan nanoparticles a novel strategy for the treatment of arsenic toxicity. *Chemico-Biological Interactions*, *199*(1), 49–61.

Yadav, V. R., Suresh, S., Devi, K., & Yadav, S. (2009). Effect of cyclodextrincomplexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. *American Association of Pharmaceutical Science and Technology*, *10*(3), 752–762.

Yen, F.-L., Wu, T.-H., Tzeng, C.-W., Lin, L.-T., & Lin, C.-C. (2010). Curcumin nanoparticles improves the physicochemical properties of Curcumin and effectively enhances its antioxidant and antihepatoma activities. *Journal of Agricultural and Food Chemistry*, *58*(12), 7376–7382.

Zhao, M., Hu, B., Gu, Z., Joo, K. I. I., Wang, P., & Tang, Y. (2013). Degradable polymeric nanocapsule for efficient intracellular delivery of a high molecular weight tumor-selective protein complex. *Nano Today*, *8*(1), 11–20.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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STUDIES ON THERAPEUTIC EFFICACY OF NANOCURCUMIN AGAINST NICOTINE INDUCED DAMAGE OF BLOOD CELLS

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ABSTRACT

Nicotine, after rapid absorption in human airways immediately goes to the blood resulting malfunction of the defence system. Studies on nicotine-induced toxicity of female population is still relevant because women are more susceptible to nicotine-induced complications. This study was an attempt to overcome the nicotine-induced genotoxicity of blood cells of female population by using nanocurcumin against nicotine-induced toxicity. Experiments were conducted on female rats exposed daily by effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of nanocurcumin (4 mg/kg body weight) for 21 days. Animals were eradicated after treatment period and several experiments were done from their blood. The molecular docking and *in vitro* interactions studies were explored to search the mechanism of action of nanocurcumin against nicotine-induced toxicity. Nanocurcumin showed its potential therapeutic efficacy against nicotine-induced complications. It increased haemoglobin (Hb) and DNA contents and reduced DNA damage very effectively ($p < 0.001$). Molecular docking predicted that nanocurcumin had stronger interaction to haemoglobin and DNA which protected those molecules from nicotine-induced toxicity. *In vitro* interaction studies also supported the molecular docking hypothesis. Nanocurcumin could be a promising therapeutic agent against nicotine-induced genotoxicities of blood cells and it could more effectively protect our health particularly, nicotine intoxicated female population.

Key words: Blood cells, DNA damage, Docking, Nanocurcumin, Nicotine.

INTRODUCTION

Nicotine, after consumption either through smoking or chewing tobacco leaves or taking snuffs, rapidly enters in the blood stream and causes disruption of the blood cells and defence system of our body. It causes several dreaded diseases like cardiac diseases [1], gastrointestinal cancer [2], pulmonary and oral cancer [3] etc. Women who are habituated in smoking, have a 25% greater increased risk of coronary heart disease than their male-smoker counterparts [4].

Nicotine has been found to alter the endocrine function, which in turn affects release of female sex-hormones [5].

In general, women suffers more than man from nicotine-induced complications due to their low

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inherent immunity [6, 7]. Scientist have already described the immunomodulation effect of nicotine and nanocurcumin in rat [8]. Studies on nicotine-induced toxicity and its amelioration of female population is still very much relevant for our society.

Nicotine reduces RBC counts and haemoglobin concentration in the blood due to which the body loses its ability for carrying oxygen to several organs in rats [9]. In fact, nicotine leads to shortness of breath and increases the pulse rate which creates a compensatory increment of the output of the heart leading to palpitations and chest pains [10]. In serious cases, the arms and legs may become swollen, and the individual may experience excessive sweating, heartburn, vomiting, bruises, and bloody stools [11]. Malenica et al. reported the aggravated effect of smoking on haematological parameter in healthy population [12]. Nicotine also enhances the reactive oxygen species (ROS) generation [5]. Enhanced ROS load and other toxic chemicals of nicotine cause peroxidative membrane damage of RBC [13]. It increases the viscosity of blood due to aggregation of RBC that results in impairment of blood flow. Nicotine also induces DNA damage in blood cells and various tissues due to its ROS generation activities [5, 14]. DNA damage inhibits M-CDKs (Mediator of Cyclin-dependent Kinases) which are a key component of progression into Mitosis [*15*]. It has also been observed that tobacco smoke induces aldehyde-DNA adducts in mice and humans, inhibits DNA repair activity and reduces repair proteins in mouse lung [16].

Curcumin derived from turmeric (*Curcuma longa*) possesses a wide range of pharmacological effects like antioxidant, anti-inflammatory and hepato-protective etc. [13, 14]. It protects DNA from damage and attenuates carboplatin-induced myelo-suppression by activating the DNA repair pathway in bone marrow cells [16, 17]. The promising therapeutic capabilities of curcumin is still restricted in use as therapeutic agent due to its poor aqueous solubility and limited bioavailability [18]. Encapsulated curcumin, liposomal curcumin etc. have been tried to increase its use in the recent past but due to large particle size, its effectiveness has been limited [19]. The concentration of curcumin is found to be very low in human blood because of its fast metabolic turnover in the liver and intestinal wall which results limited distribution in the tissues following oral dosing [20, 21]. Also, there are some reports concerning curcumin toxicity due to higher effective dose in the body [22]. Several studies were designed to formulate nanoparticles of curcumin through enhancing its bioavailability and bio-distribution activity for its therapeutic efficacy in drug delivery [23-28].

The current work was focused to evaluate the improved efficacy of nanocurcumin and its significant protective action against nicotine-induced damages of blood cells so that nanocurcumin could become the better replacement of curcumin for therapeutic uses in several diseases. An attempt was also made to find out the possible mechanism of action of nanocurcumin against nicotine-induced complications.

MATERIALS AND METHODS

Raw materials and Chemicals

Nicotine hydrogen tartrate, curcumin and powder haemoglobin (Hb) were purchased from Sigma Chemicals Company, St Louis, USA. All other analytical grade chemicals were supplied by SpectroChem Pvt. Ltd. India and Merck India. Nanocurcumin was prepared in our lab. The

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preparation and characterization of nanocurcumin was elaborately discussed in the previous paper [24].

Diets and Treatments

A total of 36 female albino rats of Wistar strain (*Rattus norvegicus*), 60 - 75 days old, weighing 140-150 g were procured from the Animal housing facility and maintained according to the guidelines of the Institutional Animal Ethics Committee of the Jadavpur University, Kolkata, India (Ref. No.: AEC/PHARM/1502/14/2015, Dated: 30/07/2015). The animals were divided into 6 groups, each containing 6 animals as shown in Table 1.

Table 1: Animal groups and treatment

Name of the group	Diet and treatment of animals for 21 days
Control (C)	Normal protein diet and received subcutaneous injection of 0.2 ml physiological saline only.
Nicotine treated (NT)	Normal protein diet and received subcutaneous injection with effective dose of nicotine (2.5 mg/kg body weight).
Nicotine treated and curcumin supplemented (NTCS)	Normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of curcumin (80 mg/kg body weight) orally.
Nicotine treated and nanocurcumin supplemented (NTNCS)	Normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of nanocurcumin (4 mg/kg body weight) orally.
Curcumin supplemented (CS)	These rats were fed with normal protein diet and supplemented with effective dose of curcumin (80 mg/kg body weight) orally.
Nanocurcumin supplemented (NCS)	These rats were fed with normal protein diet and supplemented with effective dose of nanocurcumin (4 mg/kg body weight) orally.

Nicotine hydrogen tartrate salt was dissolved in normal saline water (2.5 g/mL) and required concentration of drug solution (based on the body weight of an animal) was added to distilled water to make the final volume of 0.2 mL which was injected subcutaneously to that particular animal. The effective doses of nicotine, curcumin and nanocurcumin were chosen from previous studies. Both curcumin and nanocurcumin dispersed in water were supplemented orally at 1 h after nicotine treatment once daily. After 21 days of treatment, animals were kept under fasting condition for 12 h and eradicated on the next day after mild anaesthesia. Blood samples were collected from the heart immediately after eradication and stored in both with or without anticoagulant (heparin) containing containers for the required experiments.

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Haemoglobin (Hb) Estimation

The haemoglobin percentage in rat blood was determined by using Standard Sahli's Haemoglobin meter as described earlier [9].

Estimation of Total DNA Content

The total DNA content from 300 µL of whole blood cells of each sample was estimated by using the protocol as described by Banerjee et al. [9]. The concentration and purity of the prepared DNA was determined spectrophotometrically by noting the absorbances at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}) respectively as describes earlier [9].

Comet Assay For DNA Damage

DNA damage study of whole blood sample was performed by comet assay as described by Bandyopadhyay et al. [29]. The measurement of the comet head diameter, tail length, tail moment and percentage of DNA damages were done accordingly. A total of 50 cells were screened per slide using a fluorescence microscope (Leica 300-FX with 20 x magnification of Objective). Quantification of DNA damage for each cell was determined by using the Perceptive Comet software version-4.

Molecular Docking

Molecular docking was performed to predict the mode of the interaction of DNA and haemoglobin with nicotine and nanocurcumin using GEMDOCK, a program for computing a ligand conformation and orientation relative to active site of the receptor and UCSF CHIMERA 1.13.1, an extensive molecular modeller system used for docking study. Protein Data Bank file for tumour suppressor P53 complex with DNA (PDB ID: 1TUP) and haemoglobin (PDB ID: 1IRD) was used as receptor molecule and nicotine (PubChem CID:89594) and nanocurcumin (PubChem CID:969516) was taken as ligand molecule for docking. The chemical structure of nanocurcumin was same as that of curcumin revealed by Bhawana et al. [30]. So the same PubChem ID of curcumin was taken into consideration for further studies.

Interaction Studies of Nicotine vs. Haemoglobin and Nanocurcumin

Solutions of nicotine hydrogen tartrate (10 mM) and haemoglobin (3 mg/ml normal saline) were prepared. Haemoglobin solution was taken in a quartz cuvette (1 mL) and its absorbance spectrum was recorded (300 nm – 500 nm) by using an UV-Visible spectrophotometer against normal saline (blank). Different concentrations of nicotine solution were added to the haemoglobin solution (final concentration of nicotine was varied from 50 to 500 µM in the solution), mixed well and the spectrum of each mixture was taken similarly against the blank which contained normal saline with respectively added nicotine. The concentration of nicotine in the interaction between

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nicotine vs. Hb was varied till the absorbance peak of Hb was suppressed completely. Next, freshly synthesized nanocurcumin was added gradually to nicotine (500 µM) + Hb solution (final concentration of nanocurcumin was varied from 10 to 50 µM in the solution), mixed well and absorbance spectrum was recorded at 300 nm – 500 nm against the blank containing normal saline with respective concentration of added nicotine and nanocurcumin to the solution.

Interaction Studies of Nicotine vs. DNA and Nanocurcumin

A solution of whole blood DNA (30 µg/mL in TE buffer) was prepared. DNA solution (1 mL) was taken in a quartz cuvette and its absorbance spectrum was recorded (240 nm – 320 nm) against TE buffer by using an UV-Visible spectrophotometer. Different concentrations of nicotine solution were added to the DNA solution (final concentration of nicotine was varied from 50 to 250 µM in the solution), mixed well and the spectrum of each mixture was taken similarly against the blank containing respective concentration of nicotine. The concentration of nicotine in this case was till the absorbance peak of DNA was suppressed sufficiently by nicotine. Next, freshly synthesized nanocurcumin was added gradually to nicotine (250 µM) + DNA solution (final concentration of nanocurcumin was varied from 10 to 50 µM in the solution), mixed well and absorbance spectrum was recorded at 240 nm – 320 nm against the blank which contained TE buffer with respectively added nicotine and nanocurcumin.

The binding constant between haemoglobin and nicotine molecular interaction was calculated by Binding-Isotherm plot using the following equation [31].

$$1/\Delta A_c = 1/A_m + [1/A_m \times K] \times 1/C$$

Where, ΔA_c was the change of the absorbance intensity of the Hb molecule, A_m was the initial maximum absorbance intensity of the Hb molecule, C was the concentration of the nicotine (quencher) molecule and K was the binding constant of the nicotine with the Hb molecule. A graph was plotted between $1/\Delta A_c$ vs. 1/C. The binding constant K, of the quencher was determined from the value of the intercept ($1/A_m$) and the slope ($1/A_m \times K$) of the curve $1/\Delta A_c$ vs. 1/C.

STATISTICAL ANALYSIS

The experimental setup was repeated twice and all data were averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied highly significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p<.01$) and ## implied highly significant ($p<.001$) of the data when compared with the data of nicotine + curcumin treatments.

RESULTS

Nicotine decreased the concentration of haemoglobin whereas, native curcumin and nanocurcumin both increased the haemoglobin concentration of the blood in the supplemented condition (Table 2). The total DNA contents in the whole blood cells was drastically reduced by nicotine as seen in nicotine treated rats (Table 2). Nanocurcumin showed significant ($p < 0.001$) ameliorative effect against nicotine on DNA content than native curcumin ($p < 0.01$). Nicotine treatment caused intense DNA damage (> 39%) of the whole blood cells where as the DNA damage in control group was 2.76% only (Table 3). The DNA damage caused by nicotine was effectively reduced by curcumin (>20%) and significantly by

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nanocurcumin (>30%) (Table 3). Supplementation of curcumin or nanocurcumin alone did not produce any negative effect on Hb and DNA content of blood cells rather they showed healthy condition of blood cells (Tables 2 and 3).

In-silico docking of nicotine vs. haemoglobin molecule (Fig. 1A) and nanocurcumin (here curcumin) vs. haemoglobin molecule (Fig. 2A) clearly depicted the formation of complex structures of nicotine-haemoglobin molecule and nanocurcumin-haemoglobin molecule respectively. Out of 10 ligand conformations, the best binding energy between nicotine vs. haemoglobin was found to be around (-) 70.98 kcal and that of curcumin vs. haemoglobin be (-) 97.41 kcal respectively. In-silico docking of nicotine vs. nanocurcumin (here curcumin) suggested that binding energy between nicotine vs. nanocurcumin was (-) 51.29 kcal (Fig. 5A). Similarly, molecular docking experiment between p53 tumour suppressor DNA and nicotine showed the formation of nicotine - DNA complex molecule (Fig. 3A) and nanocurcumin (here curcumin) vs. DNA interactions showed complex structure of nanocurcumin – DNA molecule (Fig. 4A) respectively. Here also the best binding energy between nicotine-DNA interaction was determined as (-) 69.48 kcal and that of between curcumin vs. DNA was around (-) 86.35 kcal.

Table 2: Haemoglobin and Total DNA content in whole blood of animals in different group

Parameter	Groups					
	C	**NT**	**NTCS**	**NTNCS**	**NC**	**NCS**
Haemoglobin (g/dL^{-1})	13.3 ± 0.3	9.8 ± 0.6 (26.0↓)	12.2 ± 1.3 (8.1↓)**	12.4 ± 0.5 (6.6↓)**#	12.9 ± 0.2 (3.0↓)	13.2 ± 0.2 (1.0↓)
Blood DNA (µg/300µL)	113.95± 2.70	68.56±1.52 (39.8↓)	90.77 ± 5.02 (20.3↓)*	107.85 ± 4.42 (5.4↓)**##	114.72 ± 3.33 (1.0↑)	116.67 ± 2.51 (2.4↑)

The whole experimental setup was repeated twice and all data of each group was the averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant (p<.01) and ** implied more significant (p<.001) of the data when compared with the data of nicotine treatment. Similarly, # implied significant (p<.01) and ## implied more significant (p<.001) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

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Table 3: DNA damage percentage and tail moment in whole blood of different groups

Parameter	Groups					
	(C)	(NT)	(NTCS)	(NTNCS)	(NC)	(NCS)
% DNA Damaged	2.76 ± 0.30	39.97 ± 0.81	16.99 ± 1.60**	9.20 ± 0.22**##	2.32 ± 0.54	2.11 ± 0.48
Tail moment (Arbitary unit)	70.5 ± 1.6	691.3 ± 10.60	245.5 ± 9.30*	162.00 ± 3.30**#	21.02 ± 3.4	20.6 ± 4.90

The whole experimental setup was repeated twice and all data of each group was the averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant (p<.01) and ** implied more significant (p<.001) of the data when compared with the data of nicotine treatment. Similarly, # implied significant (p<.01) and ## implied more significant (p<.001) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

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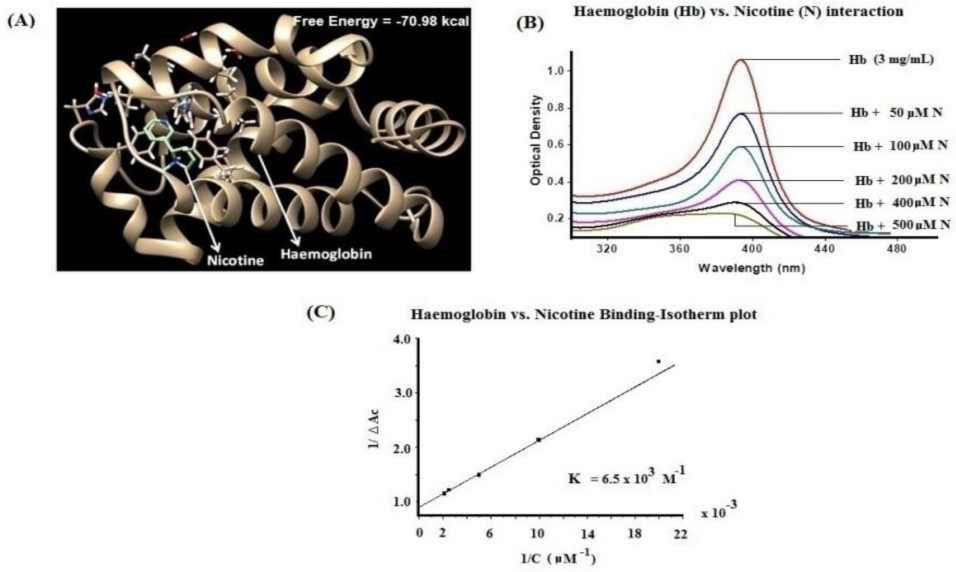

Figure 1 : Interaction studies between nicotine vs. haemoglobin (Hb).

Inset A shows the Molecular docking interaction between nicotine vs. Hb.

Inset B shows the UV-Vis spectra of interaction between nicotine vs. Hb.

Inset C shows the Binding -Isotherm Plot between nicotine vs. Hb.

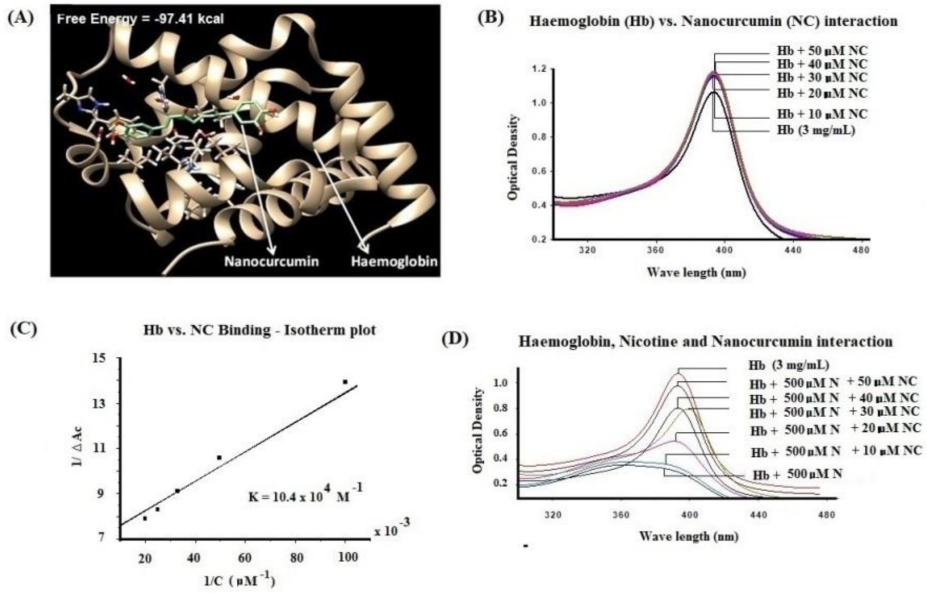

Figure 2: Interaction studies between nanocurcumin vs. haemoglobin (Hb).

Inset A shows the Molecular docking interaction between nanocurcumin vs. Hb.

Inset B shows the UV-Vis spectra of interaction between nanocurcumin vs. Hb.

Inset C shows the Binding -Isotherm Plot between nanocurcumin vs. Hb.

Inset D shows the UV-Vis spectra of interaction between nanocurcumin vs. nicotine + Hb.

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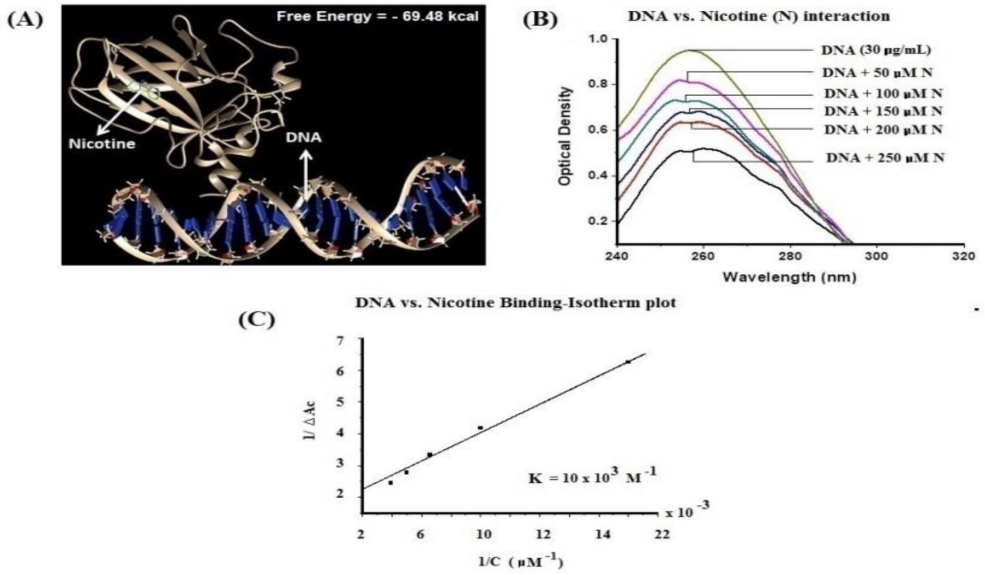

Figure 3:Interaction studies between nicotine vs. DNA.

Inset A shows the Molecular docking interaction between nicotine vs. DNA.
Inset B shows the UV-Vis spectra of interaction between nicotine vs. DNA.
Inset C shows the Binding -Isotherm Plot between nicotine vs. DNA.

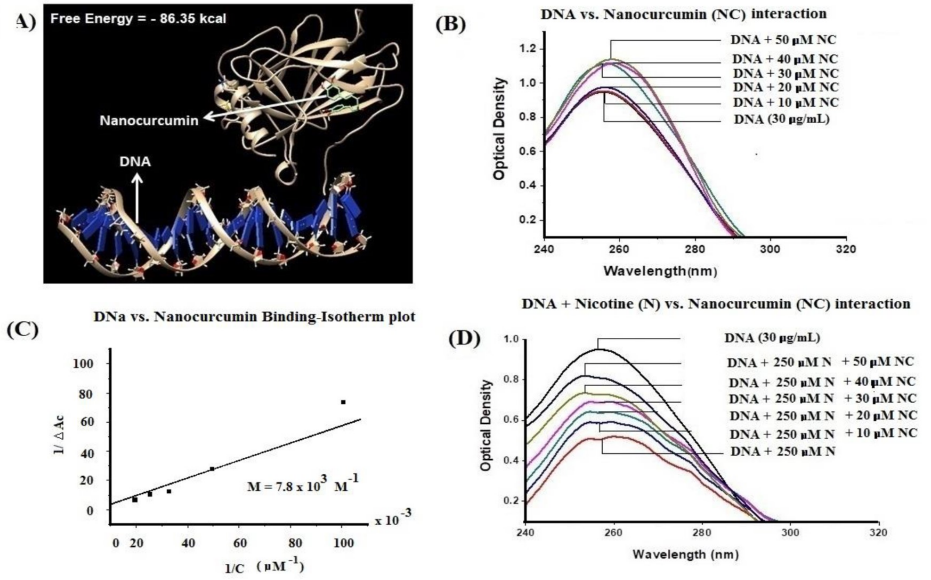

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Figure. 4:Interaction studies between nanocurcumin vs. DNA.

Inset A shows the Molecular docking interaction between nanocurcumin vs. DNA.
Inset B shows the UV-Vis spectra of interaction between nanocurcumin vs. DNA.
Inset C shows the Binding -Isotherm Plot between nanocurcumin vs. DNA.
Inset D shows the UV-Vis spectra of interaction between nanocurcumin vs. nicotine + DNA.

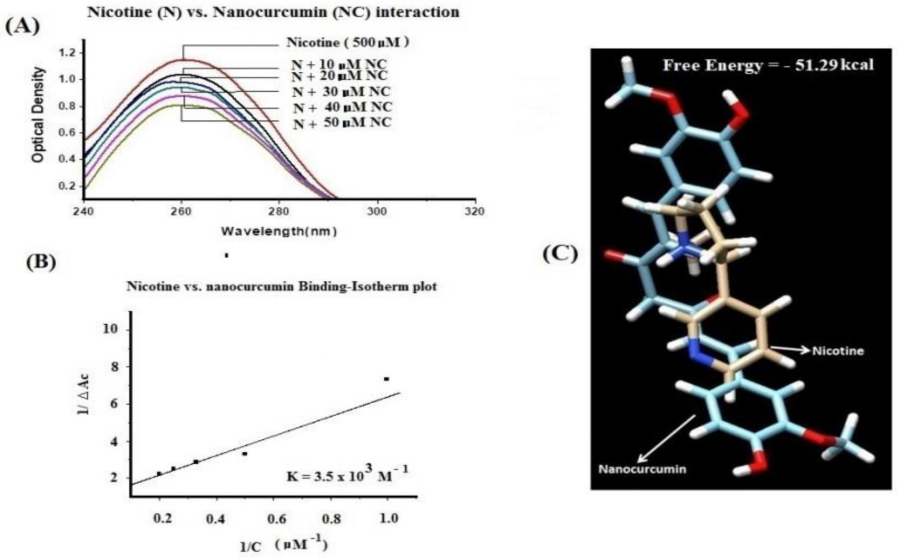

Figure 5: Interaction studies between nicotine vs. Nanocurcumin.

Inset A shows the UV-Vis spectra of interaction between nicotine vs. Nanocurcumin.
Inset b shows the Binding -Isotherm Plot between nicotine vs. NANOCURCUMIN.
Inset c shows the Molecular docking interaction between nicotine vs. nanocurcumin.

UV-Visible spectral studies between nicotine vs. haemoglobin interaction showed that nicotine bound with Hb (binding constant, K = 6.5 × 10^3 M^{-1}) and completely suppressed the characteristics absorbance peak of Hb molecule at 400 nm (Fig. 1B). Whereas, nanocurcumin showed higher binding affinity (binding constant, K = 10.4 × 10^4 M^{-1}) to Hb (Fig. 2B) and nullified the toxic effect of nicotine resulting reappearance of the characteristics absorbance peak of Hb at 400 nm.

From UV-Visible spectral analysis between nicotine vs. DNA interaction (Fig. 3B) similarly showed that nicotine bound with DNA (binding constant, K = 10 × 10^3 M^{-1}) and deformed the structural integrity of DNA resulting reduction of the characteristics absorbance maxima of DNA at 260 nm as well as produced multi absorbance maxima in the spectrum of DNA. Nanocurcumin showed similar binding affinity (binding constant, K = 7.8 × 10^3 M^{-1}) with DNA (Fig. 4B) and reduced the toxic effect of nicotine on DNA due to which the characteristics absorbance spectrum of DNA was reappeared (Fig. 4B). Nanocurcumin also showed binding affinity to nicotine (K = 3.5 × 10^3 M^{-1}).

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Nanocurcumin did not show any negative effect on the characteristics absorbance maxima of Hb and DNA both rather it increased the absorbance maxima of Hb and DNA as seen from Fig. 2B and Fig. 4B respectively.

DISCUSSION

The broad range of pharmaceutical activities of curcumin is still not converted into clinical benefits because of its limited bioavailability and undesirable pharmacokinetics [18, 32]. Increased bioavailability of curcumin using a novel dispersion technology system has been demonstrated by Briskey et al. [33]. Increased efficacy of curcumin by formulation of nanocurcumin against nicotine-induced toxicity at cellular levels in rats is reported earlier [24]. The present study is an another attempt to explore the superior efficacy of nanocurcumin against nicotine-induced genotoxicity on whole blood cells of female rats.

The study shows that nicotine causes a significant decrease in haemoglobin concentration in blood at normal condition. Earlier, Thomas and Lumb [34] have shown that binding of oxygen with haemoglobin is affected by carbon monoxide exerted from tobacco smoke, due to its greater binding affinity (>300 times) than that of oxygen. Banerjee et al. [9] have reported that nicotine reduces the RBC counts due to peroxidative membrane damage of erythrocytes resulting decrease in haemoglobin content of blood. In nicotine-haemoglobin docking, the amino residues such as TYR 42, ASN 97, PHE 98 and LEU 101 of haemoglobin molecule showed hydrophobic interaction with nicotine molecule and thus strengthen the nicotine haemoglobin complex structure. Similarly, in nanocurcumin-haemoglobin docking, nanocurcumin molecule can nicely accommodate into the haemoglobin's active site by forming one hydrogen bond with the residue His 58, SER 102, SER 133 of the protein. Besides this hydrogen bond, nanocurcumin also exhibited few hydrophobic interactions with the amino acid residues such as LYS 61, VAL 62, LEU 83, HIS 87, PHE 98 of haemoglobin molecule. The binding free energy between haemoglobin vs. nicotine was higher than that of nanocurcumin vs. haemoglobin. From the above docking experiment, it is clear that nanocurcumin binds more tightly with the haemoglobin molecule than nicotine did.

This study shows that nanocurcumin maintains the haemoglobin content of RBC cells against nicotine-induced stress due to its higher binding affinity to haemoglobin ($K = 10.4 \times 10^4\ M^{-1}$) than that of nicotine ($K = 6.5 \times 10^3\ M^{-1}$). It binds with Hb and resists nicotine to bind with Hb because of its higher binding affinity. Also, nanocurcumin possesses a binding affinity ($K = 3.5 \times 10^3\ M^{-1}$) to nicotine due to which it bind with the nicotine and reduces the free avaiable nicotine molecules to interact with the RBC and causes less membrane damage. This may explain the increase of Hb concentration of the nicotine treated rat which were supplemented with nanocurcumin. Our study thus is in agreement with the earlier studies which show the efficacy of nanocurcumin against nicotine-induced toxicity [24] and confirms that nanocurcumin is more effective to increases the haemoglobin concentration of the blood and shows better protective efficacy towards haemoglobin [9].

Nicotine (N2) formed a hydrogen bond with SER 241 residue of p53 tumour suppressor protein. Other amino acids such as SER 240, ARG 248, ARG 249, ARG 273, VAL 274 etc. of p53 protein associated with DNA showed hydrophobic interaction with the nicotine molecule that strengthen the stability of nicotine-DNA complex. Likewise, in nanocurcumin-DNA docking, nanocurcumin molecule can nicely accommodate into the active site of p53 protein associated with DNA by forming one hydrogen bond with the residue GLN 165, ASN 247 of the protein.

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Besides this hydrogen bond, nanocurcumin also exhibited few hydrophobic interactions with the amino acid residues such as ARG 248, CYS 176, HIS 179 of p53 protein associated with DNA molecule. The binding free energy between nicotine-DNA was determined as - 69.48 kcal, which was higher than that of nanocurcumin vs. DNA (- 86.35 kcal). This concludes that nanocurcumin binds more rigidly with the p53 protein associated DNA molecule than nicotine did and therefore nanocurcumin-DNA complex is more stable than nicotine-DNA complex. From the docking experiment of nicotine nanocurcumin interaction, it may be assumed that nanocurcumin might compete with nicotine while binding with a protein (Hb) and/or DNA and ameliorate nicotine-induced genotoxicity in whole blood cells.

The total DNA yield per 300 µL of blood (109.38 µg) under normal condition is also in good agreement with the value obtained previously [9]. The reduction of total DNA contents was due to the oxidative stress caused by nicotine. The decrease of total DNA concentration of the blood cells was more effectively ($p < 0.001$) resisted by nanocurcumin due to which the total DNA concentrations of the blood cells was almost restored to normal level in nanocurcumin supplemented condition as compared to that of native curcumin supplemented condition (Table 2). The observed minimal DNA damage of control blood cells was 2.76% (Table 2) which is acceptable, whereas, the average DNA damage in nicotine-treated rat was seen significantly higher (40% ; $p< 0.001$) in comparison to control (Table 3). Sanner and Grimsru [35] have shown that nicotine induces chromosomal aberration, sister chromatid exchange and single-strand DNA strand breaks due to oxidative stress. Banerjee et al.[9] have reported that nicotine increases the formation of free radicals and reactive oxygen species (ROS) resulting increased DNA damage in blood cells and liver tissues of female rats. They have also shown that curcumin effectively interacts with nicotine and DNA and also reduces the oxidative stress in nicotinic condition resulting less DNA damage. Here, it is observed that nanocurcumin increases the DNA content of whole blood cells against nicotine-induced toxicity. It has similar binding affinity to DNA ($K = 7.8 \times 10^3\ M^{-1}$) as nicotine ($K = 10.0 \times 10^3\ M^{-1}$). It therefore binds with DNA and resists nicotine to damage DNA. The binding affinity of nanocurcumin with nicotine is an added advantage to protect DNA from nicotinic attack. This study therefore suggests that nanocurcumin is an effective bio-molecule that can protect blood DNA against nicotine-induced genotoxicity.

From our wet lab study it is seen that nicotine has a strong interaction with Hb/DNA haemoglobin due to which the absorption peak of Hb/DNA is suppressed (Fig. 2 and Fig. 4). Nanocurcumin also shows very strong interaction with Hb (binding affinity of nanocurcumin is 10 times higher than that of nicotine when interacts with Hb) as well as it has an interaction with nicotine. These results of these interactions thus corroborate the molecular docking results and explain the protective action of nanocurcumin in Hb/DNA against nicotine treated condition. In presence of nanocurcumin, free nicotine molecules become less available to interact with Hb/DNA, as nicotine is out competed by nanocurcumin and this results in regain of structural integrity of Hb/DNA as seen from spectral analysis. The effective amelioration of nanocurcumin against nicotine-induced genotoxicity of whole blood cells of female rats thus may be described from our experimental results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest of any kind related to this work.

REFERENCES

1. Rigotti NA, Pipe AL, Benowitz NL, Arteaga C, Garza D, Tonstad S. Efficacy and safety of varenicline for smoking cessation in patients with cardiovascular disease: a randomized trial. *Circulation* 2010;121:221–229.
2. Jensen K, Afroze S, Munshi MK, Guerrier M, Glaser SS. Mechanism for nicotine in the development and progression of gastrointestinal cancers. Transl Gastrointest Cancer 2012;1:81–87.
3. Warnakulasuriya S, Dietrich T, Bornstein MM, Peidró EC, Preshaw PM, Walter C et al. Oral health risks of tobacco use and effects of cessation. Int Dent J 2010;60:7–30.
4. Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet 2011;378(9799):1297–305.
5. Jandikova H, Duskova M, Starka L. The Influence of Smoking and Cessation on the Human Reproductive Hormonal Balance. Physiol Res 2017; 66 (3): S323-S331. doi: 10.33549/physiolres.933724.
6. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol 2014; 35: 347–369.
7. Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet 2011;378(9799):1297-305.
8. Maiti M, Chattopadhyay K, Verma M, Chattopadhyay BD. Curcumin protects against nicotine-induced stress during protein malnutrition in female rat through immunomodulation with cellular amelioration. Mol Biol Reports 2015;42(12): 1623 -1637.
9. Banerjee S. Bandyopadhyaya G, Chattopadhyhay K, Chattopadhyhay BD. Amelioration of nicotine-induced damage of blood cells in protein malnourished female rats by curcumin. Int J Pharmacol 2010; 6(4):609–620.
10. Zeng Q, Shen LJ, Li S, Chen L, Guo X, Qian CN et al. The effects of haemoglobin levels and their interactions with cigarette smoking on survival in nasopharyngeal carcinoma patients. Cancer Med 2016;5(5):816–826.
11. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood 2018;131: 505–514.

ISSN 2046-5114

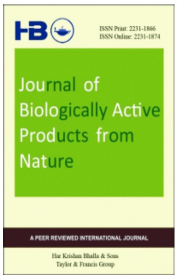
12. Malenica M, Prnjavorac B, Bego T, Dujic T, Semiz S, Skrbo S et al. Effect of Cigarette Smoking on Haematological Parameters in Healthy Population. Med Arch 2017; 71(2): 132–136
13. Padmavathi P, Vaddi DR, Kavitha G, Maturu P, Varadacharyulu NC. Chronic cigarette smoking alters erythrocyte membrane lipid composition and properties in male human volunteers. Nitric Oxide 2010; 23(3): 181-186.
14. Banerjee S, Chattopadhyay K, Chhabra JK, Chattopadhyay BD. Protein dependent fate of hepatic cells under nicotine induced stress and curcumin ameliorated condition. Eur J Pharmacol 2012;684:132–145.
15. *Giglia-Mari G, Zotter A, Vermeulen W.* DNA Damage Response. *Cold Spring Harb Perspect - Biol 2011;3(1): a000745.*
16. Weng M, Lee HW, Park SH, Hu Y, Wang HT, Chen LC et al. Aldehydes are the predominant forces inducing DNA damage and inhibiting DNA repair in tobacco smoke carcinogenesis. PNAS 2018; 115 (27): E6152–E6161.
17. Chen X, Wang J, Fu Z, Zhu B, Wang J, Guan S et al. Curcumin activates DNA repair pathway in bone marrow to improve carboplatin-induced myelosuppression. Sci Rep 2017;7:17724.
18. Her C, Venier-Julienne MC, Roger E. Improvement of Curcumin Bioavailability for Medical Applications. Med Aromat Plants 2018; 7(6):326-340.
19. Feng T, Wei Y, Lee RJ, Zhao L. Liposomal curcumin and its application in cancer. Int J Nanomed 2017; 12: 6027-6044.
20. Schiborr C, Kocher A, Behnam D, Jandasek J, Toelstede S, Frank J. The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. Mol Nutr Food Res 2014; 58(3): 516-527.
21. Metzler M, Pfeiffer E, Schulz SI, Dempe JS. Curcumin uptake and metabolism. Biofact 2013; 39(1): 14-20.
22. Burgos-Moron E, Calderon-Montano JM, Salvador J, Robles A, Lopez-lazaro M. The darkside of curcumin. Int J Cancer 2010; 126: 1771-1775.
23. Lopez-Lazaro M. Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. Mol Nutn Food Res 2008; 52 (S1):S103–S127.
24. Chattopadhyay K, Samanta A, Mukhopadhyay S, Chattopadhyay BD. Potential amelioration of nicotine-induced toxicity by nanocurcumin. Drug Dev Res 2018;79:119–128.
25. Das RK, Kasoju N, Bora U. Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomed 2010;6(1):153–160.
26. Danafar H. Study of the composition of polycaprolactone/poly (ethylene glycol)/polycaprolactone copolymer and drug-to-polymer ratio on drug loading efficiency of curcumin to nanoparticles. Jundishapur J Nat Pharmaceut Products 2017; 12(1): e34179.
27. Montalbán MG, Coburn JM, Lozano-Pérez AA, Cenis JL, Víllora G, Kaplan DL. Production of Curcumin-Loaded Silk Fibroin Nanoparticles for Cancer Therapy . Nanomat 2018; 8: 226-243.

ISSN 2046-5114

International Journal of Pharmaceutical Research and Innovation, Volume 14, 2021,12-26

28. Nosrati H, Sefidi N, Sharafi A, Danafar H, Manjili HK. Bovine Serum Albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug. Bioorg Chem 2018; 76: 501–509.
29. Bandyopadhyaya G, Sinha S, Chattopadhyay BD, Chakraborty A. Protective role of curcumin against nicotine induced genotoxicity on rat liver under restricted dietary protein. Eur J Phrmacol 2008;588:151–157.
30. Bhawana, Basniwal RK, Buttar HP, Jain VK, Jain N. Curcumin Nanoparticles: Preparation, Characterization, and Antimicrobial Study. J Agri Food Chem 2011; 59(5): 2056–61.
31. Lakowicz J. Principle of Fluorescence Spectroscopy. Plenum Press, New York 1983.
32. Manjili HK, Ghasemi P, Malvandi H, Mousavi MS, Attari E, Danafar H. Pharmacokinetics and in vivo delivery of curcumin by copolymeric mPEG-PCL micelles. Eur J Pharmaceut Biopharmaceut 2017; 116: 17–30.
33. Briskey D, Sax A, Mallard AR, Rao A. Increased bioavailability of curcumin using a novel dispersion technology system (LipiSperse®). Eur J Nutr 2019; 58:2087-2097.
34. Thomas C, Lumb A. Physiology of haemoglobin, Continuing Education in Anaesthesia and Critical Care. Pain 2012;12(5): 251–256.
35. Sanner T, Grimsru TK. Nicotine: Carcinogenicity and Effects on Response to Cancer Treatment – A Review. Front Oncol 2015; 5:196.

ISSN 2046-5114



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Promising Efficacy of Nanocurcumin in Comparison to Curcumin Against Nicotine-induced Complications

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Original Article

Promising Efficacy of Nanocurcumin in Comparison to Curcumin Against Nicotine-induced Complications

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Abstract: Long-term exposure to nicotine severely affects the immune system. Nicotine-addicted women suffer more to diseases because of their history to several disorders or susceptibility to nicotine-related morbidity and mortality. The beneficial uses of nano-curcumin in nicotine-addicted women are still unknown, possibly due to its poor pharmacokinetics and limited bioavailability. Nanocurcumin is administered to study the immune-protective effects in nicotine exposed female rats. Female albino rats of Wistar strain (*Rattus norvegicus*) are nicotine injected (2.5 mg/kg bodyweights s.c., daily) for 21 days. Three hours later nanocurcumin is administered by oral gavages (4 mg/kg bodyweights/day). The dose and period of nanocurcumin is adopted from our earlier publications elsewhere. Animals are eradicated after the treatment period and immune-related cytokines, apoptotic markers, sex hormones and other parameters are measured from individual serum. Molecular docking, as well as, *in vitro* interactions of nanocurcumin with nicotine and some specific cellular proteins are measured for understanding the mechanism of action of nanocurcumin. Nanocurcumin reduced protein oxidation, augmented the immune system by regulating the expression of immunity-related cytokines and apoptotic markers. It helps maintaining the healthy levels of sex hormones and protein content of cells under nicotine-stressed condition. Molecular docking and *in vitro* interactions reveal that nanocurcumin possesses higher binding ability to nicotine and several cellular proteins. Cytokines, apoptotic markers and steroidogenic hormones are severely affected by nicotine exposure. Bio-soluble nanocurcumin acts as significant immune-modulator, which offers better protection than curcumin to female rats from nicotine-induced complications. Heightened therapeutic effects of nanocurcumin are discussed.

Keywords: Apoptotic molecule, Cytokines, Female hormones, Nicotine, Nanocurcumin.

Introduction

Intake of nicotine through tobacco (smoking or through any routes) generates oxidative stress [1]. It disrupts the immune system resulting cardiac disorders [2], and various cancers [3]. Tobacco consumptions in different forms like smoking of beedi, leaf tobacco chewing, consumption of zarda pellets are a common addiction in female population of India, particularly women who are socio-economically handicapped. Females are seen to differ in strength and nature of immune responses against various diseases [4]. Females are more susceptible to immunopathology, especially autoimmune disorders compared to males and smoking-related complications and mortality [5]. Nicotine-addicted women have greater increased risk of coronary heart disease [6].

Nicotine affects blood cells and hampers

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the cellular and immune responses due to the generation of reactive oxygen species [7]. It reduces the haemoglobin concentration and therefore body loses its normal ability to supply oxygen in the vital organs leading to shortness of breath and others complications [8]. In serious cases, the symptoms like inflammation of arms and legs, excessive sweating, heartburn, vomiting etc. are noted in some individuals. Nicotine also induces DNA damage in the tissues [9].

Oxidative stress causes oxidative damage in the tissues (e.g., liver, kidney, ovary etc.) leading to several diseases. Nicotine abuse elicits acute phase response in liver by activating monocytes and macrophages and thereby causes acute liver injuries [10]. In addition, nicotine enhances the productions of interleukin-4 (IL-4) and interleukin-6 (IL-6) [11]. IL-4 induces differentiation of native helper Th0 cells to Th2 cells. Increased production of Th2 cells is associated with allergies and various types of cancers [12]. Higher expression of IL-6 is associated with the development of encephalitis in children and asthma [13]. Aberrant expression of IFN-γ, an important activator of macrophages associated with auto-inflammatory and autoimmune diseases, inhibits viral replication directly and possesses immune-stimulators and immune-modulators characters [14]. Exposure to tobacco smoke leads to the over-expression of tumour necrosis factor alpha (TNF-α) [15]. Nicotine also affects the Bcl-2 vs. Bax ratio [16]. These two molecules are important apoptotic proteins. The pro-apoptotic proteins in the Bcl-2 family (including Bax) normally act on the mitochondrial membrane to promote permeability. The activity of pro-apoptotic protein is inhibited by the action of Bcl-2 and its relative-protein Bcl-Xl [17].

In addition, nicotine alters the function of endocrine system and affects the release of female hormones, which are very important agents for the protection of women health [18]. Deficiency of estrogens results in the irregularity of periods, infertility, bone weakness, hot flashes, depression, and urinary tract infection [19]. Similarly, deficiency of progesterone affects the menstrual cycle, pregnancy, and embryogenesis of humans and other species [19].

Curcumin possesses a wide range of pharmacological properties [20]. It works against DNA damage also [9]. The promising therapeutic uses of curcumin are limited because of its poor water solubility and limited bioavailability [21]. Curcumin is well adapted by individuals but it exhibits toxicity in our body at high dosages [22]. Piperine has been used to enhance bioavailability of curcumin without altering the mechanism or magnitude of effect [23]. Several studies are designed to formulate nanocurcumin for enhancing its bioavailability [24]. The current work is an attempt to improve bio-distribution by increasing the bioavailability of curcumin which may help for better functioning of the immune system. We hypothesize that nanocurcumin may combat more effectively than curcumin against nicotine-induced immunological disruption and will lead to a healthy individual, particularly for nicotine-addicted female population.

Material and methods

Chemicals

Nicotine hydrogen tartrate, curcumin, α-Lactalbumin (α-LA), Cytochrome complex (Cyt-c) and Haemoglobin (Hb) proteins are purchased from Sigma Aldrich Co. (St. Louis, MO, USA). All other analytical grade chemicals are supplied either by SpectroChem Pvt. Ltd. (India) or Merck (India). PureGene AG (Zeiningen, Switzerland) made ELISA kits supplied by Genetix Biotech Asia Pvt. Ltd. (India) are used for the detection of cytokines, apoptotic protein and steroidogenic hormones. Nanocurcumin used in the study is prepared in our laboratory following the methodology published elsewhere [24].

Animals and treatments

Female albino rats of Wistar strain (*Rattus norvegicus*), 30 in number, 60-75 days old, weighing 140-150 g are procured from the Animal Housing Facility and maintained with normal protein diet according to the guidelines of the Institutional Animal Ethics Committee of the Jadavpur University, Kolkata, India (Ref. No.: AEC/PHARM/1502/14/2015, Dated:

30/07/2015) [24]. The animals were maintained with normal protein diet and divided into 6 groups, each containing 5 animals as described below -

Control group (C) - Animals injected with 0.2 ml physiological saline.

Nicotine treated group (NT) - Animal injected by effective dose of nicotine.

Nicotine treated and curcumin supplemented group (NTCS) - Animal injected by effective dose of nicotine followed by supplementation of effective dose of curcumin orally.

Nicotine treated and nanocurcumin supplemented group (NTNCS) - Animal injected with effective dose of nicotine followed by supplementation of effective dose of nanocurcumin orally.

Curcumin supplemented group (CS) - Animal supplemented of effective dose of curcumin orally.

Nanocurcumin supplemented group (NCS) - Animal supplemented of effective dose of nanocurcumin orally.

A solution of nicotine hydrogen tartrate salt (2.5 g of nicotine salt dissolved in per mL of normal saline water) is prepared as stock solution. The nicotine solution is diluted by normal saline water to make a final concentration 2.5 µg/µL. Before use, the required volume of nicotine solution (based on the bodyweights of the animal) is taken in a tube to which normal saline water is added to make a volume of ~ 0.2 ml, which contains effective dose of the drug and is injected subcutaneously daily to the respective animals. Both curcumin and nanocurcumin are separately dispersed in sterilized distilled water and administered by oral gavages after 3 h of nicotine treatment to the respective groups of animal. The effective doses of the drug i.e., nicotine (2.5 mg/kg bodyweights/day), curcumin (80 mg/kg bodyweights/day) and nanocurcumin (4 mg/kg bodyweights/day) are determined in our previous study [24]. Injection of effective dose of nicotine and oral supplementation of effective doses of curcumin and nanocurcumin respectively are continued for 21 days. After 21 days, animals are kept under fasting condition for 12 h and eradicated on the next day after mild anaesthesia. Blood samples are collected immediately from the heart and stored in without anticoagulant (heparin) containing containers. Sera are separated and stored at - 20ºC for future analysis. Vital organs are dissected out and stored for future studies in vacuum desiccators at -20ºC to prevent auto-oxidation.

Preparation and characterization of nano-curcumin

The synthesis of nanocurcumin is described earlier [24]. In brief, a solution of 0.018 M curcumin is prepared by dissolving curcumin in dichloromethane (DCM). The solution is added to warm (50ºC) Milli-Q ultrapure water with a flow rate of 0.2 mL/min for 10 min under ultrasonic-pulse sonication subjected to constant stirring (Hielscher Ultrasonic Processor- UP100H, Germany). The solution containing nanocurcumin turned yellow and was separated by centrifugation. It is then sterilized by autoclaving, freeze-dried at -80ºC followed by lyophilization (Eyela-FDU- 2000, Japan). A dry orange coloured powder of nanocurcumin is obtained. The details of characterization of the nanocurcumin are discussed earlier [24] where, the morphology of the nanoparticles is observed by Field Emission Scanning Electron Microscopy (FE-SEM, FEI INSPECT F50, The Netherlands), and characterized by Fourier transform infrared spectroscopy (FTIR) (Perkin-Elmer FTIR-1600, USA). The crystalline nature of the nanocurcumin powder is determined by X-ray diffractometer (Bruker AXS, Inc., Model D8, WI).

Total protein estimation from tissues and protein oxidation from serum

The total protein content of various tissues from different vital organs (liver, kidney and ovary) are estimated according to the method of Lowry *et al.* [25].

Protein oxidation is determined by measuring the carbonyl content of serum as described by Levine *et al.* [26]. Here, serum was first diluted with phosphate-buffered saline (10 mM sodium phosphate, pH 7.4, and 0.14 M NaCl) in the ratio 1 : 40, and centrifuged at 12,000 rpm for

10 min to remove all the particulate materials. Cold trichloroacetic acid (TCA, 20% final concentration) was added to the serum to precipitate the serum proteins by centrifugation similarly for 5 min. The precipitated protein was then dispersed to a solution containing 10 mM DNPH in 2 N HCl and kept in the dark at room temperature for 1 h with repeated vortexing in every 15 min. The protein solution was then mixed with cold TCA (final concentration 10%) and centrifuged at 12000 rpm for 5 min. After discarding the supernatants, the protein pellets were washed with 10% TCA through centrifugation similarly and 1 ml of ethanol/ethyl acetate (in the ratio of 1:1, v/v) was added to it to eliminate any free DNPH. Samples were then suspended in 6 M guanidine hydrochloride and kept at 37°C for 15 min with vortex mixing. Carbonyl contents present in the protein was estimated from the absorbance at 366 nm by using a molar absorption coefficient of 22,000 M^{-1} cm^{-1}.

ELISA of cytokines, apoptotic proteins and steroidogenic hormones

Nicotine, cucumin and nanocurcumin induced expression of different cytokines (IL-4, IL-6, TNF-α, and IFN-γ) and apoptotic regulating proteins (Bcl-2 and Bax) in the serum of rats under different groups are determined as per the protocols outlined in Quantikine Immunoassay Kits (PureGene).

PureGene ELISA Kits for Progesterone (PG-6770R) and for 17-β Estradiol (PG-5600) are used for detection of the total unconjugated form of steroidogenic hormones.

Molecular docking study

Human serum proteins, α-LA and Cyt-c are chosen for the Molecular docking studies. The docking experiments are performed by using GemDock, a program for computing a ligand conformation and orientation relative to active site of the receptor and Ucsf Chimera 1.13.1., which is an extensive molecular modeller algorithm. From protein data bank, α-LA protein (PDB ID: 1A4V) and Cyt-c protein (PDB ID: 5EXQ) are used as receptor molecules and nicotine (PubChem CID: 89594) and nanocurcumin (PubChem CID:969516) are taken as ligand molecules for docking. Human serum protein has 73% homology with the serum protein of *Rattus norvegicus* and thus adopted for molecular docking study. The chemical structure of nanocurcumin and curcumin being same, the PubChem ID of curcumin is considered for further analysis [27].

***In vitro* interaction study**

Earlier, it is observed that addition of 500 μM of nicotine completely suppressed the absorbance peaks of different cellular proteins or bio-molecules (50 μg/ml water) [8]. Therefore, the initial absorbance spectrum of nicotine hydrogen tartrate solution (500 μM) is recorded from 230 nm to 300 nm by using a UV-Visible spectrophotometer. Freshly synthesized nanocurcumin is then gradually added to the nicotine solution (final concentration of nanocurcumin to the mixture solution ranging from 10 μM to 100 μM), incubated for 15 minutes at ambient temperature and the absorbance spectrum of each addition is recorded from 230 to 300 nm. All the absorbance data of the interacting solutions are taken against its corresponding reference or control solutions.

In nicotine-treated α–LA protein vs. nano-curcumin interaction, at first absorbance spectrum of α–LA protein solutions (50 μg/ml water) is recorded from 230 nm to 500 nm. Nicotine (500 μM) is then added to the α-LA protein solution and absorbance spectrum of the mixture solution is recorded similarly from 230 nm to 500 nm. Various concentrations of nanocurcumin are added then gradually to the nicotine-treated protein solution so that the final concentration of nanocurcumin in the solution mixture ranges from 10 μM to 100 μM and the absorbance spectra are recorded similarly from 230 nm to 500 nm. Similar experiments are performed by using nicotine-treated Cyt-c protein vs. nanocurcumin.

The fluorescence spectra of α-LA (50 μg/ml water) or Cyt-c protein (50 μg/ml water) and nicotine (500 μM)-treated α-LA (50 μg/ml water) or Cyt-c protein (50 μg/ml water) are studied by intrinsic fluorescence spectroscopy. The excitation wavelengths are fixed at 255 nm

for α-LA protein and 420 nm for Cyt-c protein. The emission spectra for both are recorded from 300 to 600 nm. The spectra of nicotine (500 µM)-treated α-LA (50 µg/ml water) or Cyt-c protein (50 µg/ml water) vs. different concentrations of nanocurcumin are recorded similarly. The final concentrations of nanocurcumin are attained between 10 µM to 100 µM in the nicotine-treated protein solutions.

Statistical analysis

The experimental set up is repeated twice and the data (N = 10) are tabulated as a mean ± standard deviation (S.D.). The statistical analyses are done by one way analysis of variance (ANOVA), where * indicates $p < 0.01$ and ** indicates $p < 0.001$ of the data in comparison to nicotine treatment. Similarly, # is $p < 0.01$ and ## is $p < 0.001$ of the data compared to nicotine + curcumin treatment. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

Results

Nicotine treatment decreased the total protein contents of liver, kidney and ovary tissues (Table 1). The protein contents of those tissues are increased more significantly by supplementation of nanocurcumin ($p < 0.001$) than curcumin ($p < 0.01$). Almost 4 times more carbonyl contents are detected in the serum proteins of nicotine-treated rats. In contrast, both curcumin or nanocurcumin supplementation showed a reduction of carbonyl content in the serum protein of nicotine-treated animals. The effect of nanocurcumin against protein oxidation is more pronounced than curcumin (Table 1).

The levels of IL-4, IL-6, TNF-α and IFN-γ in the serum are increased in nicotine treated rats (Table 2), while a significant decrease of cytokine levels are observed in rats supplemented with either curcumin or nanocurcumin. The reduction is highly significant ($p < 0.001$) for nanocurcumin than that of curcumin ($p < 0.01$). The low level of apoptotic molecule Bcl-2 due to nicotine stress is increased effectively ($p < 0.01$) by curcumin and more effectively ($p < 0.001$) by nanocurcumin (Table 3). The increased concentration of Bax in the nicotine-treated serum of rats is decreased significantly ($p < 0.01$) by supplementation of curcumin and nanocurcumin both. Interestingly, nanocurcumin supplemented female rats restored effectively ($p < 0.001$) the steroidogenic hormonal levels (estrogen and progesterone) of nicotine-treated female rats (Table 4).

In-silico docking studies of nicotine vs. α-LA protein and nanocurcumin vs. α-LA protein clearly demonstrate the formation of complex structures between nicotine and α-LA protein (Fig. 1A) as well as between nanocurcumin and α-LA protein (Fig. 1B). Out of 10 ligand conformations, the best free binding energy between nanocurcumin

Table 1. Total protein content in wet tisssue (mg/g) of animals in different groups

Parameter	Groups					
	(C)	(NT)	(NTCS)	(NTNCS)	(CS)	(NCS)
Liver	23.9 ± 0.10	17.8 ± 1.0 (25.5↓)**	19.8 ± 1.8 (17.2↓)**	21.2 ± 1.6 (11.5↓)**#	24.1 ± 0.1 (1.0↑)	25.3 ± 0.1 (5.8↑)
Kidney	20.6 ± 3.2	16.2 ± 2.0 (21.5↓)**	17.9 ± 0.3 (13.2↓)**	19.1 ± 1.6 (7.3↓)*##	22.1 ± 0.5 (7.2↑)	22.7 ± 0.5 (10.2↑)
Ovary	20.9 ± 1.1	15.8 ± 1.6 (24.3↓)**	17.8 ± 1.9 (15.0↓) **	18.7 ± 1.8 (10.7↓)*#	21.1 ± 1.4 (1.0↑)	22.1 ± 1.4 (5.7↑)

The experimental setup was repeatd twice and all data were averaged over n =12 animals and given mean ± S.D. Significance levels were determined by usuing ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the nicotine treatmentl. Similarly, # implied significant ($p < 0.1$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotinxe + curcumin tratment. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control

Table 2. ELISA of cytokine molecules

Parameter	Groups					
	(C)	(NT)	(NTCS)	(NTNCS)	(CS)	(NCS)
IL-4 (pg/mL)	33.6 ± 1.1	51.9 ± 3.5 (54.5↑)**	41.7 ± 2.1 (24.1↑)*	36.5 ± 1.0 (8.6↑)$^{**\#\#}$	32.1 ± 1.2 (4.5↓)	32.8 ± 1.2 (2.4↓)
IL-6 (pg/mL)	136.8 ± 5.9	303.5 ± 3.5 (121.8↑)**	201.0 ± 1.7 (46.9↑)*	154.1 ± 7.1 (12.6↑)$^{**\#\#}$	146.4 ± 17.1 (7.0↑)	144.4 ± 17.1 (5.6↑)
TNF-α (pg/mL)	172.8 ± 6.0	403.4 ± 6.5 (133.4↑)**	217.4 ± 3.4 (25.6↑)*	188.5 ± 4.2 (9.1↑)$^{**\#\#}$	150.6 ± 5.6 (12.8↓)	156.4 ± 5.6 (9.5↓)
IFN-γ (pg/mL)	92.0 ± 6.4	136.0 ± 6.0 (47.8↑)**	110.2 ± 6.4 (19.8↑)**	97.0 ± 7.7 (5.2↑)$^{*\#\#}$	89.7 ± 10.3 (3.0↓)	90.3 ± 10.3 (1.8↓)

The experimental setup was repeatd twice and all data were averaged over n =12 animals and given mean ± S.D. Significance levels were determined by usuing ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the nicotine treatment. Similarly, # implied significant ($p < 0.1$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotine + curcumin tratment. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control

Table 3. Apoptosis regulator proteins

Parameter	Groups					
	(C)	(NT)	(NTCS)	(NTNCS)	(CS)	(NCS)
BCL-2 (ng/mL)	1.99 ± 0.01	0.56 ± 0.05 (71.8↓)**	0.93 ± 0.11 (53.3↓)**	1.42 ± 0.04 (28.6↓)$^{**\#\#}$	2.08 ± 0.20 (4.5↑)	2.14 ± 0.20 (7.5↑)
BAX (ng/mL)	1.02 ± 0.06	1.68 ± 0.21 (64.7↑)**	1.44 ± 0.02 (39.3↑)**	1.40 ± 0.02 (37.3↑)$^{**\#}$	0.98 (3.9↓)	0.94 ± 0.20 (1.1↓)

The experimental setup was repeatd twice and all data were averaged over n =12 animals and given mean ± S.D. Significance levels were determined by usuing ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the nicotine treatment. Similarly, # implied significant ($p < 0.1$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotine + curcumin tratment. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control

Table 4. ELISA of Steroidogenic hormonal levels

Parameter	Groups					
	(C)	(NT)	(NTCS)	(NTNCS)	(CS)	(NCS)
Steroidogenic hormones						
Estradiol (pg/mL)	81.1 ± 3.3	61.7 ± 2.4 (23.9↓)**	69.4 ± 2.4 (14.4↓)*	77.8 ± 4.7 (4.1↓)$^{*\#\#}$	85.0 ± 1.7 (1.0↑)	87.2 ± 1.7 (7.5↑)
Progesterone (ng/mL)	12.41 ± 0.05	8.98 ± 0.02 27.6↓)**	9.53 ± 0.09 (23.2↓)**	11.2 ± 0.22 (9.7↓)$^{*\#\#}$	12.87 ± 0.80 (5.7↑)	13.68 ± 0.80 (10.2↑)

The experimental setup was repeatd twice and all data were averaged over n =12 animals and given mean ± S.D. Significance levels were determined by usuing ANOVA, where * implied significant ($p < 0.01$) and ** implied more significant ($p < 0.001$) of the data when compared with the control. Similarly, # implied significant ($p < 0.1$) and ## implied more significant ($p < 0.001$) of the data when compared with nicotine tratment. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control

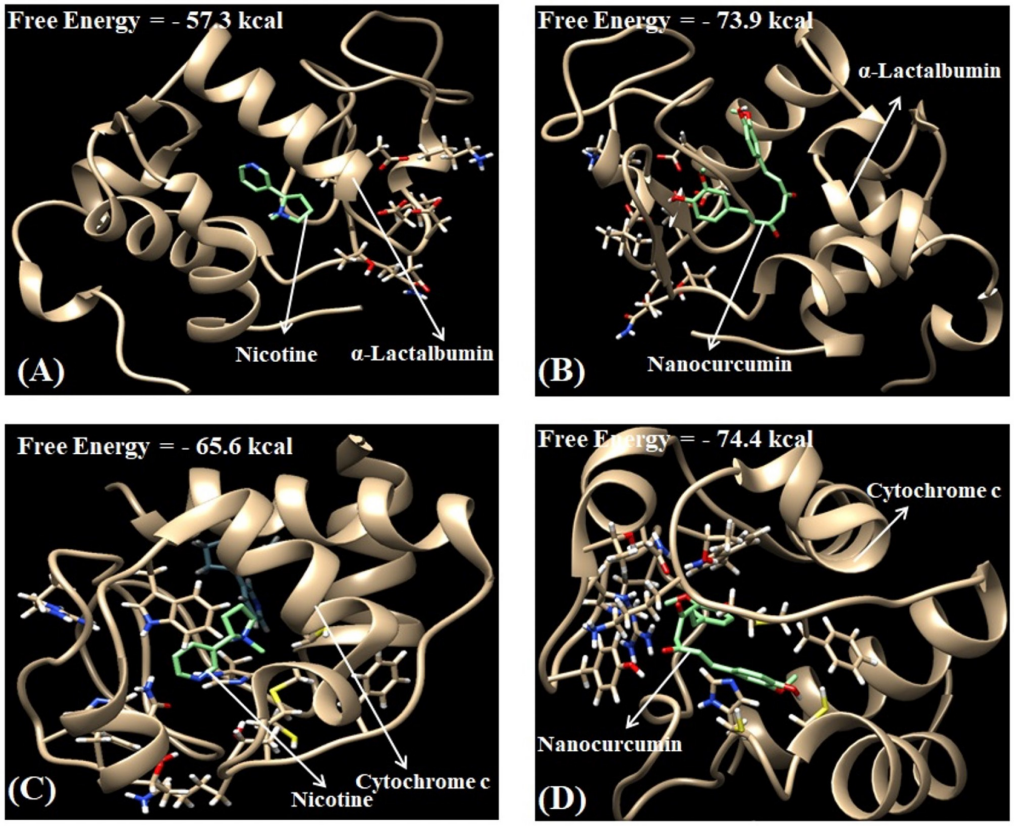

Figure 1. Molecular docking studty between nicotine/nanocurcumin vs. α-LA/Cyt-c protein. Inset A shows the docking interaction between nicotine vs. α-LA; Inset B shows the docking interaction between nanocurcumin vs. α-LA; Inset C shows the docking interaction between nicotine vs. Cys-c, and Inset D shows the docking interaction between nanocurcumin vs. Cyt-c protein

and α-LA protein is around -73.9 kcal. The free binding energy between α-LA vs. nicotine seems to be -57.3 kcal. Similar docking experiment on nicotine vs. Cyt-c and nanocurcumin vs. Cyt-c demonstrate that the probability of complex formation between nanocurcumin vs. Cyt-c is more favourable (free energy -74.4 kcal) than nicotine vs. Cytc complex formation (binding free energy - 65.6 kcal).

The UV-Visible spectral studies indicate that nanocurcumin causes significant suppression of the characteristic absorption peak (λ_{max} = 260 nm) of nicotine with the gradual addition of nanocurcumin (10 µM to 100 µM) to the nicotine solution (500 µM) (Fig. 2A). Similarly, the characteristic absorption peak of α-LA (λ_{max} = 280 nm) and Cyt-c (λ_{max} = 410 nm) proteins are suppressed substantially by nicotine (Figs. 2A and 2B). Gradual addiction of nanocurcumin reversed the quenching action of nicotine on these proteins.

The intrinsic fluorescence emission spectrum of α-LA with emission maxima at λ_{max} = 345 after excitation at 255 nm is shown in Fig. 3A. The blue shift of maxima (λ_{max} = 330 nm) of α-LA protein occurred due to the presence of nicotine. Gradual addition of nanocurcumin to the nicotine-treated protein solution, the fluorescence intensity of the treated protein is seen to increase and wavelength maxima overlap the native protein. Similar interaction is observed between nanocurcumin vs. nicotine-treated Cyt-c protein (Fig. 3B).

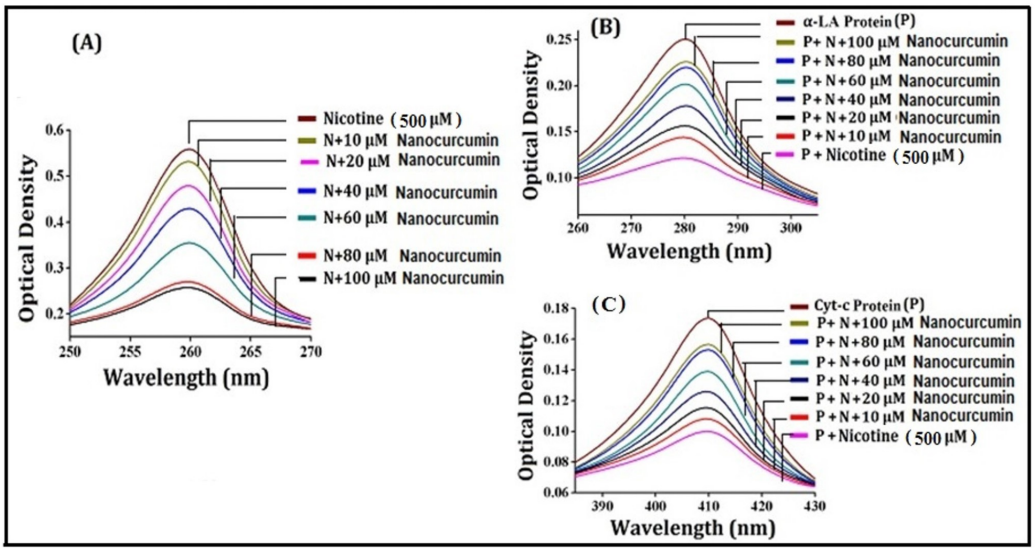

Figure 2. UV-Vis study of nicotine-nanocurcumin interaction. Inset A - Nicotine hydrogen tartrate solution (500 µM) was taken in a cuvette and its absorbance spectrum was recorded (230 nm - 300 nm) using UV-Visible spectrophotometer. Different concentration of freshly synthesized Cur-NPs was added to nicotine solution gradually (final concentration of Cur-NPs was 10 µM to 100 µM), incubated 15 min at ambient temperature and absorbance spectra of each interaction was recorded at 230 - 300 nm; Inset B shows the interaction absorption spectra of nanocurcumin vs. nicotine (500 µM) mediated pure protein of α-LA (50 µg/ml water); Inset C shows the interaction absorption spectra of nanocurcumin vs. nicotine (500 µM) mediated pure protein of Cyt-c (50 µg/ml water)

Discussion

Curcumin is a well-known potent anti-oxidative, anti-carcinogenic, and anti-inflammatory agent [22,24]. Inadequate bioavailability and adverse pharmacokinetics limit the use of curcumin against various complications [21]. Scientists working on curcumin are trying to increase its usage against several diseases through the formulation of nanocurcumin, because it is more water soluble and has higher physical stability [24].

It is known that the constituents of cigarette smoke (mainly nicotine) and the systemic inflammatory mediators enhance proteolysis and inhibit protein synthesis, leading to a loss of muscle mass. Nicotine causes oxidative damage in the tissues (e.g. liver, kidney, ovary tissues etc.) leading to several diseases. Our investigation shows that nicotine causes a decrement (20 to 25%) of tissue protein (liver, kidney and ovary), either by decreasing protein biosynthesis or increasing protein catabolism (Table 1). Depletion of tissue protein certainly declines the self-protective efficacy of the body due to the structural and functional deformity of the cells. Curcumin protects our body by regulating the action of other targets, such as apoptotic proteins, cell cycle regulators, growth factors, receptors, protein kinases, and transcription factors [9,28]. Thus, curcumin or nanocurcumin acts by blocking nicotine-induced stress in different tissues (Table 1). Increased oxidative stress by nicotine aggravates protein oxidation. It leads to increase in carbonyl contents of the tissues resulting dysfunction of biological enzymes, hormones, and immune system [7]. The anti-oxidative nature of curcumin reduces carbonyl content in the tissues by inhibiting the protein oxidation [26,29]. Our results show that nanocurcumin, being more bio-available molecule, has a better self-protective ability against protein oxidation than that of curcumin (Table 1).

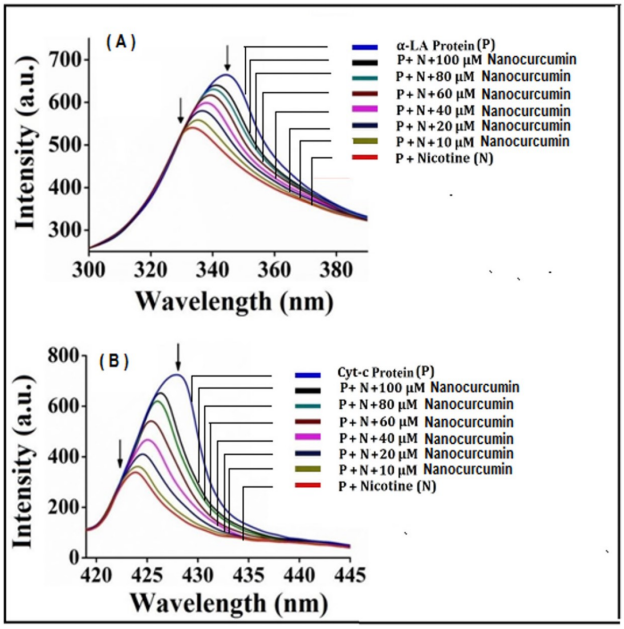

Figure 3. Fluorescence study of protein-nicotine-nanocurcumin interaction. The Fluorescence measurements of protein(s) (50 µg/ml) under different conditions of nicotine (500 µM) and nanocurcumin (10 µM to 100 µM) were studied by intrinsic fluorescence spectroscopy. The excitation wavelength was fixed at 255 nm for α-LA protein and 420 nm for Cyt-c protein respectively. The emission wavelength windows were recorded at 300-600 nm. At first nicotine was added to the protein solution and spectrum was taken similarly. Next, to the nicotine added protein solution, curcumin nanoparticles (final concentration 10 µM to 100 µM) was added gradually and absorption spectrum was recorded again

At the physiological level, IL-6 plays an important role in the cytokine network. However, elevated expression of IL-6 is related to fatigue scores. Higher magnitude of TNF-α can also be seen in fatigue condition. In particular, scientists have observed that childhood trauma and stress factors enhance the levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β, INF-γ) and chemokines (MCP-1) in either patients or controls [30]. Our results thus corroborate with the earlier reports in which it is shown that nicotine augments the secretion of pro-inflammatory cytokines and generates excess amount of ROS within the cells [12,14]. Furthermore, it is known that curcumin supplementation leads to simultaneous co-expression of p53 and TGF-β along with down-regulation of Th1 & Th2 cytokines and increases immunity of cells. Here we find that the elevated levels of cytokines under nicotine exposure, are normalized by curcumin or nanocurcumin supplementation (Table 2). The immune response of nanocurcumin is more prominent than that of curcumin in terms of different cytokine levels under nicotine stressed condition (Table 2).

Bcl-2 plays an important role by promoting the action of cellular survival and inhibiting the function of pro-apoptotic proteins. Damaged Bcl-2 gene is identified as a cause of a number of cancers and a possible cause of schizophrenia and autoimmunity [16]. Hardwick *et al.* have shown that the damaged Bcl-2 gene is a cause of resistance to cancer treatments also [17]. They have also shown that the apoptosis regulator protein Bax forms a hetero-dimmer with Bcl-2 and functions as an apoptotic activator. This study shows that there is a decrement of anti-apoptotic molecule Bcl-2 and increment of pro-apoptotic

molecule Bax in nicotine treated rats (Table 3). Nanocurcumin works more effectively to restore the normalcy of the levels of apoptotic molecules and plays a promising beneficial immuno-modulatory role against apoptosis.

Nicotine is seen to cause a significant reduction of 17 β-estradiol and progesterone levels in normal protein fed condition which in turn affects the reproductive system (Table 4). This is in accordance with our other findings [18,24]. 17 β-estradiol levels are decreased in nicotine treated group due to the anti-estrogenic effect of nicotine. Nicotine also decreases progesterone level by increasing PGF2α and VEGF-mRNA expressions. Here, curcumin is seen to reduce the action of nicotine on estrogens metabolism due to its high estradiol binding capacity. Nanocurcumin is seen to have better effect on increasing of 17β-estradiol and progesterone levels of nicotine treated animals because of its higher bioavailability and greater binding ability to nicotine.

Molecular docking experiments provide a theoretical interpretation about the interactions amongst the nicotine, nanocurcumin and cellular proteins (α-LA and Cyt-c protein). The docking results indicate that nanocurcumin (curcumin) binds to α-LA protein (free energy -73.9 kcal) to form a nanocurcumin - α-LA protein complex. The formation of nanocurcumin - α-LA protein complex is more favourable than that of nicotine bound α-LA protein (free energy -57.3 kcal) complex. Similarly, nanocurcumin bound Cyt-c protein (free energy -74.4 kcal) complex is more favourable than the nicotine bound Cyt-c protein (free energy -65.6 kcal) complex (Fig. 1). This implies that nanocurcumin resists the nicotine to further interact with the protein moiety due to its higher binding probability (as revealed from free energy calculations) and thereby it provides extra protection against nicotine. Our previous study showed that curcumin had a strong interaction with nicotine (free energy -51.29 kcal) [23]. Strong binding ability of nanocurcumin (curcumin) may help the molecule to be a potent immune-modulator against nicotinic effects.

The interpretations of Molecular docking experiments have been substantiated by the wet-lab interaction studies amongst nanocurcumin, nicotine and cellular proteins. Nicotine has a tendency to bind with the tryptophan residue of proteins [8], due to which the absorption intensities of the two important mammalian proteins (α-LA and Cyt-c) are quenched by nicotine (Fig. 2A and 2B). Nanocurcumin also shows strong interaction with nicotine. Thus, in presence of nanocurcumin, availability of free nicotine molecules for interaction with the protein is less in the reaction medium. Nicotine, therefore, produces lesser damage to the proteins in the presence of nanocurcumin.

The emission maximum wavelength of α-LA protein (345 nm) is seen to shift towards 330 nm (blue shift) due to interaction with nicotine. It indicates increase in absorption energy due to the binding of nicotine with the internal sites of the protein leading to the conformational change of the protein (Fig. 3A). With the gradual addition of nanocurcumin to the nicotine bound protein solution, the intensity, as well as the emission maximum wavelength, is significantly overlap the emission maxima of the native protein. Nanocurcumin thus steadily assists the protein to restore its native state by lowering the energy due to the H-bonding or π-π conjugation. A similar phenomenon is observed in case of Cyt-c emission spectra (Fig. 3B).

The bioavailability of nanocurcumin is greater than that of curcumin due to its higher aqueous solubility. The higher binding ability to nicotine as well as to cellular proteins, it protects the cells from nicotinic effects. The enhanced self-protective efficacies of the tissues related to protein content, protein oxidation, cytokines and apoptotic markers expression and hormonal concentrations are more pronounced in case of nanocurcumin supplementation in rats than curcumin-treated group. Nanocurcumin thus may be used as a significant protective and therapeutic agent against nicotine-induced complications particularly in nicotine intoxicated female population.

Authors contribution

Anwesha: Methodology, Data curation, Investigation. Krishna Chattopadhyhay: Concep-

tualization, Visualization, Writing- Original draft preparation. Somashree Biswas: Data curation, Investigation. Bhola Nath Paul: Reviewing and Editing. Brajadulal Chattopadhyhay: Conceptualization, Validation, Writing- Original draft preparation, Supervision

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Conflict of interest
The authors declare no conflict of interest of any kind related to this work.

References

1. **Padmavathi, P., Raghu, P.S., Padmakanthan, S., Reddy, V.D., Bulle, S., Marthadu, S.B., Maturu, P. & Varadacharyulu, N.C. (2018).** Chronic cigarette smoking-induced oxidative/ nitrosative stress in human erythrocytes and platelets. Mol. Cellu. Toxicol. 14: 27-34.
2. **Rigotti, N.A., Pipe, A.L., Benowitz, N.L., Arteaga, C., Garza, D., Tonstad, S. (2010).** Efficacy and safety of varenicline for smoking cessation in patients with cardiovascular disease. Circulation. 121: 221-229.
3. **Jensen, K., Afroze, S., Munshi, M.K., Guerrier, M., Glaser, S.S. (2012).** Mechanism for nicotine in the development and progression of gastrointestinal cancers. Transl. Gastrointest. Cancer. 1: 81-87.
4. **Roved, J., Hansson, B., Tarka, M., Hasselquist, D., Westerdahl, H. (2018).** Evidence for sexual conflict over major histocompatibility complex diversity in a wild songbird. Proc. Royal Soc. B- Biol. Sci. 285(1884): 20180841.
5. **Allen, A.M., Oncken, C., Hatsukami, D. (2014).** Women and Smoking: The Effect of Gender on the Epidemiology, Health Effects, and Cessation of Smoking. Curr. Addict. Rep. 1(1): 53-60.
6. **Huxley, R.R., Woodward, M. (2011).** Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet. 378(9799): 1297-1305.
7. **Phaniendra, A., Jestadi, D.B., Periyasamy, L. (2015).** Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Ind. J. Clin. Biochem. 30(1): 11-26.
8. **Zeng, Q., Shen, L.J., Li, S., Chen, L., Guo, X., Qian, C.N., Wu, P.H. (2016).** The effects of hemoglobin levels and their interactions with cigarette smoking on survival in nasopharyngeal carcinoma patients. Cancer Med. 5(5): 816-826.
9. **Banerjee, S., Chattopadhyay, K., Chhabra, J.K., Chattopadhyay, B.D. (2012).** Protein dependent fate of hepatic cells under nicotine induced stress and curcumin ameliorated condition. Eur. J. Pharmacol. 684: 132-145.
10. **Hoek, J.B., Pastorino, J.G. (2002).** Ethanol, oxidative stress, and cytokine-induced liver cell injury. Alcohol. 27: 63-68.
11. **Gour, N., Wills-Karp, M. (2015).** IL-4 and IL-13 Signalling in Allergic Airway Disease. Cytokine. 75(1): 68-78.
12. **Ul-Haq, Z., Naz, S., Mesaik, M.A. (2016).** Interleukin-4 receptor signalling and its binding mechanism: A therapeutic insight from inhibitors tool box. Cytokine Growth Factor Rev. 32: 3-15.
13. **Peters, M.C., McGrath, K.W., Hawkins, G.A., Hastie, A.T., Levy, B.D., Israel, E., Phillips, B.R., Mauger, D.T., Comhair, S.A., Erzurum, S.C., Johansson, M.W.,**

Jarjour, N.N., Coverstone, A.M., Castro, M., Holguin, F., Wenzel, S.E., Woodruff, P.G., Bleecker, E.R., Fahy, J.V. & National Heart, Lung, and Blood Institute Severe Asthma Research Program (2016). Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. Lancet Respir. Med. 4(7): 574-584.

14. **Schoenborn, J.R. and Wilson, C.B. (2007).** Regulation of interferon-gamma during innate and adaptive immune responses. Adv. Immunol. 96: 41-101.
15. **Strzelak, A., Ratajczak, A., Adamiec, A., Feleszko, W. (2018).** Tobacco Smoke Induces and Alters Immune Responses in the Lung Triggering Inflammation, Allergy, Asthma and Other Lung Diseases: A Mechanistic Review. Int. J. Environ. Res. Public Health. 15(5): 1033.
16. **Jalili, C., Salahshoor, M.R., Moradi, M.T., Ahookhash, M., Taghadosi, M., Sohrabi, M. (2017).** Expression Changes of Apoptotic Genes in Tissues from Mice Exposed to Nicotine. Asian Pac. J. Cancer. Prev. 18(1): 239-244.
17. **Hardwick, J.M. and Soane, L. (2013).** Multiple functions of BCL-2 family proteins. Cold Spring Harb. Perspect. Biol. 5(2): a008722.
18. **Sinha, S., Maiti, M., Chattopadhyay, K., Chattopadhyay, B.D. (2012).** Potential amelioration of curcumin against nicotine-induced toxicity of protein malnourished female rats. J. Pharmacol. Toxicol. 7: 166-180.
19. **Jandikova, H., Duskova, M., Starka, L. (2017).** The Influence of Smoking and Cessation on the Human Reproductive Hormonal Balance. Physiol. Res. 66(3): S323-S331.
20. **Tung, B.T., Hai, N.T., Son, P.K. (2017).** Hepatoprotective effect of Phytosome Curcumin against paracetamol-induced liver toxicity in mice. Brazilian J. Pharma. Sci. 53(1): e16136.
21. **Anand, P., Kunnumakkara, A.B., Newman, R.A., Aggarwal, B.B. (2007).** Bioavailability of curcumin: problems and promises. Mol. Pharm. 4(6): 807-818.
22. **Lopez-Lazaro, M. (2008).** Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. Mol. Nutrin. Food Res. 52(S1): S103-S127.
23. **Mhaske, D.B., Sreedharan, S., Mahadik, K.R. (2018).** Role of Piperine as an Effective Bioenhancer in Drug Absorption. Pharm. Anal. Acta. 9: 7.
24. **Chattopadhyay, K,, Samantam A., Mukhopadhyay, S., Chattopadhyay, B.D. (2018).** Potential amelioration of nicotine-induced toxicity by nanocurcumin. Drug. Dev. Res. 79: 119-128
25. **Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951).** Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1): 265-275.
26. **Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E. (1994).** Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233: 346-357.
27. **Bhawana., Basniwal, R.K., Buttar, H.S., Jain, V.K., Jain, N. (2011).** Curcumin Nanoparticles: Preparation, Characterization, and Antimicrobial Study. J. Agricul. Food Chem. 59(5): 2056-2061.
28. **Sershen, H., Reith, M.E.A., Banay-Schwartz, M., Lajtha, A. (1982).** Effects of prenatal administration of nicotine on amino acid pools, protein metabolism, and nicotine binding in the brain. Neurochem. Res. 7: 1515-1522.
29. **Alisi, I.O., Uzairu, A., Abechi, S.E. (2020).** Molecular design of curcumin analogues with potent antioxidant properties and thermodynamic evaluation of their mechanism of free radical scavenge. Bulletin Natn. Res. Centre. 44: 137.
30. **Wang, M., Jiang, S., Zhou, L., Yu, F., Ding, H., Li, P., Zhou, M., Wang, K. (2019).** Potential Mechanisms of Action of Curcumin for Cancer Prevention: Focus on Cellular Signaling Pathways and miRNAs. Int. J. Biol. Sci. 15(6): 1200-1214.