Evaluation of Hydroalcoholic Extract of *Excoecaria Agallocha* Bark in the Management of Drug-induced liver injury

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CERTIFICATE

This is to certify that Ankit Kumar has carried out the research on the project entitled **"Evaluation of Hydroalcoholic extract of *Excoecaria Agallocha* bark in the management of Drug-induced Liver Injuries"** under my supervision in the division of Pharmacology and Toxicology—Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.

He has incorporated his findings into this thesis, which has the same title as what she submitted in partial fulfilment of the requirement for the award of a Master of Pharmaceutical Technology degree from **Jadavpur University**. I am satisfied that she has carried out his thesis with proper care and confidence to my entire satisfaction.

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CERTIFICATE

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Signature of External

DECLARATION

I declare that **"Evaluation of Hydroalcoholic extract of *Excoecaria Agallocha* bark in the management of Drug-induced Liver injuries"** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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ABSTRACT/PREFACE

The present study entitled "**Evaluation of Hydroalcoholic Extract of *Excoecaria Agallocha* Bark in the Management of Drug-Induced Liver Injury**" covers original research work conducted by the author for the award of Master of Pharmacy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

Drug-induced liver injury is a major global cause of death and affects the therapy of antituberculosis drugs. The purpose of this work is to investigate the ethnomedical claim that the hydroalcoholic extract of *Excoecaria Agallocha* Bark can protect rats' livers from injury induced by isoniazid (INH) and rifampicin (RIF).AGL-HA extract (low dose 200 mg/kg and high dose 400 mg/kg) and silymarin (50 mg/kg) were examined in an induced liver injury model of oxidative stress in Wistar rats by administering RIF and INH (100 mg/kg each) orally for 35 days. Markers of oxidative stress and injury to the liver, such as ALT, AST, bilirubin, and ALP, were evaluated. Biomarkers of antioxidant status, such as catalase, GSH, and superoxide dismutase (SOD), as well as lipid peroxidation markers, malondialdehyde (MDA), have been evaluated using standard protocols. The haematological profile, serum indicators for liver function, and histological investigation were all evaluated.The haematological indices were significantly reduced and the biochemical enzyme markers (AST, ALT, bilirubin, and ALP) were elevated after intoxication with RIF and INH; antioxidant biomarkers were significantly depressed and MDA was elevated; however, pre-treatment with AGL-HA significantly alleviated this alteration and sustained the antioxidant potential. The histopathological morphology supports the biochemical evidence for hepatoprotection.The current investigation suggests that AGL-HA has potential antioxidant activity, and hepatoprotective effects, and might be used to treat RIF-INH-induced liver injury in laboratory rats.

Ankit Kumar

CONTENTS

INTRODUCTION

1. Introduction

Tuberculosis (TB), one of mankind's most ancient communicable diseases, is one of the top ten causes of death globally. This infectious disease is caused by the bacterium Mycobacterium tuberculosis. It mostly affects the lungs (pulmonary tuberculosis) but can also affect other organs. Pulmonary tuberculosis is an airborne illness. Chest X-rays, sputum, and other testing can help identify tuberculosis. (Thakur, G., et al.2021) The Global TB Report 20221 estimates 10.6 million TB incident cases in 2021. The World Health Organisation's (WHO) END TB plan seeks to eliminate 95 percent of TB fatalities and 90 percent of new TB incidents by 2035. Geographically, among the six high-burden nations in Southeast Asia, India accounts for 28% of the worldwide TB burden. According to the National TB Prevalence Survey, the crude prevalence of TBI among people over the age of 15 in 2021 was 31.3 percent (95% confidence interval (CI), 30.8–31.9). According to reports, 5–10% of TBI patients acquire clinically active tuberculosis. (Chauhan, A., et al 2023)

TB affects all nations and age categories, however adults accounted for the vast majority (90%) of infections in 2016. Eight developing nations accounted for nearly two-thirds of the cases, with India providing 27% of the 10.4 million total. In 2017, only 64% of the global estimated incident cases of tuberculosis were documented; the remaining 36% were undiagnosed, untreated, or unreported. These "missing TB cases" have received a lot of attention for the issues they pose in implementing the End TB Strategy. Many persons with tuberculosis (or TB symptoms) do not have access to an accurate initial diagnosis. In many countries, tuberculosis (TB) is still diagnosed by sputum microscopy, which has known limitations.(Thakur, G., et al.2021)

TB disease can be cured by taking an appropriate course of anti-TB medications. Anti-TB medications are classified into two categories: first-line drugs (isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin) and second-line therapies (para-aminosalicylic acid (PAS), ethionamide, cycloserine, amikacin, and capreomycin). The current recommended treatment plan for pulmonary tuberculosis consists of two months of co-administration of four medications (isoniazid, rifampicin, ethambutol, and pyrazinamide), followed by a four-month maintenance phase needing just isoniazid and rifampicin (WHO, 2019). When this treatment regimen is completely followed, it has a cure rate of 85%. Nonetheless, according to the most recent treatment result statistics, a considerable proportion (15%) of tuberculosis infections are still being treated unsuccessfully. (Sankar, M., et al.2015) Isoniazid (INH) and rifampicin (RIF) have been routinely used to treat tuberculosis (TB) since 1968; they kill more than 99% of tubercular bacilli within two months of starting therapy (Iseman and Madsen 1989). They are regarded as the most essential first-line antitubercular medications; when used in combination, the period of therapy for TB is reduced from 18 months to 6 months (Somoskovi et al., 2001). Although both medications are highly effective against tuberculosis, they are both known to be hepatotoxic. (Saad, E. I., et al.2010) These medications, however, cause drug-induced liver damage. The biggest incidence of DILI occurs during the first stage of TB therapy. Overall, hepatotoxicity caused by anti-TB medicines has been observed in 5% to 28% of those treated. However, it's unclear how many of these cases fall within the more current worldwide consensus definition of drug-induced liver damage (DILI). In most reports, hepatotoxicity has been diagnosed by measuring elevated levels of alanine (ALT) or aspartate transaminase (AST) by three times the upper limit of normal range (ULN) when symptoms (such as

abdominal pain, nausea, vomiting, unexplained fatigue, or jaundice) are present, or by five times the ULN of ALT or AST when symptoms are absent.(Ramappa, V., &Aithal, G. P. 2013).

1.1.Drug metabolism in the liver

Many medicines are metabolised and eliminated mostly through the liver. Although some medications cause hepatotoxicity by directly targeting particular organelles, such as mitochondria or nuclei, the majority of hazardous drugs need metabolism to generate toxic metabolites. The liver undergoes three steps of drug metabolism. In phase I, medicines are metabolised by cytochrome P-450 enzymes. This process can produce hazardous electrophilic compounds and free radicals. During phase II, the parent drug or metabolites are conjugated with glutathione (GSH), sulphate, or glucuronide to form water-soluble molecules. As a result, the body might eliminate these chemicals in bile or urine. Excretory transporters in the hepatocyte, canalicular, and sinusoidal membranes primarily control the elimination pathway (phase III). Individual differences in phase I, II, and III drug metabolism can be explained by genetic polymorphisms or environmental factors, such as concurrent use of drugs and alcohol. These factors may also influence an individual's susceptibility to idiosyncratic drug-induced liver injury by affecting hepatic exposure to hazardous metabolites. (Gunawan, B. K., &Kaplowitz, N. (2007))

1.2.Biochemical events of INH+RIF leading DILI

1.2.1. Isoniazid

INH is metabolised and eliminated mostly in the liver. The risk of hepatotoxicity is determined by two major enzymes in the metabolic pathway: N-acetyltransferase 2 (NAT2) and microsomal enzyme cytochrome P4502E1. NAT2 is responsible for the conversion of isoniazid to acetyl isoniazid, which is then hydrolysed to acetyl hydrazine. Later, it may be oxidised by CYP2E1 to make N-hydroxy-acetyl hydrazine, which then dehydrates to produce acetyl diazine. Acetyl diazine may be the hazardous metabolite, or it may degrade to reactive acetyl onium ion, acetyl radical, and ketene, all of which can bind covalently to hepatic macromolecules and cause liver damage. The enzyme NAT2 is also in charge of further acetylating acetyl hydrazine to non-toxic diacetyl hydrazine. As a result, delayed acetylation causes the buildup of both the parent molecule and mono-acetyl hydrazine. INH further inhibits acetyl hydrazine acetylation. Furthermore, direct hydrolysis of INH without acetylation yields hydrazine, which might cause liver damage.(Scales, M. D. G., &Timbrell, J. A. (1982))This minor pathway increases INH metabolism tenfold in slow acetylators, particularly when combined with rifampicin. (Sarma, G. R., et al. 1986)

1.2.2. Rifampicin

Rifampicin is effectively absorbed from the stomach and metabolised in the liver to desacetyl rifampicin, while a distinct hydrolysis process creates 3-formyl rifampicin. Desacetyl rifampicin is more polar than the parent molecule and has microbiological activity. The metabolite is responsible for the bulk of the bile antibacterial action. (Nakajima, A et al.2011) Rifampicin is nearly equally eliminated in both bile and urine. These metabolites are not harmful. Rifampicin is linked with a DILI hepatocellular pattern and frequently enhances the

hepatotoxicity of other anti-TB medicines (Huang YS, et al. 2003). The xeno-sensing pregnane X receptor (PXR) belongs to the nuclear receptor superfamily of ligand-dependent transcription factors and may be activated by a range of medicines, including rifampicin. Activated PXR interacts to response sites in promoters, increasing the transcription of phase I and II drug-metabolising enzymes such as cytochrome P450 (CYP), glutathione S-transferases (GSTs), and transporters (phase III). Rifampicin stimulates various metabolic enzyme pathways, including the cytochrome P450 (CYP3A4) system, via the hepatocyte PXR (Burk, O., et al. 2004). This activation of CYP3A4 causes enhanced metabolism of isoniazid, resulting in toxic metabolites, which explains rifampicin's potentiating impact on anti-TB drug-induced hepatotoxicity. Rifampicin also activates isoniazid hydrolases, resulting in increased hydrazine synthesis, particularly in slow acetylators, which increases toxicity when combined with isoniazid. (Ramappa, V., &Aithal, G. P. (2013))

Toxic compounds from drug metabolism can then directly disrupt the biochemistry of liver cells, resulting in cell death or an immune-mediated assault on the liver. Drug metabolites can bind to proteins, lipids, and DNA, causing cell death through metabolic processes such as oxidative stress, GSH depletion, redox alterations, and lipid peroxidation. These events may have a direct impact on the mitochondria, endoplasmic reticulum, microtubules, cytoskeleton, and nucleus, resulting in a significant direct insult. On the other hand, these occurrences might cause signalling kinases, transcription factors, and gene expression proteins to be activated or inhibited. This could make hepatocytes or cholangiocytes more vulnerable to the harmful effects of the innate immune system, like cytokines and chemokines, which are triggered by the initial liver injury. (Gunawan, B. K., &Kaplowitz, N. (2007))

1.3.The Role of Oxidative Stress in Liver Regeneration

There is evidence that the balance between damage and regeneration determines the prognosis of an acute liver injury, particularly following exposure to hepatotoxic substances. In general, oxidative stress causes harm to the liver during damage. However, fresh data suggests that Regeneration may be stimulated by oxidative stress. A redox-responsive nuclear transcription factor called nuclear factor (erythroid-derived 2)-like 2 (NFE2L2/Nrf2) binds to DNA sequences called antioxidant response elements (AREs) upstream of antioxidant genes, therefore triggering the production of those genes. The cysteine-rich protein Kelch-like ECH-associated protein 1 (Keap1) generally prevents Nrf2 from becoming activated in the cytosol. When oxidative stress occurs, two key cysteine residues in Keap1 oxidise, leading it to dissociate from Nrf2. Nrf2 can then get to the nucleus. It was discovered that Nrf2-deficient animals exhibited slower liver regeneration following partial hepatectomy. It was determined that the loss of Nrf2 and the subsequent loss of antioxidant response gene activation resulted in increased oxidative stress in these animals, which led to a decrease in insulin and insulin-like growth factor 1 signalling. While this shows that high oxidative stress hinders regeneration, keep in mind that this study was conducted using Nrf2 mutant mice. More recent research has demonstrated that in normal wild-type mice, the gene for augmentation of liver regeneration (ALR) is downstream of an ARE, and that activating Nrf2 results in enhanced expression of ALR, which can improve liver regeneration. Thus, it appears that oxidative stress is vital in regulating the balance of damage and healing pathways.

Pharmacological Nrf2 activators may be employed in the treatment of ALF in the future. (McGill, M. R., &Jaeschke, H. (2015))

1.4.Diagnosis

Currently, a thorough clinical history, a systematic drug exposure assessment, the exclusion of prevalent causes of hepatic injury based on the clinical context and the pattern of abnormalities in the liver from laboratory tests, and the application of clinical assessment scales to evaluate the reaction's likelihood are all used to diagnose DILI. DILI patients exhibit a wide variety of unexplained symptoms, including tiredness, nausea, stomach discomfort, pruritus, and jaundice, which are prevalent in other liver illnessesSeveral threshold criteria for diagnosing a case of DILI have been set by the European Association for the Study of the Liver (EASL) and an international consensus committee: Serum ALT or AST levels greater than five times the upper limit of normal (ULN) in the absence of symptoms, serum ALP levels greater than two times the ULN, or a combination of AST or ALT levels greater than three times the ULN and a concurrent elevation of total bilirubin (TBL) levels greater than two times the ULN in the presence of symptoms (abdominal pain, nausea, vomiting, unexplained fatigue, or jaundice) suggest liver injury.(Verma, S., &Kaplowitz, N. (2009)). Because liver biopsy is not often performed for DILI diagnosis, liver biochemistry is utilised to determine the kind of liver injury. The R-ratio of blood indicators of liver damage (ALT/ALP) during DILI presentation can be used to distinguish between the various laboratory patterns. Hepatocellular is defined as ALT > 5 × ULN alone or R-ratio ≥5, whereas cholestatic is defined as ALP ≥ 2 × ULN alone or R-ratio ≤2. Mixed liver damage is defined as ALT ≥ 3 × ULN, ALP ≥ 2 × ULN, and an R-ratio of 2-5. Hepatocellular injury is marked by an increase in serum transaminases due to hepatocyte damage caused by the toxin, and it is more likely to be linked with a poor result. (Villanueva-Paz, M et al.2021)

1.5.Management of DILI

Various free radicals can be reduced by N-acetylcysteine (NAC), and its clinical efficacy is enhanced by its early use. To prevent certain severe adverse reactions, it is recommended that NAC be administered to adult patients at a dosage of 50–150 mg/kg per day for a minimum of three days. The rate of intravenous infusion should be rigorously controlled. Currently, NAC is recommended for the treatment of early-stage ALF in patients in China through integrated therapy. Future studies are necessary to figure out whether NAC is therapeutically effective for individuals with moderate or severe DILI. In order to treat DILI brought on by APAP in the US, the FDA only authorised NAC as an antidote in 2004. NAC is advised for use exclusively in patients with ALF associated with non-APAP medications, with the exception of APAP-induced DILI. A prospective controlled trial by an American ALF research group over eight years in the twenty-fourth medical centres showed that NAC may improve the survival rate of individuals in the early stages of DILI-related ALF who have not had a liver transplant. ALF produced by non-APAP drugs was present in 173 of the study's subjects. In 2011, recommendations for ALF were established by the American Association for the Study of Liver Diseases (AASLD). According to these recommendations, NAC should be used to treat ALF brought on by medicines and toadstools. In 2014, the ACG released guidelines for the clinical diagnosis and management of IDILI. According to this

recommendation, individuals with early ALF should be treated with NAC. Nevertheless, a randomised, controlled treatment trial in children with ALF caused by non-APAP agents did not provide evidence for a therapeutic role for NAC. Consequently, NAC is not advised for the treatment of ALF in children, particularly those under the age of two that is caused by non-APAP agents.There are currently no randomised controlled trials on the therapeutic impact of glucocorticoids on DILI, even though they are occasionally used to treat very severe DILI. Given the numerous adverse effects of glucocorticoids, they should be taken with care. In theory and in practice, glucocorticoids may be given to people who clearly demonstrated hypersensitivity or autoimmunity but whose biochemical indicators did not significantly improve—in fact, they may have become worse—after stopping hepatoxic drugs. Based on results from randomised controlled studies, the Chinese Food and Drug Administration (CFDA) has authorised magnesium isoglycyrrhizinate to treat acute DILI, including acute hepatocellular damage and mixed liver injury with considerably high serum ALT levels. (Verma, S., &Kaplowitz, N. (2009)).

Bicyclol and glycyrrhizic acid might be considered empirically for the treatment of mild to moderate drug-induced hepatocellular damage or mixed liver damage; silymarin could be considered to treat individuals with mild liver inflammation. Therapeutic effects of ursodeoxycholic acid (UDCA) and S-adenosyl methionine (SAMe) are claimed to be present in individuals with cholestatic DILI. Prospective, randomised, and controlled trials will help to confirm the definite therapeutic benefits of these medications. (Yu, Y. C., ET al.2017)In recent years, natural plant-based medicines have shown promising therapeutic effects against drug-induced liver injury for example: garlic, Gallic acid, Resveratrol, etc. However, natural herbs can be the ultimate solution with fewer side effects for DILI.

1.6.Natural phytochemicals

Phytochemicals extracted from plants are a valuable natural source of inspiration for medicine production. These compounds, which are designed for specialised use, can serve as a natural template for the development of new drugs. A minimum of 12,000 such compounds have been found, representing less than 10% of the total. The chemical variety of these compounds has the potential to serve as the foundation for future medications. Over the last 25 years, there has been a revived interest in phytochemicals and their potential as a drug discovery platform (Newman et al., 2007). Approximately 60% of all medicines used in clinical settings worldwide are derived from natural resources, with medicinal plants accounting for 25% of all drugs (GuribFakim, 2011). More than 28% of newly announced chemical entities in the market have their origins in natural substances. According to plant research, there are between 250,000 and 350,000 plant species found, with around 35,000 being utilised medicinally globally (Kong et al., 2003). According to the World Health Organisation (WHO), 65% of the world's population and 80% of developing countries utilise traditional plant-based medicines. This highlights the relevance of plant-based medicinal systems (Cragg& Newman, 2013). People in rural regions can access primary healthcare through medicinal plants. According to a 2004 poll, 40 to 50 percent of people in wealthier countries utilise traditional medicines, whereas 80% of people in third-world countries rely

on them for their basic healthcare needs. These traditional remedies are mostly plant-based therapies (Oyebode, 2016).

Literature Review

2. Literature Review

2.1. **[Alongi, D.D. 2001]** Plants produce a wide range of secondary metabolites, which can perform several biological functions and serve as useful sources of medicinal or pharmaceutical products. Plants have been used medicinally throughout history, and many modern drugs are derived from them. Morphine, the first pharmaceutically active molecule extracted from the plant Papaversomniferum, was discovered in 1805. The discovery of morphine heralded a new age in which plant-derived drugs were refined, studied, and delivered in precise doses. Recently, interest in alternative medicines and the therapeutic use of natural commodities, particularly plant-derived pharmaceuticals, has grown, with plants accounting for around 25% of all prescription prescriptions globally.

2.2. **[Kaliamurthi, S., & G. Selvaraj (2016)]** E. agallocha, the milky mangrove, may be found throughout India's coastline. The leaf, stem, latex, and root extracts include around 50 volatile compounds, 15 terpenoids, and 20 polyphenols. An ethanolic leaf extract provided a wide range of compounds. E. agallocha has high amounts of terpenoids and polyphenols, which have been linked to antibacterial, anticancer, and anti-diabetic activities, as well as the treatment of endemic, epidemic, and endocrine diseases.

2.3. **(Mondal et al., 2016)** E. agallocha contains compounds including sterols, phenolic acids, tannins, triterpenoids, diterpenoids, and flavonoids. E. agallocha's roots, bark, wood, leaves, and stems mostly include diterpenoids. Labdane, artisane, beyerane, daphnane, tigliane, isopimarane, or kaurane may all be used to categorise diterpenoids (Li et al., 2009; Mondal et al., 2016). Among the most commonly isolated diterpenoids are agallochaexerins, agallochaols, agallochains, excoeagallochaols, and excoecarins. Reports indicate for triterpenoids, phenolic acids, flavonoids, sterols, and tannins in E. agallocha leaves and stems. Triterpenoids fall into oleanane, taraxerane, friedelane, cycloartane, and lupane forms.

2.4. **Sofia & Teresa, 2016** Research has indicated that when evaluated using reputable assays, the bark **(Subhan et al., 2008)** and leaf (**Laith et al., 2016**) Antioxidant properties of *E. agallocha* extracts are notable. Based on the scavenging of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH) in diverse plant components extracted using different solvents.

2.5. **Thirumurugan** et al. investigated the antidiabetic properties of E. agallocha leaves in alloxan-induced diabetic rats. The findings revealed that an ethanolic leaf extract at a dose of 500 mg/kg had a significant hypoglycemic impact in both normal and alloxan-induced diabetic mice. . [Thirumurugan G, 2010].

2.6. **(Konoshima et al. 2001)** According to research, when examined using recognised assays, the bark (Subhan et al., 2008) and leaf (Laith et al., 2016) extracts of E. agallocha demonstrate significant antioxidant activity. Based on 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging in diverse plant components extracted using different solvents (Sofia and Teresa, 2016),Latex has traditionally been used to poison fish in Southeast Asian countries. In Thailand, its bark and wood are used in traditional medicine to treat flatulence (Karalai et al., 1994). The plant has long been utilised in South China's coastal areas to treat ulcers, rheumatism, leprosy, and paralysis (Li et al. 2012). In

Okinawa, Japan, resinous E. agallocha wood has been utilised to replace agarwood (jinko) as an incense.

2.7. **(Nagababu and Rao, 2017)** Recent investigations have revealed antibacterial activity in E. agallocha bark extract (500 µg/disc) (Rajeswari K and Rao TB, 2015) and silver nanoparticles from E. agallocha fruit extract.

2.8. **(Babuselvam et al., 2012)** Diterpenoids, like agallochaols, found in E. agallocha stems and twigs have been proven to have anti-inflammatory activities (Li et al., 2010). Preventing NF-kB and AP-1 activation successfully reduced TNF-α and IL-6 produced by lipopolysaccharide (LPS) in murine macrophage RAW 264.7 cells. In another study, E. agallocha was tested for anti-inflammatory and analgesic activities. As a result, the primary purpose of this research is to investigate the scientific foundation for therapeutic use and then evaluate the biological potential of these plants.

2.9. **Anwer, T., et al. 2023** studied that Hepatocellular injury occurs when isoniazid and rifampin are used to treat TB. Isoniazid and rifampin (100 mg/kg) were used to induce hepatotoxicity, with diosmin serving as a control. The study evaluated liver function markers (ALT, AST, ALP, and bilirubin), inflammatory cytokines (TNFα, IL-6, and IL-1β), apoptosis (caspase-3), oxidative stress indicators (LPO, GSH, CAT, and SOD), and histological alterations in the liver in normal, hepatotoxic control, and treatment groups. Isoniazid and rifampin treatment led to elevated levels of liver dysfunction indicators (ALT, AST, ALP, and bilirubin), cytokines (TNFα, IL-6, and IL-1β), and apoptosis (caspase-3). However, daily diosmin administration drastically lowered these indicators of liver malfunction, inflammatory cytokines, and apoptosis to near-normal levels. Furthermore, hepatic oxidative stress indicators were considerably changed, as seen by increased LPO levels and reduced endogenous antioxidants such as GSH, SOD, and CAT in the isoniazid- and rifampin-treated hepatotoxic group. It was discovered that diosmin therapy lowered high levels of LPO and significantly improved antioxidant levels. Histological examinations of the liver confirmed our biochemical findings, which showed that diosmin therapy protected against hepatocellular deterioration and inflammation. The current investigation found that diosmin can protect the liver from isoniazid and rifampin-induced hepatotoxicity. Thus, this article indicates that diosmin can be used in conjunction with anti-tubercular medications (isoniazid and rifampin) in TB patients to mitigate their hepatotoxic side effects.

2.10. **Jahan I.A et al 2014** studied that Theethanolic extract of Excoecaria agallocha stem bark was tested for antioxidant activities and bioactive polyphenol quantitation using HPLC-DAD. The ABTS test, total antioxidant capacity, reducing power, and total phenolic and flavonoid contents were utilised to assess antioxidant activity. In the ABTS experiment, the extract demonstrated 74.11% inhibition at 10 µg/ml and an IC50 of 4.80 µg/ml, which was much higher than ascorbic acid (12.20 µg/ml). The antioxidant capacity was determined as 686 mg of ascorbic acid per gramme of extract. The reducing power assay yielded a maximal absorbance of 0.73 at 250 µg/mL. The overall phenolic and flavonoid levels were shown to be considerable (375.46 mg/g gallic acid and 22.44 mg/g quercetin equivalent, respectively). In the HPLC experiment, catechin hydrate had the highest concentration (713.91 mg/100 g dry extract). The results indicate that Excoecaria

agallocha stem bark extract has antioxidant properties due to its high catechin hydrate content.

2.11. **Babuselvam M et al. 2012** showed that the anti-inflammatory and analgesic activity of ethanol with water in the ratio of 3:1 extracts was obtained from different parts of Excoecaria agallocha, viz., leaves, seeds, and latex. The latex was sequentially soxhlated with petroleum ether and methanol-dried latex for anti-inflammatory processes and analgesic activity in two concentrations (250 mg/kg and 500 mg/kg). Preliminary phytochemical analysis showed that the maximum presence of alkaloids, flavonoids, and saponins was found in the seed extract. Acute inflammatory studies showed that latex, leaf, and seed extracts of both concentrations of the chosen plant produced significant inhibition of carrageenin-induced rat paw oedema at the 3rd hour ($p<0.005$) as compared to the control, causing 63.15%, 62.15%, and 69.69%, respectively. In addition, the seed extract at a concentration of 500 mg/kg showed maximum inhibition at 57.03% as compared to the control in the cotton pellet-induced granuloma test. The analgesic activity of seed extract at a dose of 500 mg/kg. Caused significant inhibitions in the acetic acid-induced writhing. Moreover, in the tail immersion model, seed extract was concentrated at a concentration of 500 mg/kg. Possess maximum activity (80.29%) as compared to control.

2.12. **Hossain, S. J., et al. 2009** studied that Hexane (Hex), chloroform (Chl), ethyl acetate (EtA), ethanol (Eth), and water (DW) fractions have been extracted from the dried powder of the bark of Excoecaria agallocha L. (Euphorbiaceae) to evaluate their polyphenol content and anti-oxidative and anti-histamine releasing properties. DW has the greatest total polyphenol content (348 mg GAE/g), followed by Eth (160.5 mg GAE/g). DW and Eth displayed higher anti-oxidative and anti-histamine-release properties than other fractions, as determined by DPPH free radical scavenging, reducing power, total antioxidant activity, and ionophore A23187-induced histamine-release tests. TLC indicated that the Eth and Chl fractions include ellagic acid-like and lupeol-like chemicals, respectively. At 100 µg/mL testing standards, lupeol exhibited a little inhibitory impact (24.5%) on histamine release, but ellagic acid had no effect at all. However, our findings indicated that the bark of E. agallocha, particularly the DW and Eth fractions, might be a potential source of anti-oxidative and anti-histamine natural compounds.

2.13. **(Nwidu, L. L., &Oboma, Y. I. (2019)** T. occidentalis pulp extract (TOPE) (125-500 mg/kg) and silymarin (50 mg/kg) were tested in an induced hepatotoxicity model of oxidative stress in Wistar rats by administering RIF and INH (100 mg/kg each) orally for 60 days. Silymarin (50 mg/kg BW) was utilised as the conventional therapy for INH+RIF-induced liver damage. Both the plant extract and the STD medications reduced the AST, ALT, and ALP. TOPE showed preventive effect against drug-induced hepatitis by increasing endogenous antioxidants and free radical scavenging capacity.

Aim, objective, and rationale

3.1. Aim

To evaluate the Pharmacological activity of *Excoecaria agallocha* (AGL) plant extract for the management of Drug-Induced Liver Injuries.

3.2. Objective

- To assess the hepatoprotective effect of excoecariaagallocha L. in the experimental model**.**
- To observe the histopathological changes in different groups of rats.
- To evaluate the anti-oxidant effect of excoecariaagallocha L. (AGL).
- To evaluate the therapeutic effect on the liver by measuring such biomarkers like – Aspartate aminotransferase (AST), Alanine transaminase (ALT), and total bilirubin

3.3. Rationale

INH and RIF are essential first-line drugs on the WHO's list of essential medicines for TB, but they show liver injury as an adverse effect following a few weeks' dose regimen. During TB treatment, if the ALT or AST level crosses more than 5 times the ULN without symptoms and the ALT or AST level crosses more than 3 times the ULN with symptoms, Then anti-TB drugs will be stopped until the liver biomarkers reduce to normal values, which affects the treatment of TB patients. And there are no specific approved drugs for the management of DILI. Natural herbs can be the ultimate solution with fewer side effects for DILI. Scientific literature suggests that AGL shows antioxidant and anti-inflammatory action. So AGL may be an alternative medicine for managing DILI by improving oxidative and inflammatory markers.

Plant profile

Plant profile

4.1. Scientific name:*Excoecaria agallocha L*

Fig1: *Excoecaria agallocha*Plant

4.2. Taxonomical distribution

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Magnoliopsida
- Order: Malpighiales
- Family: Euphorbiaceae
- Genus: Excoecaria
- Species: E. agallocha

4.3. Synonyms

ExcoecariaaffinisEndl.,ExcoecariacamettiaWilld.

4.4. Common names:

a. Sanskrit- Agaru, gangwa, gaourai
b. Hindi- Gangiva, tejbala.
c. Bengali- Gewa,
d. English- Milky mangrove, blind-your-eye mangrove, river poison tree
e. Malayalam- Komatti, Kammetti, Kannampotti

4.5. Geographical Distribution

E. agallocha L. (AGL) thrives in mangrove forests in Asia, Australia, and the Southern Pacific. Information is provided in **Table 1.**

Table 1: Geographical distribution of Excoecaria agallocha L.

Sl no	Specific Region	Countries and subarea
1.	Asia-temperate	China - Guangdong, Guangxi, Hainan; Japan - Ryukyu Islands; Taiwan
2.	Asia-tropical Indian Subcontinent	India - Andhra Pradesh, Goa, Karnataka, Kerala, Maharashtra, Orissa, Pondicherry, Tamil Nadu, West Bengal, Andaman and Nicobar; Sri Lanka Bangladesh
3.	South Eastern Asia	Cambodia; Myanmar; Thailand; Vietnam; Indonesia; Malaysia; Papua New Guinea; Philippines

4.	Australia	Australia, New South Wales, Northern Territory, Queensland, Western Australia
5.	Pacific South	Fiji; New Caledonia; Niue; Tonga

4.6.Botanical Description

E. agallocha L. is a mangrove plant that belongs to the Euphorbiaceae family. This little tree can grow up to 15 meters tall. The **Table 2** provides a detailed description of the botanical features.

Table 2:Botanical features of Excoecaria agallocha L.

Sl no	Plant part	Morphological description
i.	Flowers	Flowers: unisexual; male flowers on catkin spikes; fragrant; yellow; 2-3 mm in diameter; 3 stamens; filaments free The female flowers are in an axillary raceme, having a pedicellate shape, pale green colour, three lobes on the calyx, three cells on the ovary, and a trifid style.
ii.	Fruits and roots	Fruits: 3 lobed, 8 mm in diameter. Around 4 mm length seeds. About 1 mm length radicle
iii.	Stem	Bark exudate rapid and copious, sometimes deciduous
iv.	Leaves	The leaves are alternating, ovate-elliptic or orbicular, with a short acuminate apex, narrowing base, and entire or sinuate-crenate margins. They measure 3-8 cm×1.5-3 cm and are glabrous and petiolate.
v.	Branches	Lenticellate, glabrous
vi.	Roots	Superterranean bands create elbow-shaped pegs rather than pneumatophores as a result of horizontal roots spreading and mingling with one another.

4.7.Ethnobotanical Information

People are well aware of the plant's economic, environmental, and medicinal benefits. In addition to its traditional usage as an emetic and purgative, it is used to treat ulcers, lesions, and stings caused by harmful aquatic creatures. The bark oil of E. agallocha is also used to treat paralysis, leprosy, and arthritis. It has also been used to treat dermatitis, haemorrhagia, and conjunctivitis. This plant produces latex, which has been used as a purgative, an abortion inducer, and to treat rheumatism, paralysis, ulcers, and leprosy. Malaysians, Indians, and New Caledonians utilise the plant's leaves and latex as fish poison. In Sri Lanka, leprosy is healed with smoke from burning wood, and swellings of the hands and feet are treated with a ginger-mashed root. A milky sap or latex exuded by the plant can cause skin blisters and

irritation, as well as temporary blindness if it enters the eye. This highlights the plant's lethal characteristics. Because of its lethal characteristics, latex is utilised as a dart and a fish poison. A soft reddish substance termed "Tejbala," taken from the lower trunk of E. agallocha, is claimed to have been used as a putative "aphrodisiac tonic" in Indian "materiamedica." The leaves are used by Burmese people to cure epilepsy. In the Solomon Islands, coconut milk is blended with the plant's latex to produce a powerful emetic and purgative. The Malays use tree oil to treat skin diseases and inflammation. The plant's roots are used in embrocations and to treat swellings and toothaches.

4.8.Chemical constituents

The chemical constituents of E. agallocha L., include mainly diterpenoids, terpenoids, flavonoids, alkaloids, tannins, and some other compounds. The details of their names are given in Table 3.

Table 3: Compound presence in E. agallocha

Sl no	Types of compounds	Compound names	Presence in *Excoecaria agallocha*
1	Flavonoid	Quercetin	Present in leaves; known for its antioxidant properties.
		Kaempferol	Found in leaves; contributes to antioxidant and anti-inflammatory effects.
2	Phenolic Compound	Tannic Acid	Found in bark; contributes to astringency and defensive properties.
		Syringic Acid	Detected in bark; known for antioxidant properties.
		Gallic Acid	Found in bark; contributes to antioxidant and antimicrobial properties.
3	Terpenoid	Lupeol	Found in leaves and bark; has potential anti-inflammatory and antimicrobial effects.
		Betulin	
		Taraxerol	
4	Steroid	β-Sitosterol	Present in leaves; has potential anti-inflammatory and cholesterol-lowering effects.
		β–sitostenone	
5	Unique to Excoecaria	Excoecarins	Specific to the genus; may have defensive and toxicological effects
		Excoagallochaol A-D	
		Agallochaol	

Collection, identification, Phytochemical screening

5.1.Collection of the plant materials

The whole plant was collected in November 2023 from the Coastal region of Sundarban, Kakdwip, South 24 Parganas, 743347, West Bengal. The plant material was identified by taxonomist R. K. Gupta, Scientist-E & Head of Office, Central National Herbarium, Botanical Survey of India (BSI), Howrah-711103, and it has been identified as Excoecaria agallocha L belonging to the family Euphorbiaceae. A voucher specimen (JU/AB-01) of the authenticated Excoecaria agallocha L was deposited on December 21, 2023, in the herbarium of BSI.

5.2.Processing of *E. agallocha*

The bark of Excoecaria agallocha L. (AGL) has been rinsed with tap water to remove dirt and other impurities. The bark was then shade-dried till dry at room temperature (25 C), and after that, the plant parts went through grinding into fine powder for further evaluation.

5.3.Extraction procedure

The AGL bark was extracted in a Soxhlet apparatus using ethanolic and hydroalcoholic extracts (ethanol: water 50:50). The extract was filtered, and the preceding procedure was repeated twice more. A rotary evaporator was used to entirely extract the solvent at lowered pressure. Lyophilization was used to get the concentrated extract, which was then kept in vacuum desiccators at 20 degrees Celsius for future use. The yield value was around 13.76% w/w.

5.4.Soxhlet extraction

Soxhlet extraction is a heated continuous extraction technique in which a dried crude medicament is extracted many times with the same solvent. The Soxhlet extractor is primarily composed of three parts: The main chamber of the Soxhlet extractor receives condensed solvent vapour from the condenser, which is located on top of the Soxhlet. Thimble paper is placed on the inner surface of the soxhlet chamber, which contains powdered crude medicine. A round bottle flask is inserted at the bottom of the Soxhlet to hold the flowing solvent. This procedure is also known as menstruation. The round bottle flask contains solvent in at least half of its capacity. The solvent is boiled in the round-bottom flask with the help of a heating mantle. The temperature is determined based on the solvent's boiling point. The soxhlet chamber contains a tube that is linked to RBF and transports solvent vapours to the chamber, which is condensed by the condenser. Another thin tube, the syphon tube, connects to the main

Fig 2: SoxhletExtraction

chamber. When the solvent level in the chamber reaches the top of the syphon tube, transfer the entire solution to the round-bottom flask. The colour of the solvent in the capillary tube should be checked often. The extraction should continue until a tinge of colour appears in the syphon tube. When the solvent in the syphon tube turns colourless, collect the liquid with the phytoconstituents and evaporate it under vacuum. If a solid residue is visible, the extraction should proceed. Otherwise, the extraction process cannot continue. The significance of this process is that it may be done multiple times to achieve successful extraction. After extraction, a final extract is obtained by several downstream processing procedures. First, the solvent is removed using a method known as concentration. Following the concentration phase, the plant extract is analysed for phytochemicals. The preliminary qualitative phytochemical investigation reveals the presence of secondary metabolites. The current study also includes quantitative estimates of the total concentration of a specific secondary metabolite, such as TPC or TFC.

5.5.Determination of physicochemical parameters

5.5.1.Moisture content determination:

1 gm of powder was put on a flat petri dish, which was then heated in a hot air oven at 5 ℃ to remove moisture from the powdered plant. The dried samples were weighed after they had cooled to room temperature within a desiccator. The % moisture content of previously weighed powdered medicines was determined. [Ahmed et al, 2015].

$$\text{Total moisture content (\%)} = \frac{\text{Weight of dried sample}}{\text{Weight of sample taken}} \times 100$$

5.5.2. Total ash content:

Two gms of dry powder plant material were weighed in a silicon crucible. The powder material was heated in a muffle furnace at 450°C for 2-3 hours until carbon-free, then cooled in a desiccator. Next, the powder was weighed to ensure its completeness. It was again heated in the furnace for 30 minutes. Finally, it was chilled and weighed. The weight of the ash was compared to that of the powdered substance. [Ahmed et al, 2015].

$$\text{Percentage of ash} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

5.5.3. Determination of water-soluble ash:

The ash produced was digested with water. 25 ml of solvent was used, and it took 20-30 minutes to boil in a water bath. The crucible content was filtered using Whatman filter paper, which was devoid of ash. The filter paper containing residue was carefully removed without

loss, folded and placed in the same crucible, dried in a hot air oven and fired in a muffle furnace at 450 °C for 1 hour. Then it was chilled with a desiccator before the final weight was taken. The proportion of water-soluble ash was determined based on the previously weighed powdered medication. [Ahmed et al, 2015].

$$\text{Percentage of soluble ash} = \frac{\text{Weight of soluble ash}}{\text{Total weight of ash}} \times 100$$

5.5.4. Determination of acid insoluble ash value:

After bringing the previously weighed total ash to a boil for 15 minutes with 25 ml of 2N HCl, the mixture was filtered through ash-less filter paper. Following filtering, the ash-filled filter paper was put in a silica crucible and burned in a muffle furnace at 450 °C until the ash was carbon-free. The crucible was then allowed to cool to normal temperature, and the weight of the medication taken earlier in powder form was used to determine the weight of the ash that was insoluble in acid. [Ahmed et al, 2015].

$$\text{Percentage of acid insoluble ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Total weight of ash}} \times 100$$

5.5.5. Determination of extractive value:

Excoecaria agallocha dry powdered plant material was extracted using hexane, hydroalcoholic, ethanol, ethyl acetate, and chloroform. Weighed in a weighing vial, 1 g of the coarsely ground plant material was then put into a dry conical flask. After that, different solvents (15 ml) were added to the flask one at a time. After being corked, the flasks were left alone at room temperature for a whole day, shaking periodically. Whatman No. 1 filter paper was used to filter the mixtures into a measuring cylinder. It was filtered and then put into Petry plates that were weighed. The solvent evaporated entirely through the filter, resulting in dry extracts. The extraction value in percent for the hexane, hydroalcoholic, ethanol, ethyl acetate, and chloroform extracts of *Excoecaria agallocha* was calculated using the following formula.[Khandelwal K.R. 2002].

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

5.6. Preliminary phytochemical screenings of *E. agallocha*

The preliminary phytochemical screening of the hydroalcoholic extract of *Excoecariaagallocha* was carried out by various standard methods [Kokate et al, 2003, Khandelwal, 2006] which are mentioned below.

5.6.1. Tests for alkaloids: (Bruck de Souza et al., 2020)

- **Wagner's test:**Two millilitres of extract solution were mixed with 0.1 millilitres of Wagner's reagent and 2 millilitres of mild hydrochloric acid. A reddish-brown colouration showed a positive reaction to alkaloids.
- **Hager's test:**A few drops of Hager's reagent were added to 1 mL of sample solution. When a yellow precipitate appears, it contains alkaloids.
- **Mayer's test:** In 1 ml of sample solution, add a few drops of Mayer's reagent (potassium mercuric iodide). When a yellow precipitate appears, it contains alkaloids.
- **Dragendroff test:**In a test tube containing a 2mL extract solution, 0.1 mL of weak hydrochloric acid and 0.1 mL of Dragendroff's reagent were added. The formation of an orange-brown precipitate proved the presence of alkaloids.

5.6.2. Tests for glycoside

- **KellarKiliani test:** In a test tube, combine a small amount of glacial acetic acid, ferric chloride solution, and concentrated sulphuric acid. Examine the interface for colour changes and observe the reaction. (Evans, W.C. et al. 2009).
- **Bontrager's test:** A few millilitres of mild sulphuric acid were added to one millilitre of extract solution before it was filtered and extracted with chloroform. The layer was treated with 1 ml. of ammonia. The appearance of red implies the presence of anthraquinone glycosides.

5.6.3. Test for saponin:

- **Frothing test:** A dry powdered plant sample was mixed with distilled water and violently shaken. If stable froth formed, saponins were present. (Kokate, C.K et al. 2009)

5.6.4. Test for flavonoid

- **Shinnoda test:**Two millilitres of sample solution were mixed with magnesium powder and a few drops of strong HCl solution. The pink colour is caused by the presence of flavonoids. (Pethappachetty et al., 2012)

5.6.5. Tests for tannin: (Segelman et al., 1969)

- **Ferric chloride test:**A few drops of 5% ferric chloride solution were added to a 2ml sample. If it becomes blue, it could include tannin.

- **Lead acetate test:** A few drops of lead acetate solution were added to 2-3ml of sample solution, resulting in the production of a white precipitate due to the presence of tannin.
- **Gelatin test:** The gelatin solution was mixed into the 2 ml sample solution. Tannin produces the formation of a white precipitate.

5.6.6. Test for steroid:

- **Liebermann Burchard test:**2 ml of test extract was combined with 1 ml of CHCl3, 1 ml of acetic anhydride, and one drop of concentrated H2SO4 was added. If the solution turns red, blue, and eventually bluish-green, it indicates the existence of a steroidal nucleus, whereas a development of purple or red indicates the presence of a triterpenoidal nucleus.
- **Sulkowski test**: 1-2 mg of powdered drug was dissolved in 1 ml of CHCL3 solution, which was then mixed with 1 ml of concentrated H2SO4. Bluish red, cherry red, and purple colours in the chloroform layer suggest the presence of sterols, but the creation of a reddish-brown colour at the interface shows the existence of a triterpenoid nucleus**.** (Bosila and El-Sharabasy, 2009)

5.6.7. Test for carbohydrate:

- **Molish's test:**1ml of Molish reagent was mixed into 2ml of test solution. Then, 1ml of strong sulphuric acid solution was added. A violet ring develops at the junction of two liquids indicating the presence of carbohydrates.(Salwaan et al., 2012)
- **Fehling's test:**The test sample was treated with 1 ml of Fehling solution A and 1 ml of Fehling's solution B. Finally, the entire mixture was boiled. Red precipitate develops with the presence of carbohydrates.(Nielsen, S.S. et al. 2010)
- **Benedict's test:** The test solution was combined with a few drops of Benedict's reagent (an alkali solution containing a cupric citrate complex) and heated in a water bath until a reddish-brown precipitate formed, confirming carbohydrate production. (Bhandary et al., 2012)
- **Barfoed's test:**2 ml of Barfoed's reagent was combined with 1 ml of test solution. The mixture was then brought to a boil. A brick red precipitate occurs when monosaccharide is present.

5.6.8. Test for phenolic compounds

- **FeCl3 test:**The test solution got 3-4 drops of FeCl3. The production of a bluish-black hue suggests the presence of phenol. (Saxena et al., 2015).
- **Lead acetate test:**Add a few drops of 10% lead acetate solution to the test tube. The development of a white precipitate suggests the presence of phenolic chemicals.(Kumar, M. K et al. 2011).

5.6.9. Test forAnthraquinones

A test tube carries extract, chloroform, and 10% sulphuric acid. Mix completely and set aside for 10-15 minutes. A colour shift in the bottom chloroform layer, usually pink, red, or orange, indicates the presence of anthraquinones. (Harborne, J.B. et al. 1998).

5.6.10. Test for Protein test

- **Millon's Test:** Add Millon's reagent to 2-3 mL of protein solution, mix well, heat, and observe colour change (Plummer, D.T. et al. 1987).
- **Xantho Protein test:** In a test tube, mix 2-3 mL of protein solution with concentrated nitric acid (HNO3), heat, and cool. Add excess ammonium hydroxide solution and observe any colour change (Sadasivam, S et al. 1996).

5.7. Preparation and characterization of tincture

A total of 21 amber-coloured bottles with a 10 mL size were used. To this, 10 ml of alcohol with varying strengths—40, 50, 60, 70, 80, and 90 percent (V/V)—and absolute alcohol were added. Each twenty-one vial held 1g of Excoecaria agallocha L. bark powder. Each batch was allowed to macerate for a set number of days (7, 14, and 21). After the appropriate maceration period, the materials were filtered through a sintered glass crucible (G4) at decreased pressure. Colour, pH, specific gravity, and total solid content were some of the physical qualities evaluated. (Barman S et al. 2024).

Figure 3: **Tinctures of different alcohol concentrations after maceration for differing periods**

Material and Methods

6. Material and Methods

6.1.In vitro Antioxidant study of Plant extract

Chemicals Used

Standard Ascorbic acid(C_6H_8O), Gallic acid ($C_7H_6O_5$), Quercetin ($C_{15}H_{10}O_7$), dibasic sodium phosphate, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Sodium phosphate dibasic dihydrate ($Na_2HPO_4.2H_2O$), Potassium dihydrogen phosphate (KH_2PO_4), Hydrogen peroxide (H_2O_2) (35% in water), α-glucosidase, 3, 5, di-nitro salicylic acid (DNS) and all other chemicals of analytical grade were obtained commercially.

6.1.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

Material and methods:

Preparation of 1M DPPH solution:

3.4 mg DPPH was dissolved in 100 ml methanol to produce 1M DPPH solution.

Preparation of dilutions of the plant extracts:

*E. agallocha*hydroalcoholic (AGL-HA) extract1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore). From there, the dilution was prepared with the help of methanol. The IC50 value (50% inhibitory concentration) was calculated by nonlinear regression using GraphPad Prism version 5.0 software. IC50 was determined through nonlinear regression of the percentage relative activity f(concentration) plots.

Using the following formula, the DPPH scavenging capacity was determined:

Scavenging % = ((Absorbance control-Absorbance sample)/(Absorbance control))×100

Methodology:

The antioxidant potential of AGL-HA extract was assessed using a DPPH free radical scavenging assay developed in our laboratory. 950 µL of 1M DPPH methanolic solution and an aliquot of 50 µL sample solution at different concentrations (50-100 µg/mL) were combined in this 96-well microplate. The reaction mixture was left in the dark for one hour at 37°C. The initial purple colour's disappearance showed the extracts' ability to scavenge free radicals. A UV-visible spectrophotometer was used to measure the absorbance of the reaction mixture at 517 nm (Bhatti MZ et al. 2015). The positive control in this experiment was ascorbic acid. The above formula was used to obtain the inhibition percentage.

6.1.2. NO scavenging assay

A Nitric Oxide (NO) scavenging test is a typical method used to assess the capacity of compounds to neutralise nitric oxide radicals, which are reactive species involved in several biological processes and illnesses.

Material and methods:

Preparation of 50mM Phosphate buffer (pH 7.4)

0.8899g Na2HPO4.2H2O, 0.68045g KH2PO4 were dissolved in 100ml water and maintain pH 7.4.

Preparation of Nitrous Oxide Solution:

Dissolve sodium nitroprusside (Na2[Fe(CN)5NO) in PBS to prepare a working solution. This solution releases nitric oxide in a controlled manner.

Preparation of 1mg/ml extract solution:

AGL-HA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

Griess Reagent Preparation:

Prepare the Griess reagent by mixing equal volumes of sulfanilamide and naphthylethylenediaminedihydrochloride in an acidic solution (typically phosphoric acid). This reagent reacts with nitrite to form a colored azo dye.

Methodology:

The GriessIllosvory reagent was commonly changed by substituting napthyl ethylene diamine dihydrochloride (0.1% w/v) for 1-napthylamine (5%). The reaction mixture, including 0.5 ml of saline phosphate buffer, 2 ml of 10 mM sodium nitroprusside, and 0.5 ml of either standard (Ascorbic acid) solution or hydroalcoholic (AGL) extracts (1000 µg/ml), was incubated for 150 minutes at 25°C. After incubation, 0.5 mL of the reaction mixture was combined with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and left to stand for 5 minutes to complete the diazotisation process. Subsequently, 1 millilitre of naphthyl ethylene diamine dihydrochloride was added, combined, and let to stand at 25°C for 30 minutes. The concentration of nitrate was measured at 546 nm.
(Patel A et al. 2010). The positive control was ascorbic acid. The following formula determined the scavenging activity of the extract:

NO scavenging activity % = ((Absorbance control-Absorbance sample)/(Absorbance control))×100

6.1.3. Determination of Total Phenolic Content (TPC):

Principle:
The reduction of the Folin–Ciocalteu reagent (FCR) in the presence of phenolics is the basis of the widely used Folin–Ciocalteu (F–C) test for phenolic analysis. Molybdenum-tungsten blue is produced as a consequence, and its concentration increases linearly with the concentration of phenolics in the reaction media. It is detected spectrophotometrically at 760 nm. By lowering precipitate formation and raising the assay's reactivity, lithium sulfate is

added to the reagent, which improves the FCR. The simple and popular F-C test may determine the total phenolics in fruits and vegetables. According to the technique, a test tube should be filled with 0.5 ml of deionized water, 0.125 ml of diluted fruit extracts, and 0.125 ml of FCR and left to react for six minutes—next, 1.25 millilitres of a sodium carbonate solution (7%) (Malta, L. G et al. 2014).

Material and Methods:

Bioactive substances that provide health advantages are called phytochemicals, especially phenolics in plants. These substances have various biological effects, including antioxidant activity, and may be found in edible and inedible plant components. They can scavenge because of their redox characteristics, enabling them to function as hydrogen donors, reducing agents, and singlet oxygen quenchers. In addition, natural antioxidants have antiviral, antibacterial, anti-inflammatory, antiallergic, antithrombotic, and vasodilator properties. Researchers and food makers are becoming more interested in the potential of these phytochemicals for maintaining health and protecting against cancer and coronary heart disease (Babbar, N et al. 2011).

Preparation of Folincio-calteau phenol reagent:

Dilute the Folin-Ciocalteu concentrate with deionized water. A common dilution ratio is 1:10. The solution is mixed thoroughly. Adjust the pH to around 10-11.

Preparation of standard gallic acid dilutions:

Gallic acid with a 95% purity level was used to create standard dilutions. A methanol solution containing 1 mg/ml was produced, and then six dilutions of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml were serially diluted.

Preparation of % $NaHCO_3$ solution:

Measure 7.5 grams of sodium bicarbonate.Add the sodium bicarbonate to 250 mL of deionized water. Stir until completely dissolved. This forms a 0.2 M solution.

Preparation of 1mg/ml extract solution:

AGL-HA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

Methodology:

In a 96-well plate, 0.1 mL aliquot of different extracts (4 mg/mL) was combined with 0.75 mL of Folin-Ciocalteu reagent (10 times diluted with deionized water). 0.75 mL of 6% sodium carbonate was added after the mixture had been left at room temperature for five minutes. The absorbance at 725 nm was measured after a 90-minute process. Gallic acid was used to draw the standard calibration (0–25 µg/mL) curve. Gallic acid equivalent milligrams per gram of dry weight represented the total phenolics. 0.1 mL of DMSO was used in place of the extract to provide the negative control (Bhatti MZ et al. 2015).

6.1.4 Determination of Total flavonoid content(TFC):

Principle:

Plant extracts were tested for TFC using the aluminium chloride colourimetry technique. This approach uses Al (III) as a complexing agent, forming Al(III)-flavonoid chelates. Depending on the pH level and the circumstances of the experiment, flavonoids have a considerable propensity to bind metal ions, usually at a 1:1 ratio (Shraim, A. M et al. 2021).

Preparation of NaNO2 solution:

Measure 0.1 grams of sodium nitrite. Add the sodium nitrite to 100 mL of deionized water. Stir until completely dissolved. Ensure the solution is homogeneous.

Preparation of AlCl3 solution:

Measure 0.5 grams of aluminium chloride. Add the aluminium chloride to 50 mL of deionized water. Stir until completely dissolved. This results in a 10% (w/v) solution of $AlCl_3$. Ensure the solution is well-mixed to achieve uniformity.

Preparation of NaOH solutions:

To prepare a 1 M solution, weigh 40 grams of NaOH pellets.Slowly add the NaOH pellets to 1 litre of deionized water while stirring continuously.

Preparation of 1mg/ml extract solution:

AGL-HeA extract 1mg was dissolved in 1ml methanol by the vortex. The solution was filtered through a 0.45µ filter (Millipore).

Methodology:

In a 96-well plate, 1.5 mL of methanol was combined with an aliquot of 0.5 mL of different extracts (1 mg/mL). Next, 0.1 mL of 10% aluminium chloride, 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water were added. For thirty minutes, the reaction mixture was maintained at room temperature. At 415 nm, the reaction mixture's absorbance was measured. The calibration curve (0–8 µg/mL) was plotted using rutin as the standard. The flavonoid content was given as milligrams of rutin equivalent per gram of dry weight ((Bhatti MZ et al. 2015).

6.2. In vivo Animal Study

Material and Methods:

6.2.1. Animals

The optimum study employed 6-8 weeks old Wistar albino rats of either sex weighing 140-160 g. Rats were housed in a standard laboratory environment with 12-hour light/dark cycles, a temperature of 25-28 °C, and a relative humidity of 55-60%. They were fed a normal diet and allowed access to water as needed. Before the trial, the animals spent a week acclimating to the lab environment.

6.2.2. Drugs and chemicals:

We bought isoniazid and rifampicin from HI Media Laboratories Pvt Ltd. India, and we got the standard drug silymarin from USV Private Limited in Himachal Pradesh. The reagent kits used were from Span Diagnostics Ltd. India. The suppliers of trichloroacetic acid (TCA), thiobarbituric acid (TBA), phenazoniummethosulfate (PMS), 5,5'-dithio bis-2-nitro benzoic acid (DTNB), nicotinamide adenine dinucleotide (NADH), and reduced glutathione (GSH) were SISCO Research Laboratory, Mumbai, India. The glacial acetic acid and potassium dichromate were from Ranbaxy, Mumbai.

6.2.3. Induction of Liver Injuries

RIF and INH suspensions were prepared separately in distilled water. To induce hepatotoxicity in allgroups, rats were administered with INH and RIF (each 100 mg/kg b.w.) for 35 days by oral gavage.

6.2.4. Treatment schedule:

After 7 days of acclimatization, animals would be divided into five groups (n=6).DILI will be induced by 100 mg/kg BW dose of each INH and RIF by gavage, daily for 5 weeks.In treatment groups, firstly dose of INH + RIF will be given then the plant extract dose or STD drug will be given once a day, daily for 5 weeks.

Experimental groups and dose schedule:

- Group 1(NC): Normal control (Rat on a normal diet) for 5 weeks
- Group 2 (DC): 100 mg/kg BW. Of each, INH + RIF given orally for 5 weeks.
- Group 3 (Low): INH+RIF (100 mg/kg BW, each) + 200 mg of AGL for 5 weeks, orally
- Group 4 (High): INH+RIF (100 mg/kg BW, each) + 400 mg of AGL for 5 weeks, orally
- Group 5 (STD): INH+RIF (100 mg/kg BW, each) + 50 mg/kg BW of silymarin for 5 weeks, orally

6.2.5. Serum Liver Function Test

Numerous serum biochemical parameters, such as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALT), serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST), Bilirubin, and ALP were measured in the collected blood. Commercially accessible kits from Span Diagnostics Ltd., India were used for all of the analyses.

6.2.6. Sacrifice and collection of livers

The deaths occurred during the conclusion period for each group. The rats were fasted for eight hours before being humanely killed with an overdose of ketamine/xylazine at the end of the intervention period. Their livers were removed and cleaned with regular saline. Each liver

had a piece of around 5 mm removed, which was then preserved in 10% neutral buffered formalin for histology. Five millilitres of sodium phosphate buffer (pH 7.5; 50 mM) containing 10% Triton X100 was used to homogenize 0.5 grams of each heart that was removed. After homogenization, the tissue samples were centrifuged for 10 minutes at 4°C at 15,000 rpm. After being decanted into Eppendorf tubes with labels, the supernatants were kept at -20◦C until further analysis could be performed.

6.2.7. Histopathological Studies

Hematoxylin and eosin staining, oil-o-red, and standard paraffin tissue processing were applied to liver tissues that had been preserved in 10% normal saline. A Digital Bright-field Microscope (OMAX, USA) was used to view the slides, and digital photomicrographs were taken.

6.2.8. Estimation of Liver Antioxidant Stress

Catalase activity, lipid peroxidation, and reduced glutathione (GSH) levels were among the oxidative stress indicators measured in tissue supernatants.

6.2.9. Reduced glutathione (GSH) level

The GSH level was ascertained using Ellman's method. To summarise, 100 µL of the tissue supernatant was deproteinized with 300 µL of 10% TCA, and the resulting combination was centrifuged at 3500 rpm for five minutes. After that, a 96-well plate was pipetted with 200 µL of the deproteinized samples and 50 µL of Ellman's solution, and it was incubated for five minutes. At 415 nm, the absorbance was measured. Utilizing a GSH standard curve, the GSH level was determined. (ELLMAN GL et al. 1959).

6.2.10. Catalase activity

The technique that was previously developed was used to determine the catalase activity. In summary, 340 µL of 50 mM sodium phosphate buffer (pH 7.0) was combined with 100 µL of the supernatants. After that, the mixture was mixed with 150 µL of 2 M H_2O_2. For three minutes, absorbance was measured at 240 nm at one-minute intervals (Aebi, H. et al. 1984).

6.2.11. Lipid peroxidation levels

The thiobarbituric acid reactive substances (TBARS) were measured and expressed as malondialdehyde (MDA) levels using the previously mentioned procedure. In short, 100 µL of the supernatant was mixed with 100 µL of 8.1% SDS solution, 375 µL of 20% acetic acid, and 1 mL of 0.25% thiobarbituric acid (TBA) in a reaction mixture. After one hour of boiling, 200 µL of the heated liquid was pipetted onto a 96-well plate, and the absorbance was measured at 532 nm. An MDA standard curve was used to extrapolate MDA values (Chowdhury P et al. 2002).

6.2.12. Statistical Analysis

Each result is displayed as mean ±SEM. Graph Pad Prism 10.3.0 (Graph Pad software, USA) was used to analyze the data for statistical significance using one-way analysis of variance (ANOVA) and post hoc Dunnett's test. P values were deemed statistically significant when they were <0.05.

Results

7.Results

7.1. Physicochemical evaluation

The physicochemical evaluation of plants involves assessing both physical and chemical properties to understand their composition, quality, and potential applications. The below results evaluate the potential physiochemical parameters of *Excoecaria agallocha*.

Table 4: Physicochemical evaluation of *Excoecaria agallocha*

Standardization parameters	**Value (%w/w)**
Moisture content	43.73 ± 1.26
Ash value	
Total ash value	16.00
Acid insoluble ash value	2.75
Water soluble ash value	13.35

Extractive value (%w/w):-	
Solvent	**Extractive value (gm/Kg)**
Ethyl Acetate	51.39
Hydroalcoholic	146.50

Values are mean ± SD, (n= 3)

Table 5: Preliminary phytochemical screening of *Excoecaria agallocha*hydro-alcoholic extract:

S.NO		PLANT EXTRACT	Hydro-alcoholic Extract	methanolic Extract
1.	Alkaloids	Mayer's reagent	Positive	Positive
		Hager's reagent	Positive	Positive
		Wagner's reagent	Positive	Positive
		Dragendorff's reagent	Positive	Positive
2.	Phenol phenolic	$Fecl_3$	Positive	Positive

	compounds	Lead acetate test	Positive	Negative
3.	Saponin	Frothing test	Positive	Positive
4.	Carbohydrate	Molisch test	Positive	Positive
		Fehling's solution	Positive	Positive
		Benedict's test	Positive	Positive
		Barfoed's test	Positive	Positive
5.	Protein and amino acids	Millon's test	Negative	Negative
		Xantho Protein test	Negative	Negative
6.	Glycosides	Bontrager's test	Positive	Positive
		KellarKiliani test	Positive	Positive
7.	Flavonoids	Lead acetate solution test	Positive	Positive
		Shinoda test	Positive	Positive
8.	Phytosterols	Liebermann-Burchard test	Positive	Negative
9.	Tannins	Ferric chloride test	Positive	Positive
		Lead acetate test	Positive	Positive
10.	Anthraquinones		Positive	Negative

7.2. Observation of absorbance in Total Phenolic estimation

The absorbance of the Standard and test solution was represented in **table5** The total phenolic content in terms of mg Gallic Acid Equivalent (GAE) of Hydroalcoholic extract was found to be 131.18 mg/g. These results suggest that the higher presence of phenolic components was responsible for the levels of antioxidant activity. The standard curve for gallic acid is shown in the **figure 6**.

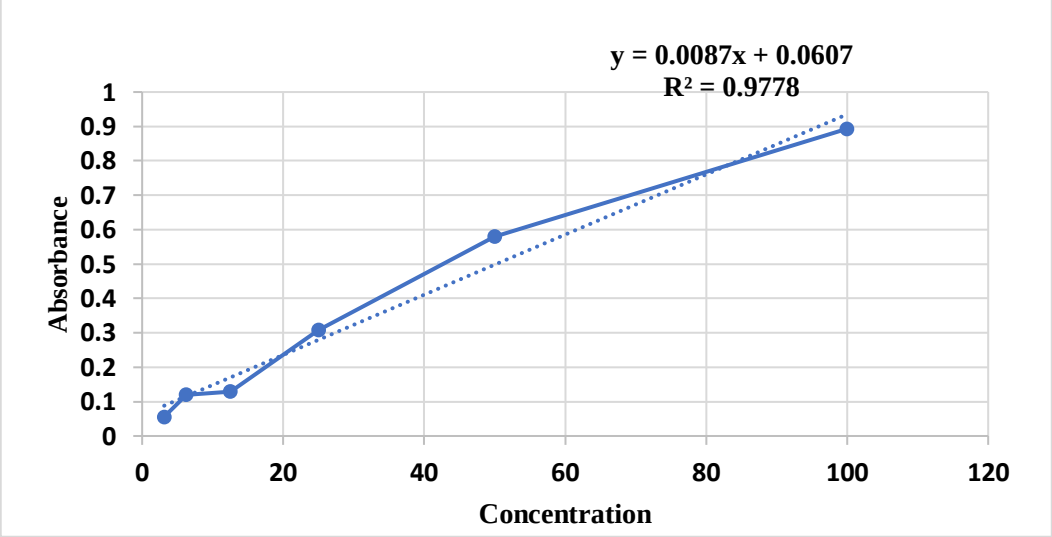
Table 6: Observation table for TPC

Sl No.		**Concentration(µg/ml)**	**Absorbance**
1		3.125	0.055

2	STANDARD (Gallic acid)	6.25	0.012
3		12.5	0.012
4		25	0.030
5		50	0.057
		100	0.893
1	SAMPLE (Hydroalcoholic extract)	1000	1.202

Values are mean ± SD, (n= 3)

Figure 4: Standard curve of Gallic acid



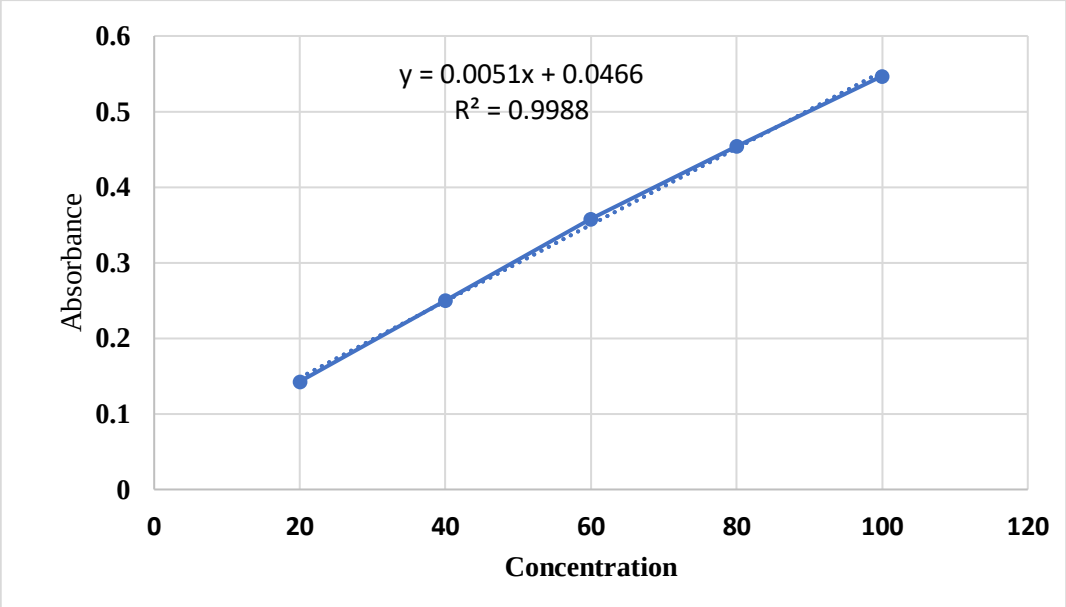
7.3. Observation of absorbance in total flavonoid estimation

The content of the flavonoid compound in the Hydroalcoholic extract of *Excoecaria agallocha* barkwas measured by aluminium chloride reagent in terms of quercetin equivalent. It was found to be 46.20mg/g. The absorbance of the Standard and test solution is represented in **Table6**. It is well known that flavonoids have significant antioxidant activity and have a positive impact on human nutrition and health. The ethyl acetate extract contains a substantial quantity of flavonoids, which may contribute considerably to the plant's antioxidant activity. The Standard curve of Quercetin is shown in **Figure 5**.

Table7:

Sl No.		Concentration(µg/ml)	Absorbance
1	STANDARD(QUERCETIN)	1000	0.145
2		500	0.085
3		250	0.048
4		125	0.025
5		62.5	0.019
1	SAMPLE (Hydroalcoholic extract)	1000	0.281667

Figure 5: Standard curve of Quercetin



7.4. Characterization of tincture

The different characteristics of the prepared tincture are shown in table 8, 9 and 10. The pH of the prepared tincture to varying strengths of alcohol is represented in table 8. The study shows that greater alcohol concentration in the tincture increases the acidity of the tincture, while storage for a more extended period also slightly increases acidic properties. The solid content of the tincture also decreases with time, as seen from table 9, while greater alcohol

concentration also imparts diminished solid content. Finally, as seen in table 10, the specific gravity of the prepared tinctures does not vary substantially with time.

Table 8: pH of the tincture

Alcohol Strength	pH		
	7 Days	14 Days	21 Days
40%	6.03	5.65	5.33
50%	5.90	5.79	5.64
60%	5.31	5.66	5.49
70%	5.73	5.82	5.50
80%	5.70	5.60	5.45
90%	5.35	5.56	5.69
Absolute(100%)	5.35	5.23	5.09

Table 9:Total Solid Content of Tincture

Alcohol Strength	7 Days	14 Days	21 Days
40%	19.76	17.23	14
50%	17.02	15.34	12.53
60%	16.90	15.39	12.30

70%	16.97	14.83	10.38
80%	15.69	13.75	10.41
90%	12.34	13.88	10.16
Absolute(100%)	9.92	7.14	5.50

Table 10: Specific Gravity of Tincture

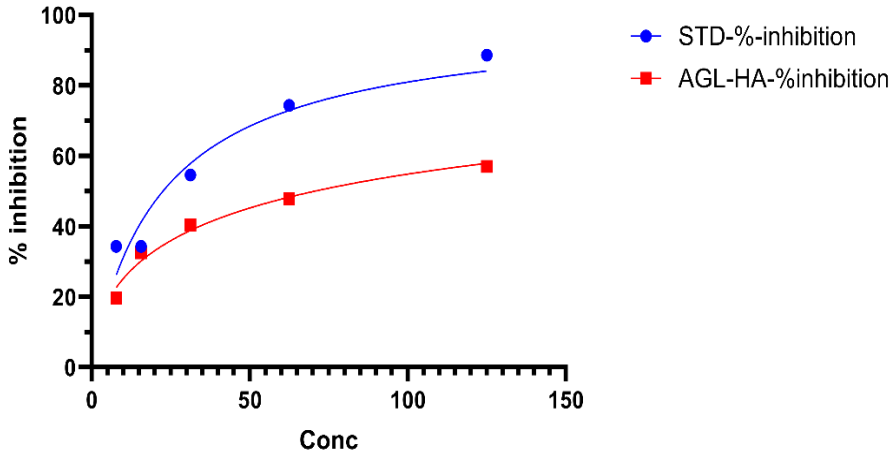
Alcohol Strength	7 Days	14 Days	21 Days
40%	0.972	0.943	0.954
50%	0.932	0.914	0.964
60%	0.900	0.912	0.994
70%	0.778	0.893	0.991
80%	0.887	0.895	0.901
90%	0.844	0.888	0.921
Absolute(100%)	0.779	0.799	0.802

7.5. Percentage Inhibition of DPPH radicals:

The decrease in DPPH absorbance at 517 nm caused by antioxidants was utilised to determine its capability for reduction. When DPPH free radicals were present in large concentrations, the extract demonstrated its highest capacity to donate hydrogen. The extract has an IC50 value of 70.52µg/ml, indicating antioxidant activity. On the other hand, the DPPH radical showed an IC50 value of 22.56 µg/ml for the well-known antioxidant ascorbic

acid. When compared to ascorbic acid, E. agallocha exhibited considerable scavenging effects with increasing concentration. The results of the DPPH scavenging activity are shown below in Figure 6, representing the DPPH radical scavenging assay of standard and test sample.

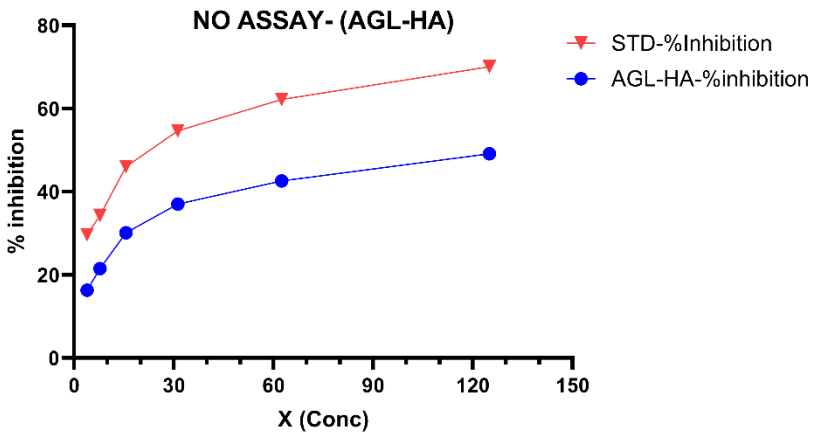
Figure 6: DPPH radical scavenging assay



7.6. Percentage Inhibition of Nitric oxide radicals

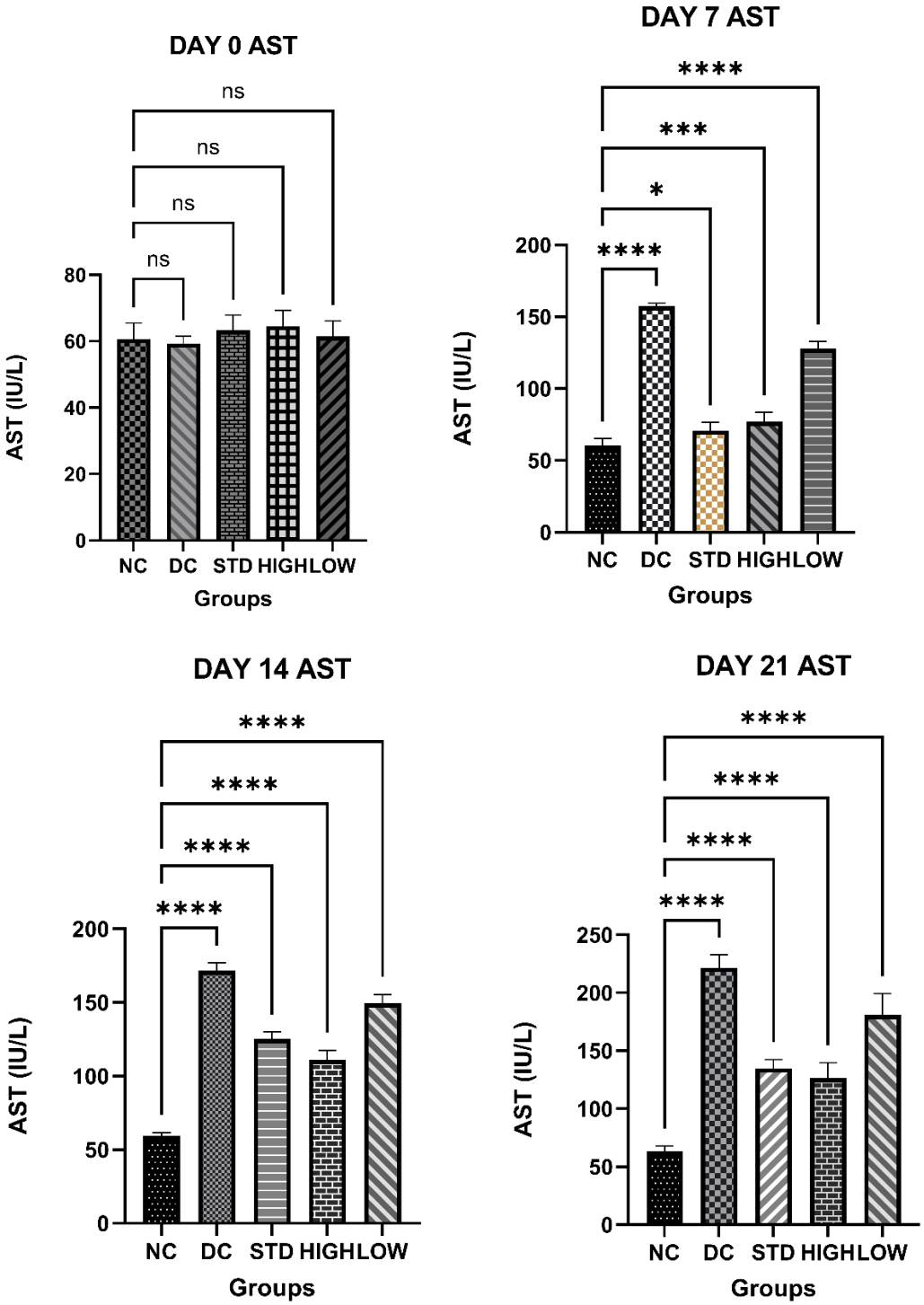
In an aerobic environment, nitric oxide is a volatile species. Using NO2, N2O4, and N3O4 as intermediates, it is combined with O2 to yield stable products such as nitrates and nitrite. With the Griess reagent, an estimate is obtained. Nitrous acid concentration falls in the presence of a test chemical, a scavenger. The degree of reduction corresponds to the degree of scavenging.The IC50 values of AGL-HA extract and ascorbic acid were 120.6 µg/ml and 23.06 µg/ml, respectively. The results showed that the test sample was adequate for scavenging Nitric oxide in a dose-dependent manner. The outcome is comparable to that of the ascorbic acid reference standard. Figure 7

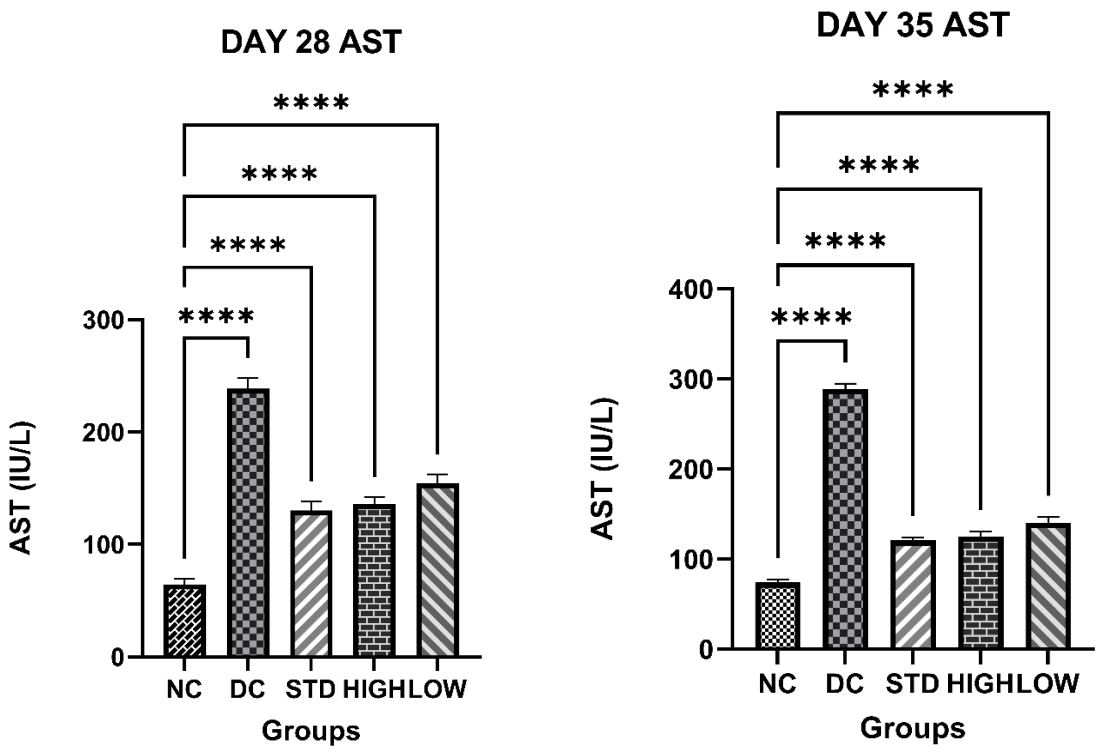
Figure 7: Inhibition graph of Nitric Oxide radical scavenging ASSAY



7.7.Effect of AGL-HA extract on AST

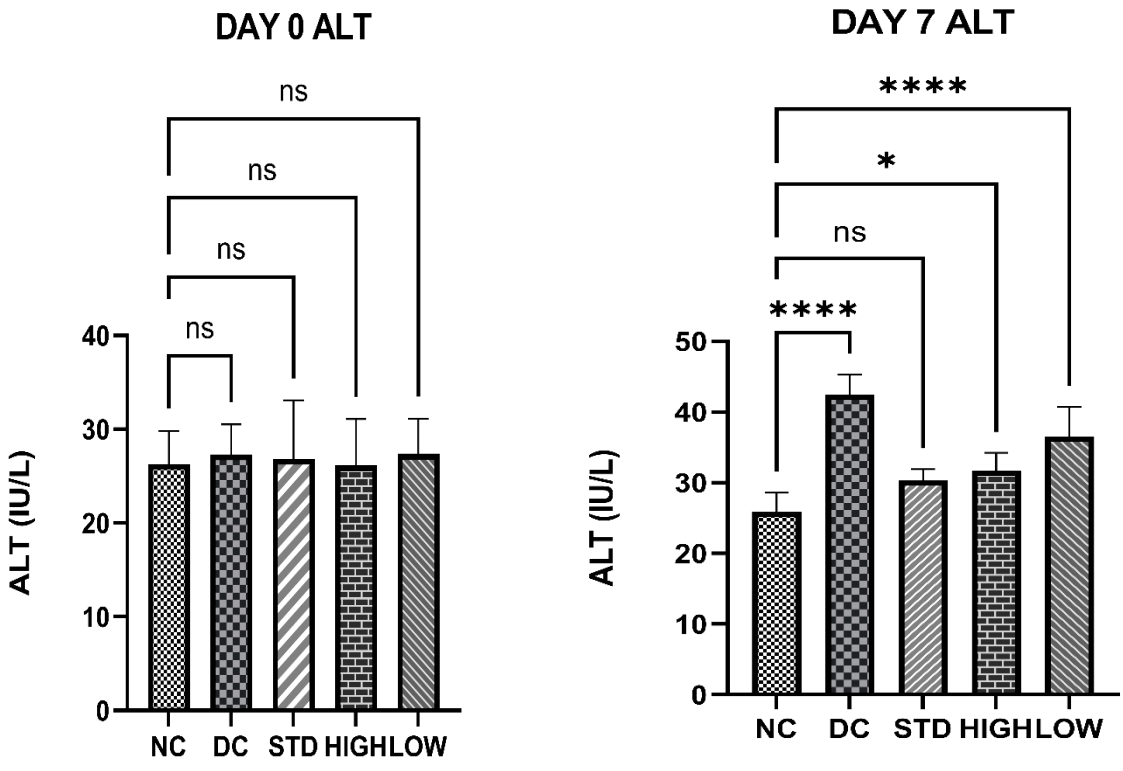
Fig 8: Significant difference in AST level at different days

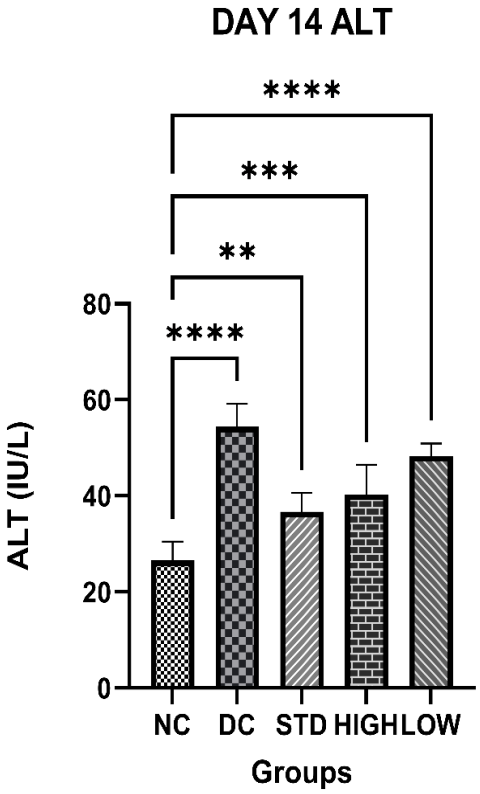


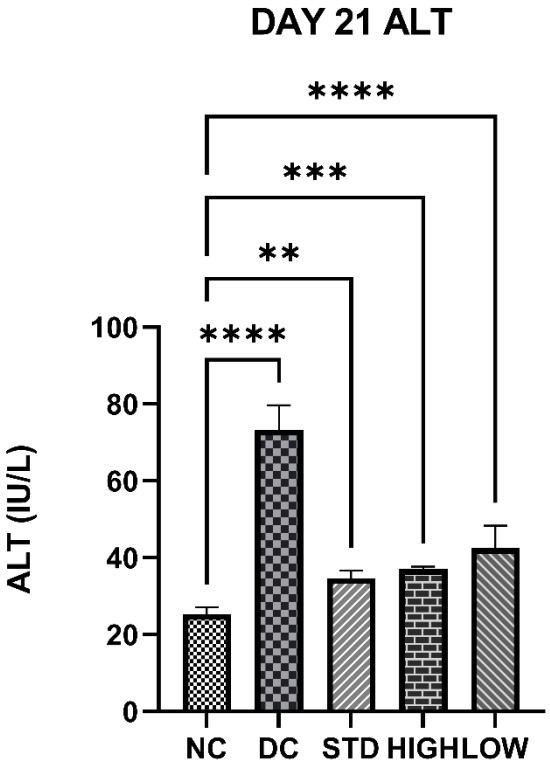


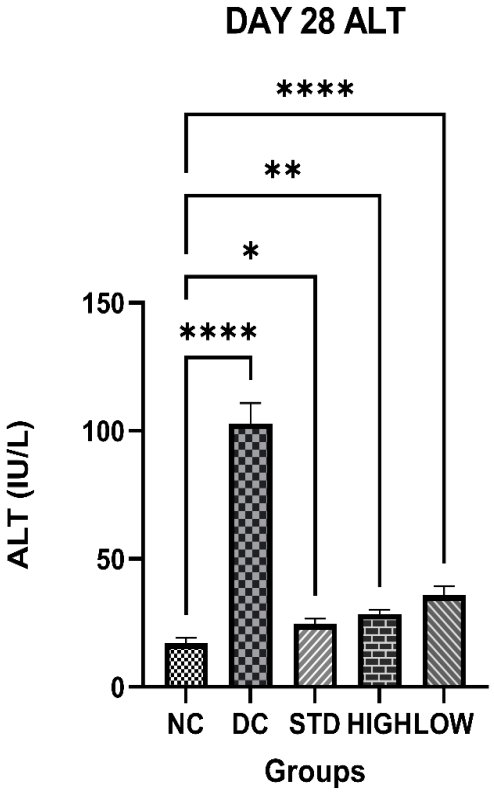
7.8. Effect of AGL-HA extract on ALT

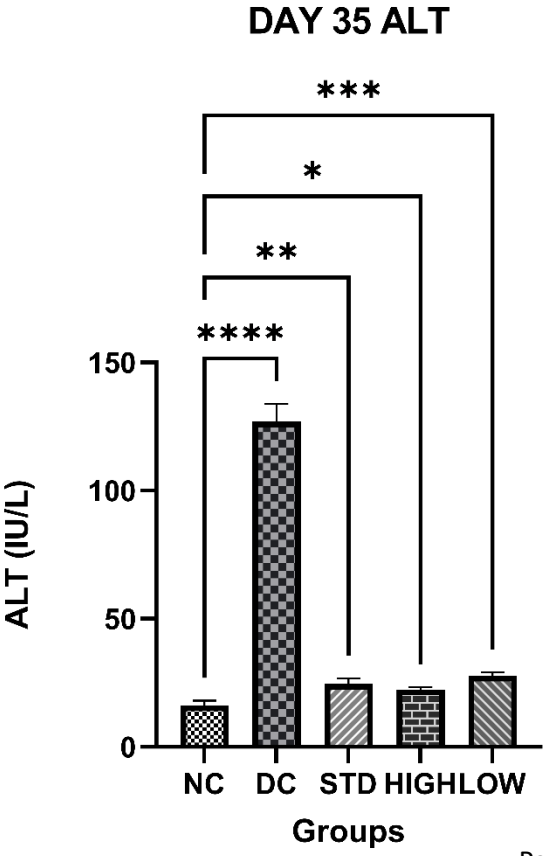
Fig 9: Significant difference in ALT level at different days




DAY 14 ALT
****
***
**
****
80
60
40
20
0
ALT (IU/L)
NC
DC
STD
HIGH
LOW
Groups

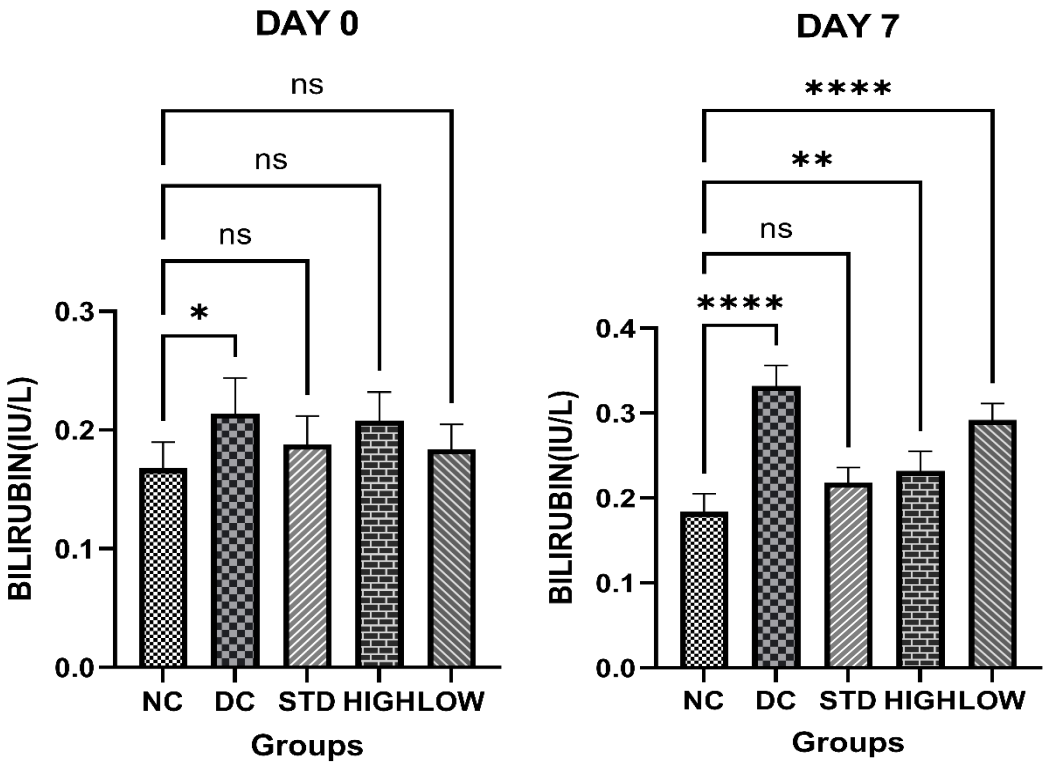

DAY 21 ALT
****
***
**
****
100
80
60
40
20
0
ALT (IU/L)
NC
DC
STD
HIGH
LOW

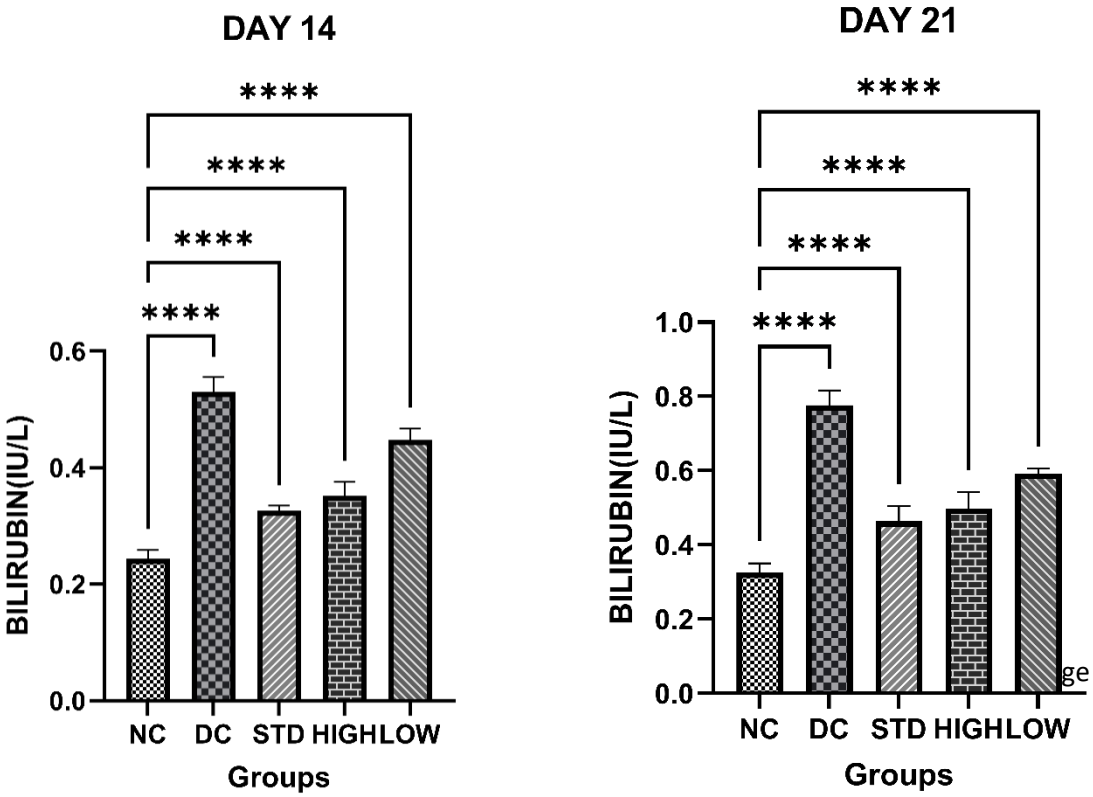

DAY 28 ALT
****
**
*
****
150
100
50
0
ALT (IU/L)
NC
DC
STD
HIGH
LOW
Groups

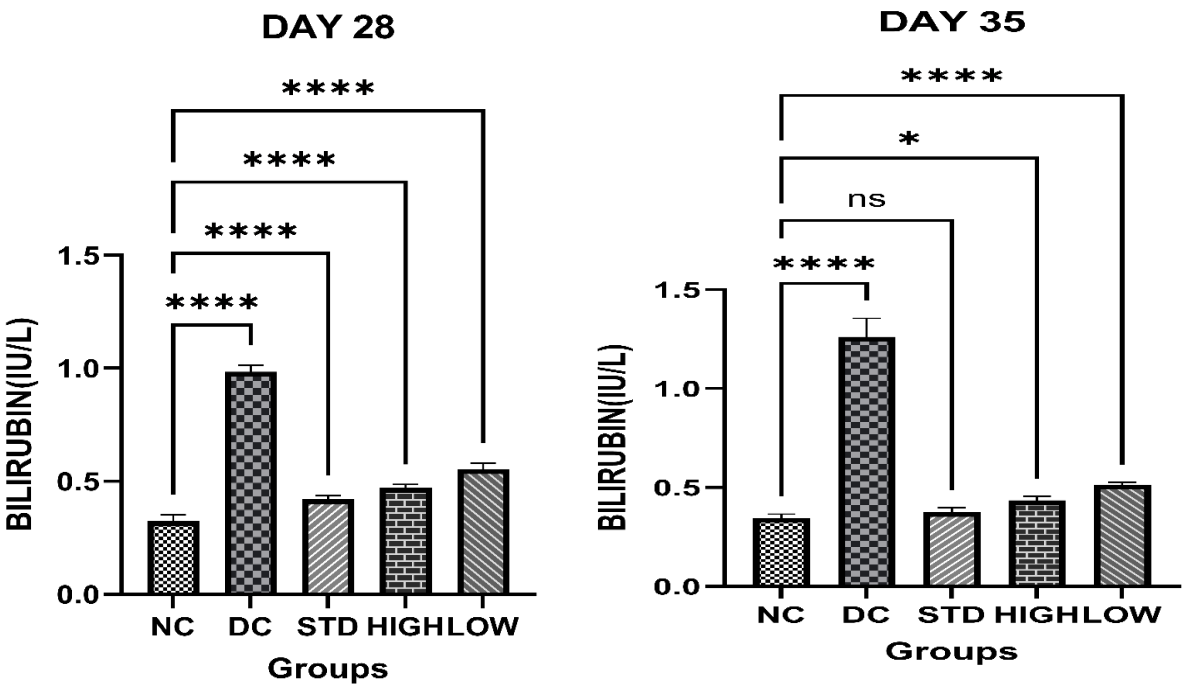

DAY 35 ALT
***
*
**
****
150
100
50
0
ALT (IU/L)
NC
DC
STD
HIGH
LOW
Groups

7.9. Effect of AGL-HA extract on Bilirubin

Fig 10: Significant difference in Bilirubin level at different days

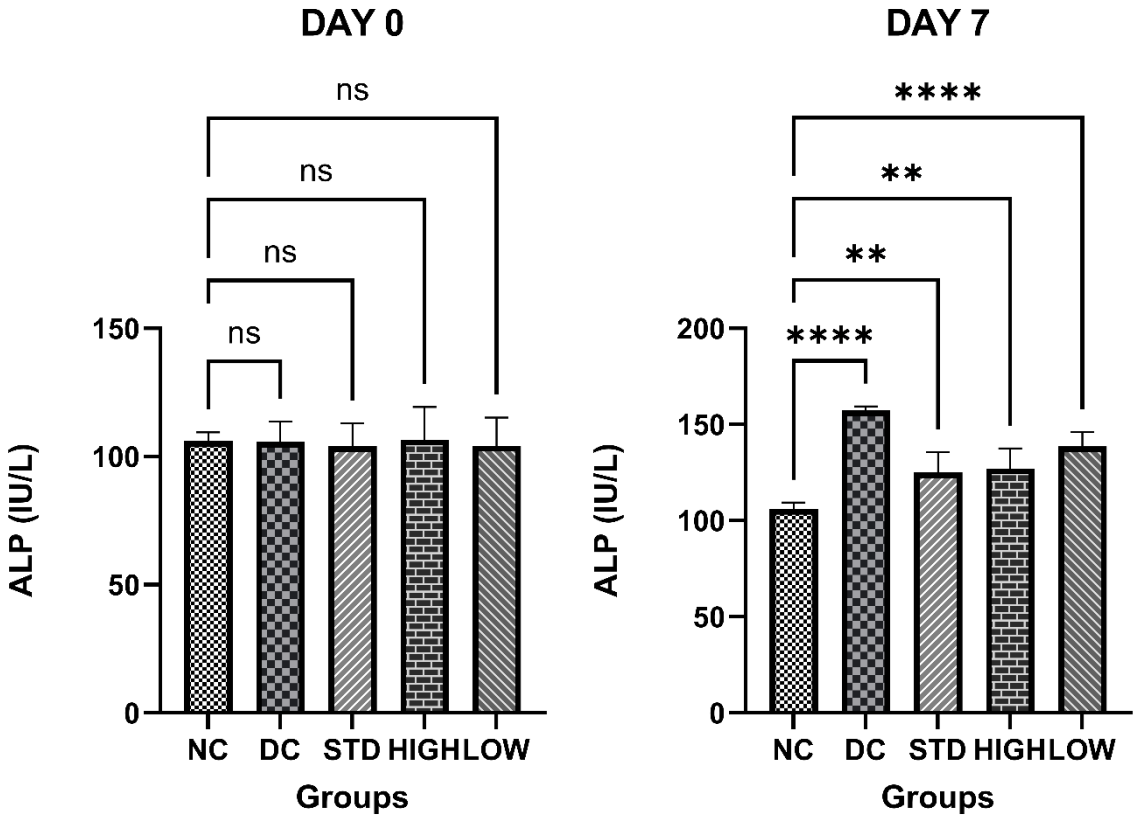


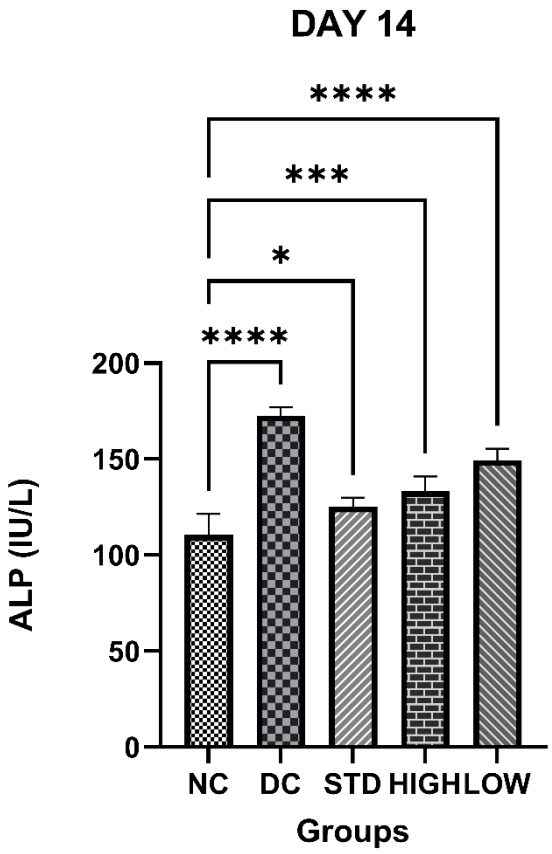


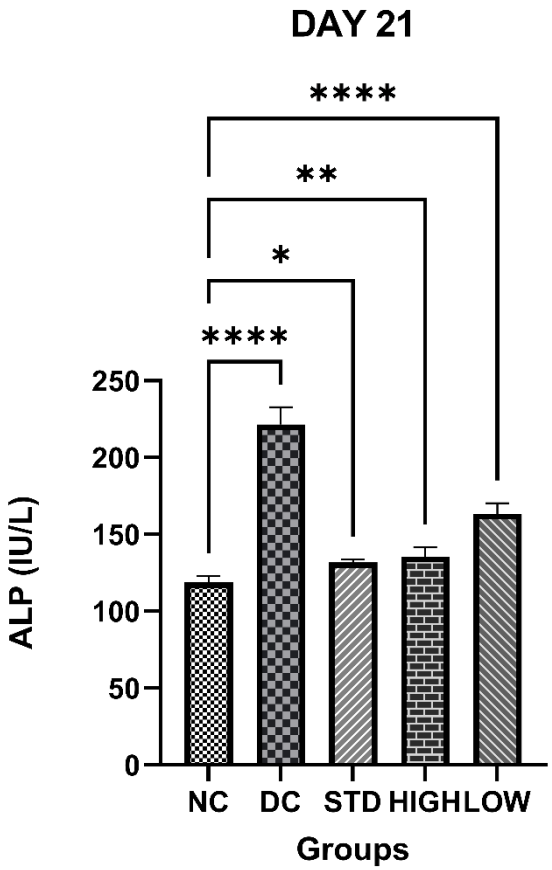


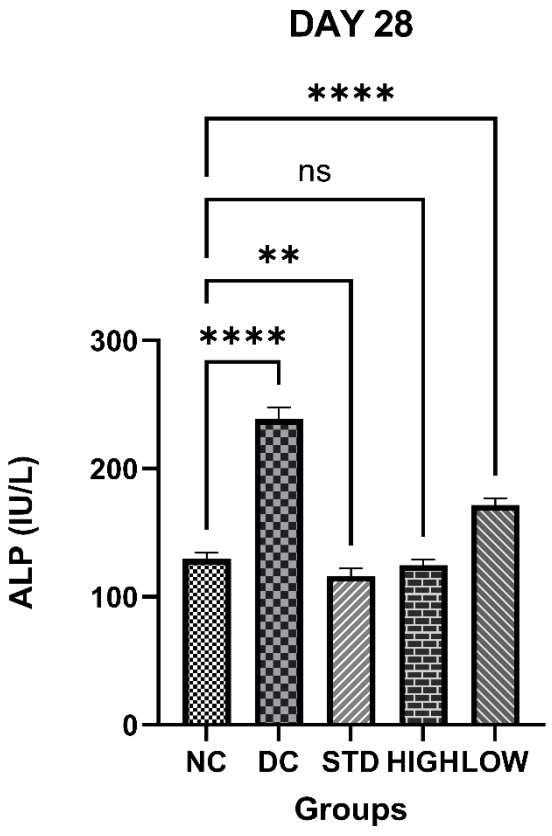
7.10. Effect of AGL-HA extract on ALP

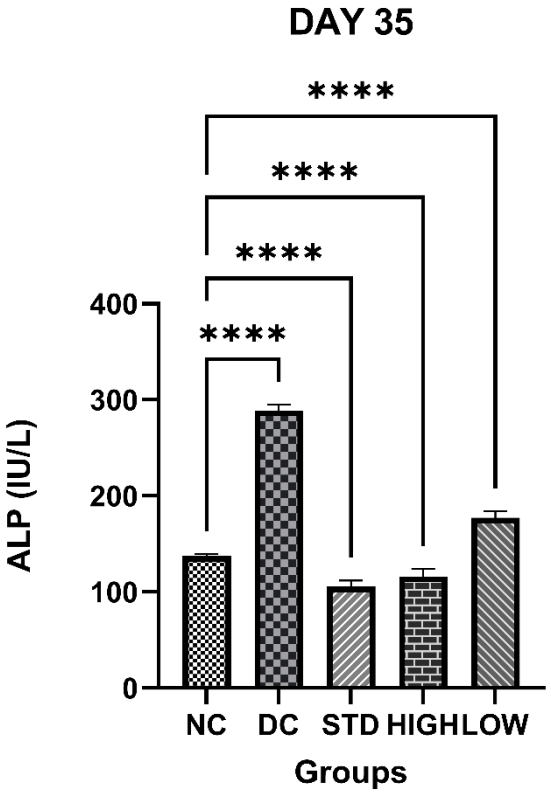
Fig 11: Significant difference in ALP level at different days




DAY 14
****
***
*
****
200
150
100
50
0
ALP (IU/L)
NC
DC
STD
HIGH
LOW
Groups


DAY 21
****
**
*
****
250
200
150
100
50
0
ALP (IU/L)
NC
DC
STD
HIGH
LOW
Groups


DAY 28
****
ns
**
****
300
200
100
0
ALP (IU/L)
NC
DC
STD
HIGH
LOW
Groups

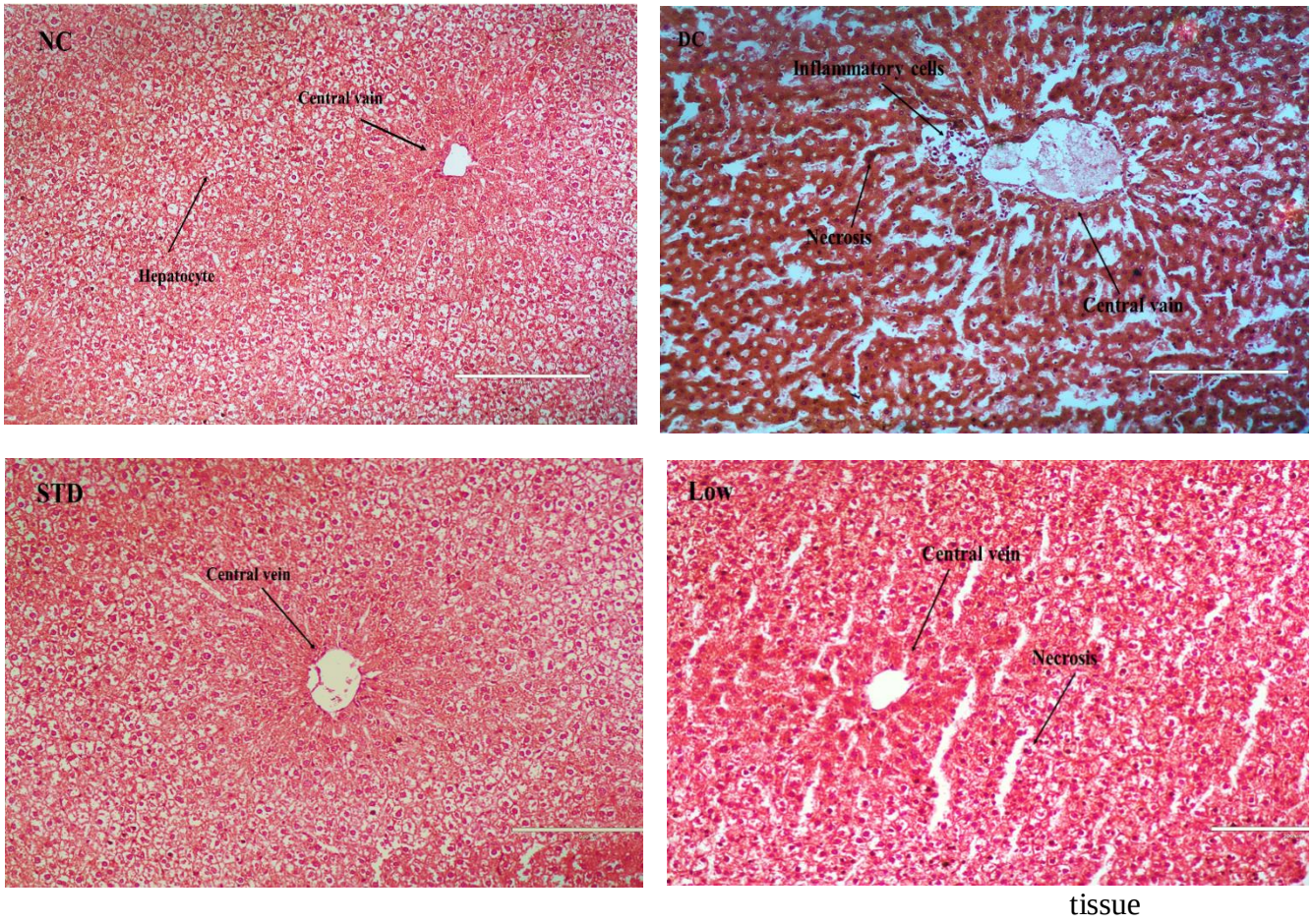

DAY 35
****
****
****
****
400
300
200
100
0
ALP (IU/L)
NC
DC
STD
HIGH
LOW
Groups

7.11. Histopathological studies:

Hematoxylin and Eosin staining (H&E staining)

The histological study of the liver in Disease group rats revealed significant deterioration of the liver tissue. Treatment with a High dose of AGL-HA extract (400 mg/kg) enhanced the cellular structure, with visible central veins surrounded by hepatocytes and well-organised hepatic ducts, indicating that the plant extract has hepatoprotective properties

Fig 12: H&E histology of different liver tissue



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7.12. Estimation of liver anti-oxidation level:

The hepatic levels of GSH and catalase activity were significantly ($p < 0.05$) reduced upon induction of Oxidative stress, and the MDA level was exacerbated concurrently. Following treatment with AGL-HA Extract, these levels and activity were markedly restored, with rats receiving the high dosage (400 mg/kg bw) showing even better activity compared to those receiving metformin. Hepatic oxidative indicators were either hardly or not affected when the infusion was given to normal animals.

Fig 13: Significant difference in GSH level at 35th days:

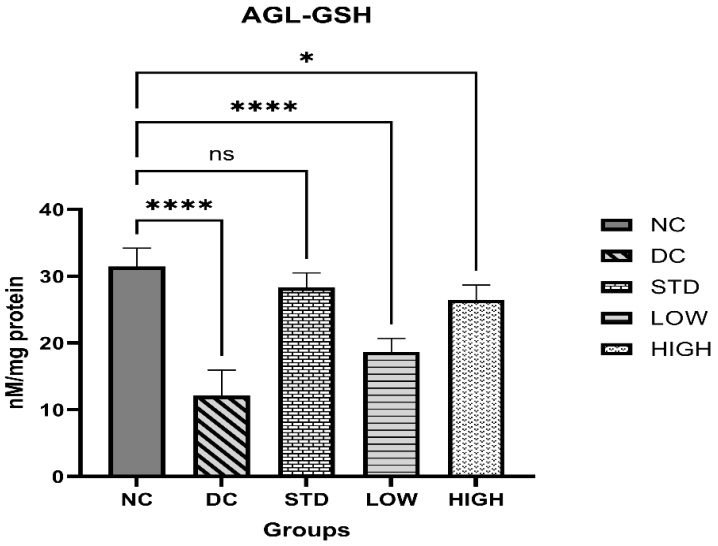


Fig 14: Significant difference in H202 level at 35th days:

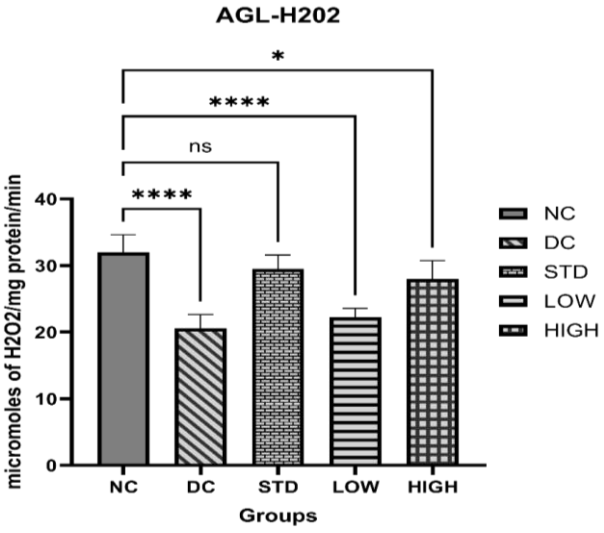


Fig 15: Effect of AGLon MDA levels at 35th day:

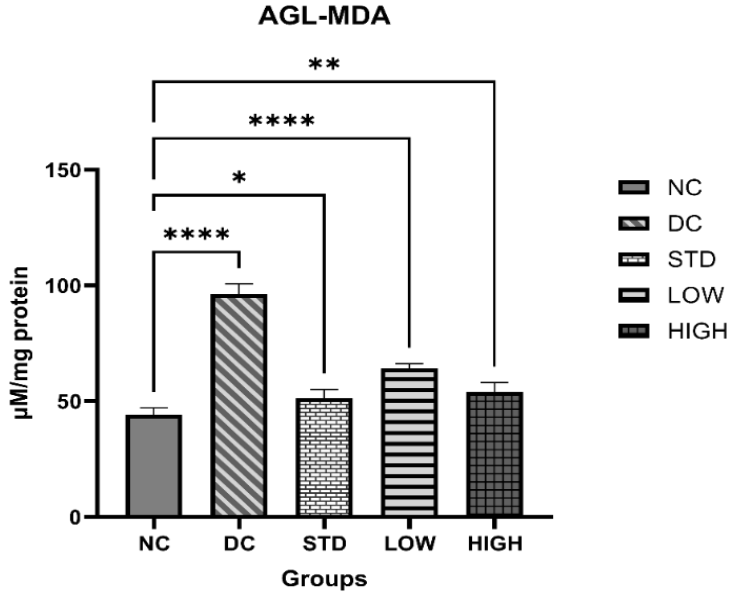
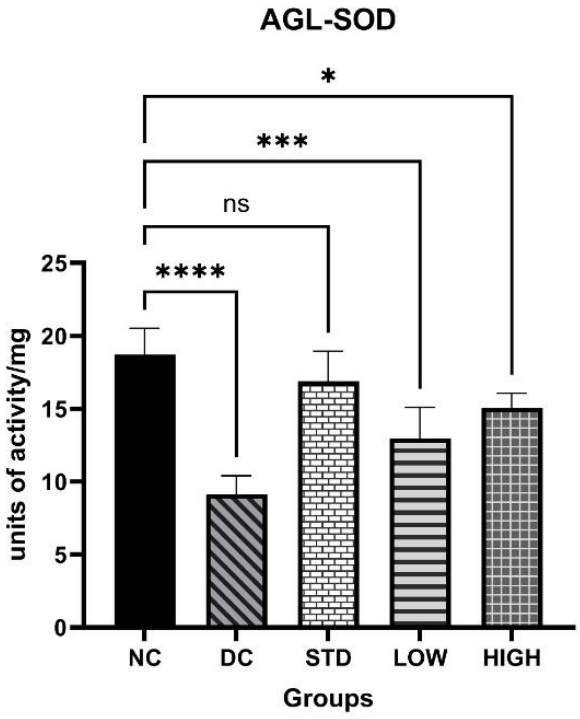


Fig 16: Effect of AGL on SOD at 35th day:



Discussion

Discussion

In this study, we investigated the antioxidant properties of the mangrove plant Excoecaria agallocha, and it demonstrated a significant result in antioxidant tests like DPPH and the Radical Scavenging Test. We also conducted various phytochemical analyses of Excoecaria agallocha, which revealed the presence of various chemical constituents like carbohydrates, glycosides, flavonoids, saponins, steroids, alkaloids, gums, fixed oils, fats, tannins, and phenols. And they showed positive results and encouraged us to conduct the in vivo animal study.

RIF-INH exposure is a commonly used hepatotoxicity model for studying natural compounds' hepatoprotective properties. Chronic liver disease is the 12th leading cause of death globally. ATT-induced hepatotoxicity has inhibited effective TB treatment. RIF and INH, the first-line antituberculosis medicines, have been shown to cause severe hepatotoxicity. RIF and INH combination treatment has been linked to a high rate of serious liver injury. This damage is prevalent when RIF is co-administered with INH. However, the mechanism behind RIF-INH-induced hepatotoxicity remains unclear. RIF is a potent inducer of liver mixed-function oxidases. When combined with drugs that promote its metabolism, such as INH, the strength of induction doubles, potentially leading to toxic metabolites that trigger immune responses and liver injury. INH, a hydrazide of hydralazine, is easily oxidised to metabolites such as hydralazine, acetyl hydralazine, and monoacetyl hydralazine by two enzymes, N-acetyltransferase 2 and amidohydrolase. The first two metabolites can be further oxidised by CYP450 to produce hazardous intermediates. Previously, it was thought that acetyl hydralazine bioactivation was the predominant mechanism of liver injury, but an increasing body of data suggests that INH auto-oxidation, with subsequent free radical formation, is the principal cause of hepatotoxicity. The biotransformation of INH into a more reactive metabolite capable of covalently interacting with hepatic proteins may activate the immune system and cause liver damage. Interindividual variations in INH acetylation rates have been highlighted as fast, moderate, and slow acetylators. The rate of acetylation of INH may influence a person's chance of developing hepatotoxicity. Both monoacetyl hydralazine and ROS production have been associated with liver injury.RIF has a synergistic effect on INH-associated hepatotoxicity because it activates enzymes involved in drug metabolism. (Nwidu, L. L., &Oboma, Y. I. (2019))

In this in-vivo study, we administered an INH+RIF dose (100 mg/kg BW, each) and we found a 2-3-fold rise in liver dysfunction indicators such as ALT, AST, ALP, and bilirubin following INH and RIF therapy. The elevated level of liver markers in blood might be attributed to their leaking from liver cells during INH and RIF-induced hepatocellular injury. An oral administration of AGL High (400mg/kg BW) and Low (200mg/kg BW) in Wistar rats for 5 weeks to check the hepatoprotective effect of this crude drug and silymarin was used as standard drug for comparison of hepatoprotective effect of AGL. AGL-HA treatment provided considerable dose-dependent protection against INH and RIF-induced liver injury by lowering elevated levels of ALT, AST, ALP, and bilirubin. Our findings support earlier studies done on a similar animal model to examine the mechanism of anti-tubercular medicines' hepatotoxicity and protection from diverse synthetic and natural compounds.

Liver enzymes are generally considered to be blood markers for INH and RIF-induced hepatocellular damage, which has been confirmed by histopathological investigations in our current study. The livers of the normal control and standard groups were histologically evaluated, and the results revealed normal hepatocytes with no inflammatory or necrotic alterations. However, the disease-control group showed hepatocyte deterioration, including vacuolation, inflammation, and necrosis. The current study found that combining AGL at a lower dosage (200 mg/kg) with INH and RIF protected the liver, as evidenced by minor degenerative alterations in hepatocytes, including inflammation and necrosis. However, a higher dose of AGL (400 mg/kg) exhibited liver protection, as seen by normal hepatocyte shape and the absence of inflammatory or necrosis alterations.

RIF-INH induces hepatotoxicity by a significant cellular change in defence systems. The toxic metabolites cause oxidative stress in the hepatocytes, resulting in hepatocellular damage. INH has been reported to activate the CYP450 system, resulting in increased metabolism, upregulation of toxic metabolites, ROS, glutathione depletion, and hepatocellular damage. In terms of antioxidant enzyme effects, intoxication with antituberculosis medications (RIF-INH) caused a substantial rise in MDA and a reduction in GSH in the disease control group as compared to the normal control group. Furthermore, the rise in MDA shows that excessive free radical generation and lipid peroxidation are involved in a variety of illnesses. Furthermore, AGL and silymarin therapy reduced MDA significantly while increasing SOD, GSH, and CAT levels as compared to the RIF-INH control group. The injection of AGL probably inhibited the formation of severe free radicals, reducing MDA generation in the liver and lipid peroxidation in rats. CAT and SOD are front-line antioxidant defence enzymes that work together. Extreme superoxide anion production inactivates SOD, resulting in CAT inactivation. In this investigation, AGL treatment reduced the generation of superoxide anion, which led to an increase in SOD and CAT antioxidant enzyme levels in the liver. As a result, administering AGL reduces hepatocyte damage and maintains GSH content, shielding the liver from oxidative stress by enhancing GSH activity. The histological examination supports the biochemical findings since AGL and silymarin at the tested concentrations reduced RIF-INH-induced hepatotoxicity. AGL reduced experimental-induced hepatotoxicity and oxidative stress in a dose-dependent manner. The unidentified bioactive compounds present in AGL may contribute to the reported hepatoprotective, cytoprotective, anti-lipid peroxidation, hepatocyte stabilisation, and anti-oxidative stress actions. Future research is needed to identify the bioactive molecules responsible for the hepatoprotective effects.

Conclusion

The study concludes that the hydro-alcoholic extract of Excoecaria agallocha bark has been demonstrated to ameliorate liver damage induced by INH and RIF. The phytochemical analysis of AGL extract revealed a rich composition of bioactive compounds, such as polyphenols, flavonoids, and terpenoids, which have significant antioxidant properties in vitro and are essential for reducing DILI-induced oxidative damage. In vivo investigations on Wistar rats demonstrated that the extract affects DILI metabolic pathways, reducing biomarkers (ALT, AST, ALP, and BILIRUBIN), improving antioxidant properties, and boosting anti-inflammatory properties. In the in-vivo study, rats treated with the extract showed improved quality-of-life ratings, less hepatic damage, and balanced liver enzyme levels compared to the placebo group. Because of its safety and tolerability, the extract may be used as a substitute to DILI treatment. However, large-scale randomised controlled trials are needed to demonstrate its efficacy and safety, with future research focused on appropriate dose regimens, long-term effects, and possible interactions. More study is needed to determine the specific mechanisms of action for AGL extract. More research is needed to fully understand the plant extract's mechanisms of action, which might include metabolic control, antioxidant, and anti-inflammatory properties. This work emphasises the need to research traditional medicinal plants as novel therapeutic agents, and it contributes to the expanding body of evidence supporting plant-based therapies for DILI.

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