HEPATOPROTECTIVE ACTIVITY OF *LACTOBACILLUS PLANTARUM* AGAINST SODIUM ARSENITE-INDUCED HEPATOTOXICITY IN RATS

Thesis submitted in partial fulfillment for the requirements of the degree of

MASTER OF PHARMACY

Submitted by,

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Certificate of Approval

This is to certify that the thesis entitled "**Hepatoprotective activity of *Lactobacillus plantarum* against Sodium Arsenite-induced Hepatotoxicity in Rats**" has been carried out by **Mr. Chiranjib Bhattacharyya** under the supervision of Prof. Saikat Dewanjee, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032. He has incorporated his findings into this thesis of the same title, being submitted by him, in partial fulfillment of the requirements for the degree of "Master of Pharmacy" of this university. He has pursued this work independently with proper attention to my entire satisfaction.

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DECLARATION

I, the undersigned solemnly declare that the project report on "**Hepatoprotective activity of *Lactobacillus plantarum* against Sodium Arsenite-induced Hepatotoxicity in Rats**" is submitted in the partial fulfillment of the degree of "Master of Pharmacy", under the supervision of Prof. Saikat Dewanjee, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032. I assert the statements made and conclusions drawn are an outcome of my research work. I further certify that the work contained in the report is original and has been done by me under the general supervision of my supervisor.

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DEDICATED TO MY PARENTS

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Preface

The presence of arsenic in drinking water and food can be harmful to both humans and animals. Aquatic animals are particularly vulnerable to arsenic exposure because they rely on water for survival. This contamination of water poses a significant risk to their existence. Arsenic can also accumulate in animals that humans consume, compromising the safety of these food sources. To treat arsenic toxicity, the primary approach is to remove arsenic from the body's tissues. Chelators have shown some effectiveness in treating acute arsenic poisoning, but their effectiveness in chronic cases is uncertain. Most chelators have side effects and limitations, so new approaches are needed. The use of phytochemicals to combat arsenic toxicity faces challenges, so it may be more reasonable to explore the use of probiotics as a primary treatment. Probiotics have been found to have positive effects on various liver diseases by reducing oxidative stress, inflammation, and fibrosis. They do this by changing the composition and function of the normal gut bacteria, which helps maintain a healthy digestive system. This leads to improved energy production and prevents negative effects on the liver. Probiotics also reduce oxidative stress in the liver by activating the body's antioxidant system. Some probiotics also activate signals that increase the production of antioxidant enzymes and substances. Additionally, probiotics have a metal-chelating effect that enhances their ability to act as antioxidants. Therefore, the goal is to use *Lactobacillus plantarum* to treat liver damage caused by arsenic and achieve better and safer treatment outcomes.

This thesis has been organized into six distinct chapters. Chapter 1 provides a concise introduction to the mechanisms of arsenic-mediated hepatotoxicity and highlights the limitations of conventional therapeutic strategies. Chapter 2 presents a comprehensive literature survey on *Lactobacillus plantarm* in its relevant context. Chapter 3 outlines the materials and methods employed to conduct the research. Chapter 4 focuses on the results and includes relevant discussions. Chapter 5 presents the conclusion of the study, while chapter 6 lists the references that contributed to the significant findings of this research.

Chapter 1

Introduction

Contents

1. Introduction

Arsenic, a toxic metalloid, has raised significant concerns regarding its impact on the health of humans and animals (Das et al., 2010a). The main natural arsenic sources are rocks, which can be released and mobilized through various natural processes (Das et al., 2018). In addition to these natural sources, industrial activities can also contribute to the release and movement of arsenic in different forms, contaminating soil, water, and air (Das et al., 2018). It is important to note that organic arsenic compounds do not pose significant health risks (Das et al., 2010b). However, inorganic trivalent arsenicals, such as arsenites (AsO_2^-), are highly toxic (Das et al., 2010c). The contamination of drinking water with arsenites is believed to be the leading cause of arsenic-related issues affecting over 140 million people in approximately 70 countries (Das et al., 2010b). When arsenic is ingested, it is absorbed by the gastrointestinal tract and accumulates in various organs (Dua et al., 2015). It can also enter the body through the respiratory system and the skin (Dua et al., 2016). Consumption of arsenites in quantities exceeding 30 μg per day can lead to arsenicosis, affecting critical organs (Das et al., 2018). Previous studies have shown that arsenic reduces the integrity of mitochondria, resulting in the production of superoxide radicals, which then trigger a series of radical reactions and enhance the production of other reactive oxygen species /reactive nitrogen species (ROS/RNS) resulting in apoptosis (Das et al., 2018). On the contrary, arsenic exacerbates oxidative stress by interacting with -SH groups, leading to the deactivation of defense mechanisms in antioxidant enzymes and the glutathione system (Das et al., 2010c). This excessive production of ROS can have detrimental effects on cells, including damage to their structural components, changes in gene expression, DNA damage, and apoptosis (Dua et al., 2015; Dua et al., 2016). Despite being a major global health threat, the development of an effective therapy for arsenic poisoning is still eluding the medical fraternity. The primary approach to treatment involves the use of chelating agents, but their clinical usefulness is limited due to the adverse effects of essential metals' removal and redistribution of arsenic (Dua et al., 2015). Additionally, the relatively short biological half-life of inorganic arsenic, approximately 10 hours, argues against the use of chelating agents as a therapeutic option (Kosnett, 2013). The increased oxidative stress caused by arsenic has been

identified as the main cause of arsenicosis, making it worthwhile to explore the potential role of probiotics in mitigating the toxic effects of arsenic.

Probiotics consist of harmless, living microorganisms, such as certain friendly bacteria, that offer significant benefits to the body and help prevent diseases when consumed in the correct amount. Probiotics primarily consist of various strains of bacteria, including *Lactobacillus, Bacillus, Bifidobacterium, Streptococcus*, and *Enterococcus*. The microorganisms in our gut have an important role in maintaining liver health. Therefore, adjusting the gut microbiota could potentially be a beneficial approach to protecting the liver. Probiotics may have a therapeutic effect in preventing liver injury by restoring the gut microbiota. Numerous studies have shown that probiotics can prevent liver damage by strengthening the intestinal wall, reducing bacterial translocation and invasion, and decreasing endotoxemia (Dewanjee et al., 2022; Twardowska et al., 2022). They can also activate the production of antimicrobial peptides and enhance the immune system (Eslamparast et al., 2013, Twardowska et al., 2022). Additionally, probiotics have been found to alleviate oxidative and inflammatory liver damage (Dewanjee et al., 2022). Various preclinical studies have demonstrated that probiotics can prevent acute liver injury caused by oxidative stress (Forsyth et al., 2009; Rishi et al 2009; Gu et al., 2019; Patel et al., 2019). Therefore, the main aim of the present research was to investigate the effects of probiotic *Lactobacillus plantarum* (*L. plantarum)* on mitigating the detrimental consequences of arsenic exposure specifically in the liver of rats.

1.1. Arsenic: a potential pollutant to cause hepatotoxicity

The liver plays a significant role in detoxifying metal contaminants, particularly arsenic (Hedayati, 2016). Methylation reactions occur in the liver, catalyzed by methyltransferases, which transform inorganic trivalent arsenic into methylarsinic acid (MMA) and dimethylarsinic acid (DMA), allowing for easy excretion in urine (Jan et al., 2015). Glutathione helps convert pentavalent arsenic to the trivalent form, facilitating these methylation reactions (Jan et al., 2015). Given the liver's abundance of glutathione, it is the primary site for arsenic detoxification (Susan et al., 2019). However, high concentrations of arsenic can significantly increase the liver's vulnerability to toxic effects from arsenic poisoning (Susan et al., 2019). The methylated forms of trivalent arsenic have been found to cause liver toxicity by interacting with DNA and protein targets in hepatocytes, which

ultimately reduces methylation efficiency and leads to arsenic accumulation in the body (Sharma et al., 2014). The DMA which formed in the liver conjugate with glutathione to form dimethylarsenic-glutathione (DMA-SG) conjugate. With the help of glutathione reductase (GR) and NADH this get conveterd to dimethylarsine (DMAS) which further gets conveted into dimethylarsine peroxy radical (DMASPO), this peroxy radical is the main reason for arsenic-mediated toxicities (**Figure 1**). Liver enlargement is a prominent indication of arsenic toxicity and is often accompanied by recurring dyspepsia that can ultimately result in liver fibrosis (Guha and Dasgupta, 2011). Chronic arsenic exposure can lead to hepatoportal sclerosis, which is often accompanied by damage to local blood vessels (Majumdar, 2020). Other conditions associated with arsenic poisoning include cirrhosis, hepatic infiltration, degenerative lesions, focal necrosis, ascites, and hepatocellular carcinoma. Studies have shown that continuous exposure to arsenic can cause hepatocellular proliferative lesions in female Swiss mice and promote the formation of malignant cell types in rat livers (Susan et al., 2019). It is believed that DNA damage caused by methylated arsenicals and impaired DNA repair mechanisms, resulting from arsenic's interactions with zinc finger proteins involved in DNA repair, contribute to the development of hepatocellular carcinoma (Muenyi et al., 2015). DNA damage caused by arsenic leads to apoptosis (**Figure 1**). Increased levels of cyclin D1 and proliferating cell nuclear antigen (PCNA) due to arsenic exposure have also been linked to arsenic-induced carcinogenesis (Liu and Waalkes, 2008). Arsenic-induced hypomethylation of the estrogen receptor-alpha can lead to abnormal signaling, ultimately resulting in hepatocellular carcinoma (Chen et al., 2004). Interestingly, hypermethylation of p16 and p53 genes by arsenic has also been suggested as potential causes for arsenic-mediated carcinogenesis (Chanda et al., 2006). Liver enzymes such as aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) have been found elevated in the bloodstream following exposure to arsenic (Das et al., 2018; Gora et al., 2014; Renu et al., 2020). This occurs due to the leakage of enzymes and damage to cell integrity. Furthermore, the stress caused by arsenic results in a significant increase in the release of lactate dehydrogenase (LDH) and apoptosis (Renu et al., 2020). The presence of markers such as nitric oxide, lipid peroxidation, ROS, AST, ALT, and intracellular calcium levels have all been shown to increase due to exposure to arsenic. The elevated

AST levels specifically harm liver cells (Renu et al., 2020). The enzyme AST, which is dependent on pyridoxal phosphate (PLP), plays a role in converting aspartate and α-ketoglutarate to glutamate and oxaloacetate. Increased levels of AST in the liver can indicate cell damage. Mice treated with arsenic also exhibited high levels of hepatic enzymes, including AST and ALT (Abhilash et al., 2018). The rise in enzyme levels indicates cellular damage and a disruption of the functional integrity of the hepatic membrane (Abhilash et al., 2018).

Arsenic impedes the production of tetrapyrroles by inducing the hemeoxygenase (HO) enzyme, resulting in increased oxidative stress and decreased heme biosynthesis (Mishra et al., 2016). Arsenic has been shown to inhibit aminolevulinate dehydratase (ALAD), a crucial enzyme in heme biosynthesis (Bhadauria and Flora, 2004). By binding strongly to the –SH group, arsenic effectively blocks the activity of ALAD, which leads to a decrease in hemoglobin synthesis and weakens red blood cells (RBCs). Additionally, ALAD dysfunction triggers excessive production of δ-aminolevulinic acid (δ-ALA), which in turn generates ROS (**Figure 1**). The presence of arsenic in the body can lead to an increase in the production of ROS, which in turn enhances lipid peroxidation in the liver (Singh et al., 2011). This can be observed through the up-regulation of markers such as thiobarbituric acid (TBAR) and malondialdehyde (Susan et al., 2019). Additionally, arsenic poisoning can result in a decrease in the activity of the enzyme glutathione peroxidase (GPx) and glutathione (GSH) levels, both of which play important roles in managing oxidative stress (Susan et al., 2019). This depletion of liver glutathione levels may occur due to various factors, such as inhibition of glutathione biosynthesis or arsenic-GSH complexation (Hall et al., 2013). Furthermore, the exposure to arsenic has been found to cause a significant decrease in the levels of glutathione-S-transferases (GSTs), which are enzymes responsible for detoxifying harmful substances in the body (Susan et al., 2019). As a consequence, the accumulation of arsenic in the body can occur. GST has also been identified as a regulator of the JNK pathway, which is involved in cell death (Laborde, 2010). The reduction in cellular GST and GSH levels can lead to a decrease in the formation of the GST-JNK complex, triggering the JNK-mediated apoptotic pathway (Townsend and Tew, 2003). Moreover, the presence of elevated levels of ROS can also disrupt the GST-JNK complex, further promoting JNK-mediated apoptosis (Townsend and Tew, 2003). Therefore, arsenic can cause

damage to hepatocytes through various mechanisms. It activates the JNK and p38 MAPK signaling pathways, leading to changes in gene expression that contribute to arsenic-associated liver cancer (Singh et al., 2011). Additionally, arsenic induces oxidative stress, apoptosis, and the up-regulation of transcription factors such as AP-1, ATF-2, and Elk-1, which are key factors in arsenic-induced liver and kidney damage (Singh et al., 2011). Furthermore, the PI3K/Akt phosphorylation pathway, which is essential for cell cycle progression, glucose metabolism, DNA repair, and protein synthesis, can be disrupted by arsenic exposure (Chen and Costa, 2018).

Superoxide dismutase (SOD) is a metalloenzyme that plays a crucial role in the cells' antioxidant defense system (Singh et al., 2017). The harmful effects of arsenic on the renal and liver tissues include a decrease in SOD activity, caused by an excessive amount of free radicals. GPx, another enzyme, also acts as an antioxidant by eliminating lipid peroxides and hydrogen peroxides. An independent study has shown that arsenic exposure promotes the production of superoxide radicals, which in turn leads to the generation of hydroxyl radicals, resulting in increased levels of ROS and genotoxicity (Jomova et al., 2011).

1.2. Effect of arsenic on Nrf2 in an imbalance redox reaction

Oxidative stress and inflammation are key contributors to the development of arsenic-related diseases. Nuclear factor erythroid 2–related factor 2 (Nrf2), which is a transcriptional factor and regulator of antioxidant response, is primarily disrupted by oxidative stress. Nrf2 and NF-κB play central roles in controlling oxidative stress and inflammation (Flora, 2011, Li et al., 2018). Targeting these regulators could lead to the development of effective treatments for diseases associated with oxidative stress and inflammation (Li et al., 2018). When Nrf2 expression is depleted, it inhibits the NF-κB pathway and increases the production of proinflammatory cytokines. Furthermore, persistent high levels of ROS/RNS can activate NF-κB, leading to the release of proinflammatory cytokines such as TNF-α, IL-1β, IL-8, and IL-6 (Mishra et al., 2021) (**Figure 1**). Chronic exposure to arsenic has been linked to increased levels of proinflammatory cytokines and NF-κB expression in various organs (Yan et al., 2020).

Nrf2 has become a crucial factor in the regulation of genes that assist in the detoxification and elimination of liver oxidative damage caused by arsenic (Thangapandiyan et al., 2019). Chronic exposure to arsenic leads to oxidative

stress, which in turn causes depletion of Nrf2 and lowers the levels of antioxidant enzymes (Ma, 2013). The level of Nrf2 was found to decrease in Chang liver cells which were exposed to arsenic for 48 hours (Vineetha et al., 2018). Additionally, the levels of NQO1, GST, and HO-1 also decreased along with Nrf2. The dysregulation of Nrf2 is the underlying cause of liver cell damage. In mice lacking Nrf2, there is a significant elevation in the levels of growth arrest and DNA damage 1 and growth arrest and DNA-damage-inducible protein, which are recognized as indicators of DNA damage and stress within the endoplasmic reticulum (Renu et al., 2020). This suggests that a deficiency in Nrf2 renders mice more susceptible to stress. The absence of Nrf2 activation ultimately leads to liver toxicity (Liu et al., 2013). Thus it can be concluded that the Nrf2 signaling pathway has a major role for the hepatotoxic effects induced by arsenic. Depletion in Nrf2 level which is caused by arsenic cause reduction in the levels of antioxidant defence enzymes and molecules (GPx, GR, SOD, CAT, GST and GSH), which leads to the formation of ROS and RNS which results in the toxic consequences (**Figure 1**).

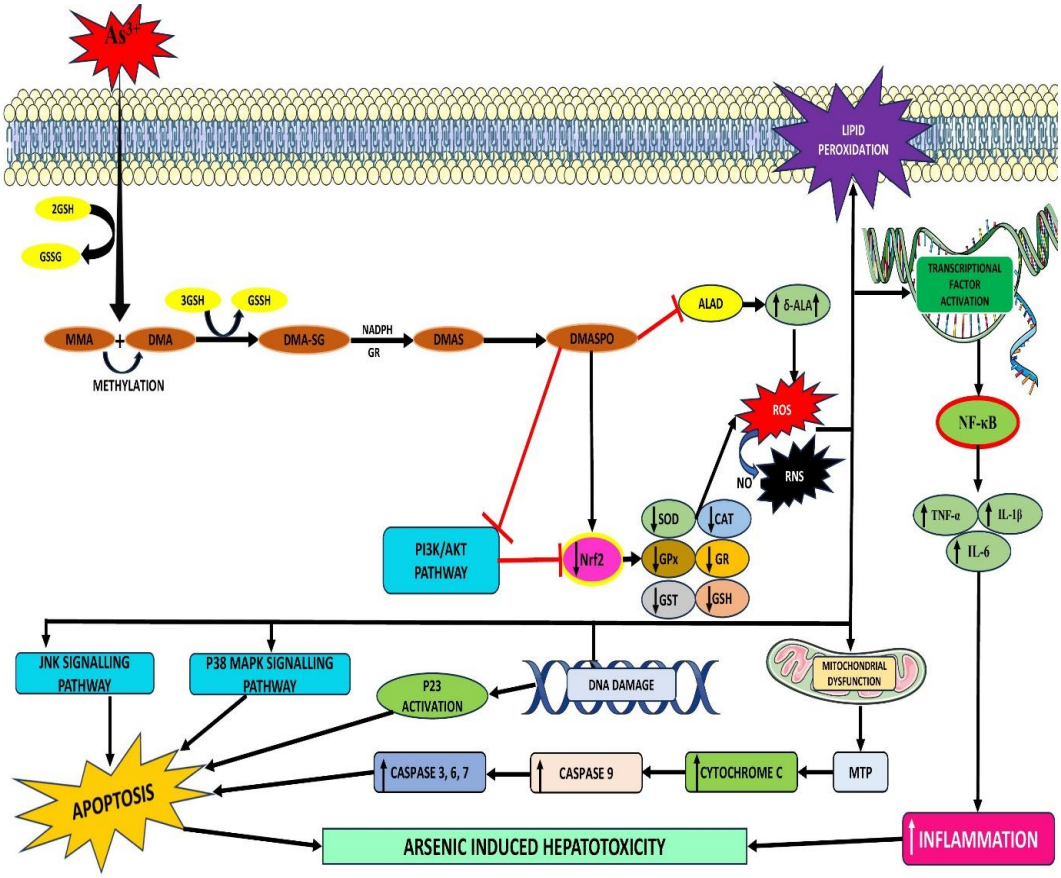

Figure 1. Mechanism of arsenic in arsenic induced hepatotoxicity

1.3. Possible therapeutic strategies against arsenic-induced toxicities

1.3.1 Chelation therapy

Since arsenic is a metalloid, researchers have looked into using ligands to form complexes with arsenic in order to remove it from the body. Chelation prevents arsenic from interacting with proteins and DNA and helps to get rid of it from the body (Susan et al., 2019). The main challenge is finding a chelating agent that is safe, can be dissolved in fat, has a strong affinity towards arsenic, and can be eliminated from the body while it is still in the complex state (Susan et al., 2019). Since complexation reactions tend to reach equilibrium, it is essential to continuously eliminate the complex formed between arsenic and the chelating ligand (Sears, 2013; Smith, 2013). Fat-soluble complexes can be more toxic because they are better absorbed by the gastrointestinal system, while water-soluble complexes are easier to eliminate (Susan et al., 2019). The brief descriptions of some synthetic chelating agents against arsenic toxicity have been shown in **Table 1**.

Table 1. Mechanisms and limitations of commercially available chelating agents against arsenic toxicity

Names	Mechanisms	Side effects	References
(BAL)	The thiol groups present in BAL complex with arsenic and the resultant chelate is eliminated through urine.	It can redistribute arsenic to the brain, potentially causing nephrotoxicity, hypertension, tachycardia, and having a poor therapeutic index. Oxidized BAL-arsenic complex release arsenic, which can be harmful to the system.	Aaseth et al., 2015; Susan et al., 2019
DPM	Can bind to arsenic using its sulfhydryl group and get excreted from the body through urine and feces.	Nausea, gastrointestinal distress, hematuria, leukopenia, hem olytic anemia and proteinuria.	Aaseth et al., 2015; Susan et al., 2019
DMSA	It binds to arsenic using two thiol groups. The use of this substance is restricted because it cannot enter the cell membrane, making it unable to remove arsenic from inside the cells	Nephrotoxicity, backache, diarrhoea, drowsiness, fever, flu like symptoms, haemorrhoids, GI-irritation, placid neutropenia, skin allergies, elevated liver enzyme level etc.	Aaseth et al., 2015; Susan et al., 2019
DMPS	It employs an organic anion transport pathway to permeate into the cell and forms an insoluble complex with arsenic.	Headache, nausea, taste impairment, pruritus and skin allergies, leukopenia etc.	Aaseth et al., 2015; Yadav and Flora, 2016.
MiDMSA	This compound effectively binds to arsenic using sulphydryl groups, dissolves in fats, and reverses arsenic's harmful effects on the body's oxidative stress and nervous system.	Depletion of the essential metal ion pool such as copper and zinc in the body and induction of mild anemia	Pachauri et al, 2013; Aaseth et al., 2015; Susan et al., 2019

BAL = British anti-Lewisite, DPM = D- penicillamine, DMSA= Meso-2,3-dimercaptosuccinicacid, DMPS =2,3-Dimercaptopropane-1-sulphonic acid, MiDMSA = Monoisoamyl DMSA

1.3.2 Anti-oxidant therapy

The use of anti-oxidant therapy has become a popular method for combating the harmful effects of arsenic, which primarily occur due to oxidative stress. Arsenic disrupts the body's natural anti-oxidant processes, so supplementing with external anti-oxidants can help reduce the oxidative stress caused by arsenic. Certain flavonoids, such as silymarin found in *Silybum marianum*, have been found to have anti-oxidant properties that can effectively treat the toxic effects of arsenic (Susan et al., 2019). In a study using CHO-K1 cells, it was observed that exposure to arsenic led to increased lipid peroxidation and decreased GGT levels, but these effects were reversed when treated with silymarin at concentrations of 5 μM and 0.125 μM (Bongiovanni et al., 2007). Melatonin and N-acetyl-5-methoxy tryptamine, which are found in mammals and certain foods, have been shown to reduce oxidative stress in the brains of animals exposed to arsenic (Lin et al., 2007). Naringenin, found in citrus plants, has been shown to mitigate liver and kidney damage caused by arsenic exposure (Mershiba et al., 2013). Epigallocatechin-3-gallate (EGCG), a compound in green tea, has been found to protect against testicular toxicity and inflammation caused by arsenic exposure in mice (Guvvala et al., 2017, Yu et al., 2017). EGCG has been shown to counteract the harmful effects of arsenic on the heart in rat models by acting as an antioxidant (Sun et al., 2016). Sulforaphane, found in vegetables like broccoli and cabbage, has been found to reduce arsenic accumulation in liver cells by activating the xenobiotic elimination pathway mediated by Nrf2 (Shinkai et al., 2006; Zheng et al., 2012). Allicin, found in garlic, and eriodictyol, a citrus flavonoid, are believed to have a similar mechanism of action on Nrf2 in conditions of arsenic-induced oxidative stress (Yang et al., 2017; Xie et al., 2017). Diallyltrisulfide, another compound found in garlic, has been shown to reverse dyslipidemia, pro-inflammatory changes, and liver damage in rats exposed to arsenic (Sumedha and Miltonprabu, 2015). Hydroxytyrosol, a compound found in olive leaves, has been shown to have antioxidant properties that can reverse the damage caused by arsenic in rat models (Soni et al., 2018). Other compounds such

as lutein, oleuropein, and ellagic acid have also been found to reduce oxidative stress caused by arsenic in rodents (Li et al., 2015, Ogun et al., 2016, Keshtzar et al., 2016). Combining the flavonoids silymarin and naringenin can significantly affect certain enzymes and reduce arsenic levels in rats (Jain et al., 2011). Vitamins C and E have been used to treat arsenic poisoning and have shown positive effects on tissue health (Susan et al., 2019). Taurine, an amino acid, can protect against arsenic damage by scavenging free radicals and protecting cell membranes (Susan et al., 2019). Nanoparticulate curcumin also effectively reduces arsenic-induced toxicity due to its strong antioxidant properties (Sankar et al., 2014). **Table 2** discusses the various phytochemicals that can be utilized to mitigate the harmful impacts and toxicity resulting from arsenic.

Table 2. A general overview of some phytochemicals against arsenic-induced toxicity

Names	Mechanisms	Limitations	References
BAC	It decrease the levels of IL-6, TNF-α, Bax, Caspase-3, TLR4, and NF-kβ. It also deactivates the NADPH activity.	Poor water solubility and low bioavailability The administration of baicalein is associated with common side effects, such as fatigue and shortness of breath.	Varsha et al., 2017; Sun et al., 2021; Tsai et al., 2021; Wen et al., 2023
CCM	It increase the activity of antioxidant enzymes, activates the Nrf2 pathway, and it also decrease chromosomal abberations and DNA damage in cells.	Common side effects like diarrhea, headache, rash, and yellow stool have been reported. Prolonged use may cause nausea and diarrhea and an increase in serum alkaline phosphatase and lactate dehydrogenase contents. Curcumin's low solubility and bioavailability are its primary drawbacks and prevent its use as a therapeutic agent.	Sankar et al., 2013; Yu and Liao, 2014; Hewlings and Kalman, 2017; Wu et al ., 2021; Damayanti et al., 2023
EGCG	Enhance the intracellular antioxidant response system by modulating Nrf2 pathway	Although EGCG has tremendous potential, it is important to note that it has a relatively short half-life, low stability, and low bioavailability. These factors	Singet al., 2021; Sarkar and Sinha., 2018; Guvvala et al., 2019; Granja et al.,

		significantly restrict its utilization in clinical settings. Hypoglycemia, hypochromic anaemia, liver and kidney failure are its major adverse effects followed by prolonged or high dose usage of it.	2016; Mereles and Hunstein, 2011
EA	It decrease the levels of IL-1β, TNF-α, Bax, Bcl2 Caspase-3,liver enzymes also increase the levels of antioxidant enzymes.	The clinical implementation of ellagic acid is currently a subject of ongoing debate due to its low solubility, limited permeability, first pass effect, and variations in gut microbial transformations, which unfortunately contribute to its poor bioavailability.	Khan et al., 2022; Zuccari et al., 2020
GA	Increase the level of antioxidant enzymes in kidney and liver tissues.	Poor stability and low bioavailability.	Hosseinzadeh et al., 2019; Bai et al., 2021
MIG	Decrease the levels of ROS, IL-1β, IL-6, TNF-α and restores the level of Nrf2 in liver.	Significant drug accumulation was observerd in pharmacokinetic studies on human. Lack of proper trials.	Sun et al., 2007; Jain et al., 2011
NG	Improves the activity of antioxidant enzymes like SOD, CAT, GSH, GST, GPx in liver and kidney.	The limited solubility and permeability of naringin, caused by its large hydrophobic ring structure, unfortunately hinder its bioavailability. As a consequence, it poses challenges in its utilization for clinical purposes. Cough, Dizziness, Headaches, Flushing sensations are its major side effects	Mershiba, 2013; Roy et al., 2014; James, 2020 ; Sharma et al., 2021
QC	Improve Nrf2 level also downregulate cytochrome c and hydroxyproline level in liver.	The most common side effects of quercetin include headaches, upset stomach, and tingling sensations in the arms and legs. It has low oral bioavailability and poor aqueous solubility.	Jahan et al., 2016; Ghosh et al., 2009; Burdeos and Davis, 2023; Nam et al., 2016

RSV	Activates Nrf2 pathway, increase the levels of antioxidant enzymes in liver and other tissues	Taking higher doses of resveratrol may potentially cause occasional episodes of mild diarrhea, nausea, hypersensitivity, and anal pruritus. The main obstacles faced by resveratrol are its poor pharmacokinetics and low potency.	Khan et al., 2022; Shaito et al., 2020; Ren et al., 2021
TA	Increase Nrf2 expression in serum and tissues	Tannic acid can be potentially harmful when inhaled or ingested. In cases of high-dose ingestion, it may cause discomfort such as nausea, vomiting, constipation, abdominal pain, and potential liver damage. Severe intoxications could lead to centrilobular liver necrosis The clinical applications of tannic acid have been limited due to its challenges in terms of poor lipid solubility, low bioavailability, off-taste, and short half-life	Jin et al., 2020; Robles, 2005; A Youness., 2021

BAC = Baicalin, CCM= Curcumin, EA= Ellagic acid, GA= Gallic acid, MIG = Magnesium isoglycerrhizinate, NG = Naringenin, QC = Naringenin, RSV = Resveratrol, TA= Tannic acid.

1.4. Origin of research problem

Arsenic, a highly toxic metalloid, is naturally distributed in the environment through both natural and human activities (Lim et al., 2014). The concern for public health increased when arsenic-related diseases became widespread in many countries (O'Bryant et al., 2011). The human body can absorb arsenic through ingestion or inhalation, but ingested arsenic has a shorter lifespan due to its increased transformation in the liver. Both ingested and inhaled arsenic is absorbed by the gastrointestinal tract, lungs, and then enters the bloodstream where it is distributed to various organs (Susan et al., 2019). Research has shown that approximately 99% of arsenic absorbed through the gastrointestinal tract binds to the hemoglobin in red blood cells and is transported throughout the body (Pr et al., 2015). Human exposure to arsenic primarily occurs through

contaminated drinking water, inhalation, and skin contact (Susan et al., 2019). The use of arsenic-contaminated herbicides and volcanic eruptions also contribute to the risk of arsenic toxicity. Arsenic-based pesticides, such as lead arsenate and sodium arsenate, are easily absorbed by plants, fruits, and sea flora, which can indirectly affect humans (Susan et al., 2019). Although these pesticides are banned in many countries, their occasional and covert use still leads to environmental contamination and associated health risks.

The intake of arsenic through drinking water or diet can lead to the accumulation of arsenic in both humans and animals. This can cause harmful effects on the organism and pose a serious threat to their safety and well-being. It is important to note that aquatic animals are particularly vulnerable to arsenic exposure compared to land animals. Since aquatic animals rely on water for survival, the widespread contamination of water with arsenic poses a significant risk to their existence (Su et al., 2023). Moreover, arsenic can also accumulate in the food animals consumed by humans, such as chickens, ducks, cattle, sheep, and fish, compromising the safety of animal-based food sources (Su et al., 2023). This issue demands our attention and concern. Therefore, our primary therapeutic approach would be to remove arsenic from the tissue, either by reducing oxidative stress or by liganding with it, in order to eradicate the toxicities caused by this metalloid. The chelators DMPS, DMSA, BAL, and D-penicillamine, when considered, show reasonable effectiveness in treating acute arsenic intoxication if administered early (within minutes or hours) (Mishra et al., 2022). Chelation can help speed up the elimination of arsenic and reduce tissue damage in chronic arsenic intoxication. However, it is still unclear how effective these various treatments are in reducing malaise and fatality rates in cases of chronic arsenic poisoning. It is evident that most chelators have various side effects and disadvantages, making them unsuitable for treating chronic arsenic toxicity (Mishra et al., 2022). Therefore, new approaches are needed to eliminate arsenic from the body with minimal negative effects. However, it is important to note that the use of phytochemicals for combating arsenic toxicity faces certain challenges. These challenges include the low bioavailability and biodistribution of phytochemicals, as well as the lack of human cohort data. Furthermore, the working mechanism of most of these phytochemicals is not fully understood, as their action is concentration-dependent and they exhibit contradictory activity in different disease models (Qi et al.,

2022). Given these factors, it would be more reasonable to consider probiotics as a major therapeutic agent against metalloid poisoning.

Probiotics have been found to have positive effects on various liver diseases by reducing oxidative stress, inflammation, and fibrosis (Sharma et al., 2013, Meng et al., 2018, Xu et al., 2019). The primary mechanism through which probiotics work is by modifying the composition and function of the normal gut flora. By maintaining a healthy microbiome in the intestine, probiotics help restore intestinal balance, leading to improved ATP production and preventing negative metabolic effects on the liver (Dewanjee et al., 2022). Organic acids produced by probiotic bacteria can enter the cell membrane, which in turn inhibits nutrient transport and ATPase activity (Chávez-Tapia et al., 2015). Additionally, probiotics aid in enhancing the integrity of the gut barrier, effectively preventing harmful bacteria from entering the liver. These pathogenic bacteria can cause damage to the liver by releasing endotoxins and other toxic substances (Meng et al., 2018). Furthermore, numerous reports have indicated that probiotics have the ability to mitigate hepatic oxidative stress, which is a common pathological occurrence in different liver diseases (Javadi et al., 2018, Hsieh et al., 2021, Wu et al., 2022). Probiotic bacteria have been discovered to effectively alleviate oxidative stress through various mechanisms (Mishra et al., 2015, Wang et al., 2017). They can activate the host's inherent antioxidant system and initiate antioxidase activities. Moreover, certain probiotic bacteria have been identified to activate the Nrf2 signaling in the host, leading to the production of host antioxidant enzymes and antioxidases (Wang et al., 2017). They produce a range of metabolites with potential for scavenging ROS, including reduced glutathione (GSH), folate, indole-3-propionic acid, and butyrate. Probiotics also inhibit the production of ROS by interfering with enzymes that produce them, such as NADPH oxidases, CYPs, and cyclooxygenases (Wang et al., 2017). Furthermore, probiotic bacteria have a metal chelating effect that enhances their antioxidant capabilities (Wang et al., 2017). A study demonstrated that probiotics can break down non-absorbable dietary phenolics into smaller molecules, which can be absorbed in the gut and have antioxidant effects (Chávez-Tapia et al., 2015). Therefore, it can be inferred that probiotics offer protection to the liver through various mechanisms.

The main objective of this research has been to investigate the protective mechanisms of probiotics against experimentally induced arsenic poisoning using appropriate preclinical assays.

1.5. Objectives of the study

The main objective of this research has been to investigate the protective mechanisms of probiotics against experimentally induced arsenic poisoning using appropriate preclinical assays. The specific objectives are stated below,

- To assess the potential hepatoprotective effects of *L. plantarum* in cases of arsenic-induced toxicity.
- To investigate the potential mechanisms through which *L. plantarum* exerts its therapeutic effects in the arsenic toxicity model.
- To assess the potential anti-inflammatory properties of *L. plantarum*.
- To assess the potential hypolipidemic effect demonstrated by *L. plantarum* in the presence of arsenic-induced toxicities.
- To evaluate the benefits and future prospects of treatment with *L. plantarum* in xenobiotic toxicity.

Chapter 2

Literature review

Contents

2. Literature review

Lactobacillus plantarum is a gram-positive lactic acid bacterium commonly found in fermented food and in the gastrointestinal tract. It is frequently used in the food industry as a potential starter probiotic. In recent times, the consumption of food along with probiotics has significantly increased. *L. plantarum* has gained attention from researchers due to its numerous applications in the medical field. Our study aims to examine the significance of *L. plantarum* in medical applications. Additionally, this report has compiled various studies that explore the applications of this promising strain. In conclusion, this study aims to attract researchers to consider this strain for the benefit of human health and medical needs. **Figure 2.** depicts various protective actions of L. plantarum in curing diseases.

2.1 Scientifically explored pharmacological activities of *L. plantarum*

2.1.1 Role in neuroprotection

In a study, it has been found that *L. plantarum* supplementation considerably increased the membrane transport ATPases system's activity in the chosen brain areas of the alzheimer's disease-induced group of rats. It was also discovered that *L. plantarum* safeguards neurons by preserving the structural and functional integrity of biological membranes by controlling the gradient of ions in their concentration, which is accomplished by its antioxidant qualities of reducing lipid peroxidation and reversing mitochondrial dysfunction (Mallikarjuna et. al. 2016). Long-term administration of *L. plantarum* supplementation significantly increased memory and learning ability by modulation of oxidative stress pathway. Moreover, it lowered Aβ accumulation and aided in proper maintenance of the arrangement of nerve cells in the hippocampus. With its antioxidant effect, *L. plantarum* slows down ageing and stop age-related cognitive decline (Lin et. al. 2021). Increased locomotion and a decrease in anxiety-like behaviours are brought on by persistent *L. plantarum* consumption. Additionally, treatment with the probiotic results in higher levels of dopamine and 5-HT in the striatum as found in mice model. According to the result, *L. plantarum* may have the ability to act as an anxiolytic agent to control the host's motor activities and mood via the gut–brain axis (Liu et. al. 2016). The protective effect on serotonin level and BDNF/TrkB/CREB pathway in the hippocampus and prefrontal cortex was

observed with continuous administration of *L. plantarum* supplementation in diabetic rats (Morshedi et. al. 2020).

2.1.2 Role in Immunomodulation

L. plantarum is a potent immunomodulator that may be employed to enhance human immunological function (Meng et al. 2018). *L. Plantarum* has the ability to normalise all parameters, enhance immunity, and lessen the harmful effects caused by toxins. Additionally, it could control the Th1/Th2 balance, boost intestinal microbial dispersal, and modify intestinal architecture to preserve the balance of the intestinal flora and improve immune function (Wang et. al. 2022). In a 2004 study, inflamed bowel tissue from seven patients was treated with L. plantarum 299v in a lab setting. The study found that L. plantarum induced the production of a cytokine called interleukin-10 (IL-10) in the tissue. This suggests that L. plantarum may have the ability to reduce inflammation in the mucosal lining by promoting the production of IL-10 (Pathmakanthan et al., 2004). A study was conducted on the effects of *L. plantarum* on Caco-2 cells incubated with tumor necrosis factor-alpha (TNF-alpha). The findings of this study revealed that *L. plantarum* effectively inhibited the induction of epithelial barrier dysfunction and interleukin-8 (IL-8) secretion caused by TNF-alpha (Ko et al., 2007).

2.1.3 Role in cardioprotection

Co-administration of probiotic microorganisms like *L. plantarum* in conjunction with modest dosages of simvastatin may result in a more significant reduction in TC and LDL level and the achievement of treatment efficacy goals in patients with dyslipidaemia (Neverovskyi et. al. 2021). Recent findings suggest that *L. plantarum* is a potential probiotic to lower the possibility of cardiovascular disease linked to a diet high in cholesterol by lowering serum total cholesterol and LDL-C concentrations and raising hepatic cholesterol and bile acid excretion (Liu et al. 2017)

2.1.4 Role in Nephroprotection

L. plantarum is found to combat chronic kidney disease by attenuating plasma urea, creatinine and other oxidative stress parameter in experimental rats. Uremic toxin levels in the blood was reduced and it was aided through antioxidant properties of the probiotic strain (Patra et. al. 2018). Systemic lupus erythematosus can manifest as lupus nephritis. Nephritis is a complicated

immunological condition. In a study, a novel strain of *L plantarum* was isolated and used in an animal model to validate the new strain's interventional effects on lupus nephritis. The findings demonstrated that *L. plantarum* reduced the signs of inflammation in the mice's blood and tissues brought on by lupus nephritis, and in particular, that probiotic strain controlled the production of TGFβ-1, which is the hallmark of lupus nephritis (Cheng et. al. 2022). *L. plantarum* effectively intrudes renal calcium oxalate stone formation. It has been observed that the probiotic ameliorates the imbalances in the gut microbiota and safeguards the intestinal mucosal barrier function. Pro-inflammatory cytokines and prostaglandin level decreases. As a result, LPS is released in the bloodstream and the inflammation is reduced. This leads to a reduction in inflammatory factors in the kidney and rise in the TLR4/NF-κB/ COX 2 signalling pathway and PGE 2 (Tian et. al. 2022).

2.1.5 Role in GI protection

L. plantarum restrain ulcerative colitis and irritable bowel syndrome by enhancing the biological barrier, chemical barrier, mechanical barrier, and immunological barrier and strengthening the intestinal mucosal barrier thereby decreasing inflammation. It acts by upregulating anti-inflammatory cytokines interleukin-1beta (IL-1β), interleukin-6 (IL-6), interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) (Wu et al. 2022). After receiving *L. plantarum* supplementation, there was a rise in the anti-inflammatory cytokine IL10 production, a decrease in IL6 production, and a general reduction in the inflammation of the stomach tissue in the alcohol-induced gastric ulcer murin model. This study confirms that the *L. plantarum* strain exhibits potential probiotic properties by regulating the gut microbiota and reducing stomach erosion (Hu et. al. 2020).

2.1.6 Role in hepatoprotection

Lactobacillus strains can enhance the antioxidant status of rats and humans and may lower the risk of ROS formation. They are responsible for markedly decreasing cholesterol, liver and kidney functional indicators (ASAT, ALT, GGT, ALP, urea, and creatinine). Hepatotoxicity and nephrotoxicity are often accompanied by a decrease in CAT and SOD activities. The toxicity caused by EDS was greatly reduced when *L. plantarum* was given orally to pregnant rats.

Nevertheless, it is still unknown exactly how free radicals are eliminated (Bouhafs et. al. 2015). The therapeutic efficacy of *L. plantarum* in lipopolysaccharide-induced hepatic injury in mice was evaluated in an experiment and it was established that the aforementioned has improved the condition. It was observed that *L. plantarum* significantly lowered the LPS-induced histological changes and the levels of serum or liver AST, ALT, TNF-α, IL-6 (Chen et. al. 2022). Pre intake of *L. plantarum* corrected the intestinal barrier damage, repaired the gut microbiota, prevented lipopolysaccharide from entering the liver through the intestinal-liver axis, and decreased the alcohol-induced high hepatic lipopolysaccharide levels. It minimises the induction of the MAPK signalling pathway that is mediated by TLR4 and lowers the onset and progression of liver inflammation (Li et. al. 2022). *L. plantarum* strains K2 and K6 reduced high fat diet-induced Non-Alcoholic Fatty Liver Disease (NAFLD) in wistar rats by controlling liver function, and influencing the expression of genes linked to lipogenesis, antioxidant enzymes, and liver function. Both strains were also successful in enhancing the diversity of the faecal microbiome (Park et. al. 2020). Increased antioxidant enzyme activity mediated through Nrf2 signalling was observed in *L. plantarum* treated rats in an experiment of Pb induced liver toxicity. It elevated hepatic levels of p-AMPK and p-AKT; which encouraged Nrf2 signalling activation. In addition to this, it enhances the elimination of toxic Pb in rats exposed to Pb (Hu et. al. 2020).

2.1.7 Anti-inflammatory activity

On intestinal epithelial cells, macrophages, and lymphocytes, the lactic acid bacterium *L. plantarum* was found to have a strong anti-inflammatory impact. This strain was able to stop S. Typhi from adhering to epithelial cells in a cell culture experiment, which in turn reduced the release of IL-8. Proinflammatory cytokine production may have decreased as a result of a small decrease in macrophage activation. The strain also increased the number of CD4+CD25+ cells and improved IL-10 production. *L. plantarum* is an excellent choice to help with the treatment of inflammatory illnesses since it shows immunomodulatory potential (Ferreira et al. 2016). A study involving 40 patients found that taking *L. plantarum* for four weeks helped relieve symptoms of irritable bowel syndrome (IBS). The study suggested that this relief may be due to the ability of *L.*

plantarum, to break down arginine and produce nitric oxide, which could improve intestinal motility (Niedzielin et al., 2001).

2.1.8 Rapid Inhibition of Pathogenic Microbes

The actions of *L. plantarum* have been studied in vitro, and it was discovered that this bacterium produces antimicrobial compounds. These compounds serve as natural preservatives with a wide range of applications in the food and agriculture industries (Niku-Paavola et al., 1999). In vitro studies have found that certain strains of the *L. plantarum* group have strong anti-Helicobacter pylori activity, which could potentially be used to treat peptic ulcers (Hütt et al., 2006). *Listeria monocytogenes* is a type of bacteria that can cause foodborne illnesses. It is worrisome because it can grow in cold temperatures and acidic environments. However, a study discovered that *L. plantarum* produces a substance called bacteriocin that can prevent the growth of *L. monocytogenes*. The bacteriocin was not affected by proteolytic enzymes, could withstand high temperatures, and remained stable in different pH levels. This is important because *L. plantarum* is commonly found in fermented foods, so it can help prevent food poisoning and preserve foods naturally, especially in tropical areas (Olasupo, 1998). Diarrhoea caused by *Clostridium difficile* infection can be recurrent, especially after taking antibiotics for a long time. In a study, *L. plantarum* was tested for its ability to increase short chain fatty acids (SCFAs) in the gut and inhibit the growth of *C. difficile*. Ten patients received antibiotics along with L. plantarum in a fruit drink, while nine patients received antibiotics and a placebo drink. The group receiving the *L. plantarum* treatment had higher levels of SCFAs and butyrate and did not experience diarrhoea, while the placebo group had decreasing levels of butyrate and continued to have recurrent diarrhoea (Wullt et al., 2007).

2.1.9 Mucoadhesive property

Research has found that *L. plantarum* is capable of sticking to different surfaces that other probiotics cannot. This strain has been discovered in samples taken from the jejunum and rectum up to 11 days after being given, suggesting that it has a strong ability to adjust to the human intestine (Connelly, 2008). *L. plantarum* has been found to stick to the lining of the gut. In healthy individuals, it adheres well, but a study on seriously ill patients who had taken antibiotics showed positive results (Connelly, 2008). The study involved 15 patients, who

underwent 240 biopsies. In the group receiving treatment, no bacterial growth was observed, while the control group had growth in five out of 32 samples. The study concluded that repeated administration was necessary, but the adherence of *L. plantarum* to the gut lining was effective (Klarin et al., 2005). The study found that *L. plantarum* adheres well to the surface of the tonsils, which has potential implications for treating tonsillitis and controlling harmful organisms in the mouth. Similar results have been observed in studies on mice, suggesting potential use in delivering oral peptides (Oh et al., 2007).

2.1.10 Increased Bioavailability of Iron

Research has shown that *L. plantarum* can improve the absorption of iron from meals that typically have low iron availability. A study conducted on 24 healthy women found that *L. plantarum* specifically increased the absorption of non-haem iron from a meal high in phytate. This suggests that the live bacteria itself, rather than just the organic acids it produces, has a positive effect on iron absorption (Bering et al., 2006). The discussion focused on the possibility that if *L. plantarum* colonizes the duodenal mucosa, it could result in higher levels of lactic and other organic acids, which would lower the pH and reduce the binding of iron, ultimately leading to increased absorption (Bering et al., 2006).

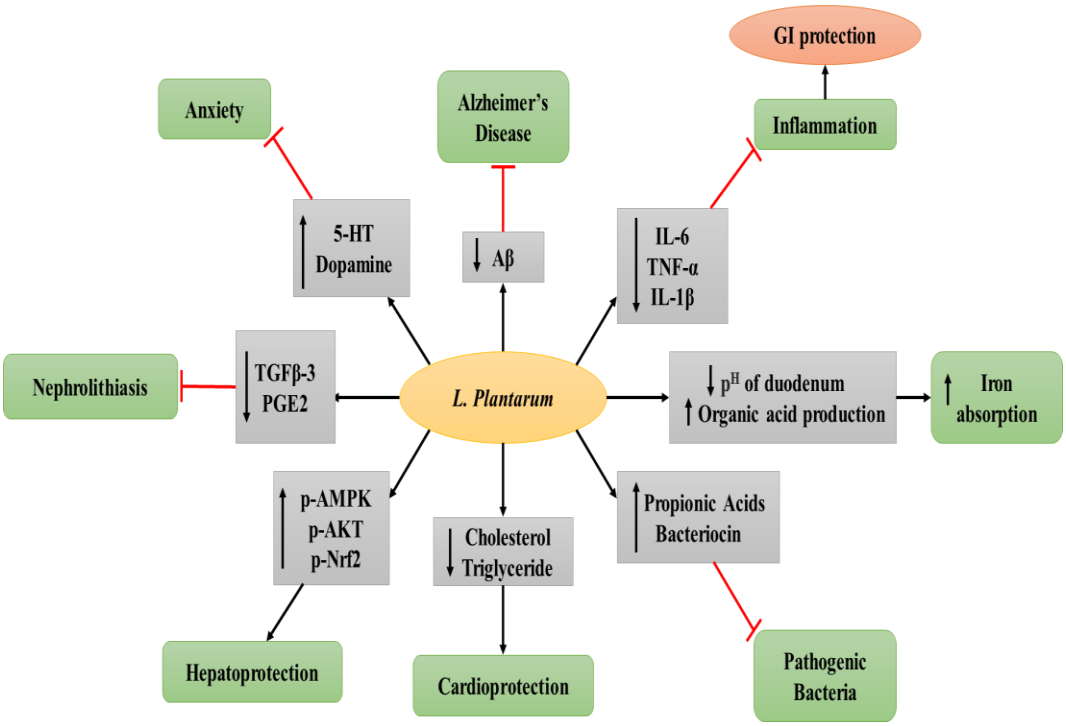

Figure 2. Protective mechanisms of *L. plantarum* in various health disorders.

Chapter 3

Materials and methods

Contents

3. Materials and methods

3.1. Chemicals

Bovine serum albumin (BSA), Bradford reagent, glycerol, hydrogen peroxide solution, hydrochloric acid solution, sodium chloride, butanol, sodium azide, and pyridine were obtained from Sigma-Aldrich, MO, USA. Kits for the measurement of different biochemical parameters were purchased from ARKRAY Healthcare Private Limited, India. Kits for pro-inflammatory cytokinin assay were acquired from Raybiotech, Georgia, USA. Lactobacillus MRS broth and tryptone soya broth were purchased from Himedia Laboratories Private Limited, India. Sodium arsenite ($NaAsO_2$), ethylenediaminetetraacetic acid (EDTA), tris Hcl, potassium dihydrogen phosphate, disodium hydrogen phosphate, 5,5'-dithiobis-(2-nitrobenzoic acid), methanol, nitro blue tetrazolium (NBT), NADH, phenazine methosulphate, GSH, 2,4-Dinitrophenylhydrazine, trichloroacetic acid, ethanol, dichlorodihydrofluorescein diacetate (DCFDA), sodium dodecyl sulfate, and thiobarbituric acid were obtained from Sisco Research Laboratory, Mumbai, India. All other reagents used were of analytical grade.

3.2. Preparation of bacterial strain

The *Lactobacillus plantarum* MTCC 5690 used in this study was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH) in Chandigarh, India. The frozen stock culture of this strain was grown on De Man, Rogosa and Sharpe agar (MRS) media for 72 hours at 37˚C. A single colony was then transferred to MRS broth and grown at 37˚C for 24 hours. Following the transfer, cells were inoculated in 250 mL of MRS broth and grown for 24 hours. The cells were harvested by centrifugation and aliquots of 10^8 Colony Forming Units (CFU) were prepared in tryptone soya agar broth with 10% glycerol and stored at -80˚C. Before administration, the cells were washed twice in sterile phosphate buffer saline (PBS) and resuspended in 1 ml of PBS. Each rat was then orally administered with 1 ml of PBS containing 10^8 CFU of this probiotic bacteria strain.

3.3 Animals

Twenty-four male Wistar rats (weighing 150-170 g) were obtained from Chakraborty Enterprise, Kolkata, India. They were then placed in standard polypropylene cages measuring 29 × 22 × 14 cm and housed in the animal house

of the Department of Pharmaceutical Technology at Jadavpur University in India. The rats were carefully provided with optimal temperature and humidity conditions, and a balanced 12-hour cycle of light and darkness (Dewanjee et al., 2013). The rats were fed standard diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee, Department of Pharmaceutical Technology, Jadavpur University (approval number JU/IAEC-22/35), Experimental procedures were carried out according to the principles of laboratory animal care (Public Health Service, 2015). The animals were given a period of 2 weeks to acclimatize before the in vivo experiment was conducted.

3.4. *In vivo* bioassay

3.4.1. Experimental setup

The in vivo experiment was performed according to the established protocol in our laboratory (Das et al., 2010, Dua et al., 2016). Twenty-four male Wistar rats (♂) were divided into four groups (n=6) and treated as follows:

Group I: Control group, rats were fed with phosphate buffer saline (PBS) (1.0 mL) via an intragastric tube once a day for 10 days;

Group II: Toxic control, rats were treated with $NaAsO_2$ (10 mg/kg body weight, p.o., once daily) via an intragastric tube for 10 days;

Group III: Rats were orally administered 1 mL of *L. plantarum* (1×10^8 CFU/mL) once daily for a period of 10 days.

Group IV: Treatment group: The rats received a daily dose of $NaAsO_2$ at a concentration of 10 mg/kg body weight, along with 1 mL of *L. plantarum* (at a concentration of 1×10^8 CFU/mL) administered orally through gavage, once a day for a period of 10 days.

The dosage of *L. plantarum* was decided based on a comprehensive literature review that examined the various therapeutic activities demonstrated by this probiotic strain (Chien et al., 2022; Chayanupatkul et al., 2022). The daily food and water intake was monitored. At the end of the experiment, rats were fasted overnight for 16 h, and on the 11th day, they were sacrificed under euthanasia. The blood samples were collected from retro-orbital venous plexus. The serum was carefully obtained through a gentle centrifugation process, with a rotation speed of 3,000 rpm for 10 minutes. The livers were removed and cleaned using

PBS. The weight of each liver was recorded. One portion of liver tissue was preserved in 10% formalin for histological examinations, while the other portion was immediately homogenized in Tris-HCl (0.01 M) + EDTA (0.001 M) buffers with a pH of 7.4. The tissue homogenate was obtained by centrifuging it at 4°C for 30 minutes at 12,000 x g. The collected supernatant was then utilized for the experiments. Samples of urine were collected from the bladder and then promptly stored at a temperature of -80°C (Khanra et al., 2017). A schematic overview of the in vivo assay has been presented in **Figure 3**.

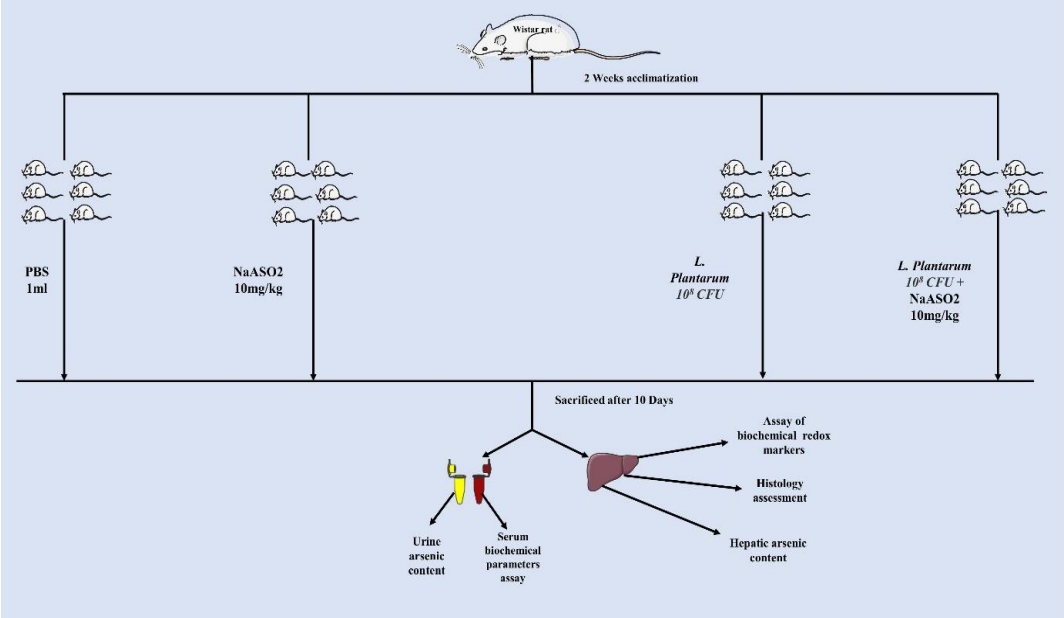

Figure 3. Schematic overview of the experimental protocol

3.4.2. Estimation of Serum Biochemical Parameters

The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine kinase (CK), and C-reactive protein (CRP) in the sera were determined using commercially available kits (ARKRAY Healthcare Private Limited, India) according to the instructions provided by the manufacturer.

3.4.3. Serum lipid profile evaluation

The levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) in blood serum were measured according to the manufacturer's instructions using commercially available enzymatic kits (purchased from ARKRAY Healthcare Private Limited, India). The calculation of low-density lipoprotein (LDL-C) levels in blood serum was done using Friedewald's formula.

3.4.4. Assays for Hepatic and Urinary arsenic

The contents of arsenic in the liver and urine of experimental animals were analyzed using the hydride generation system in an atomic absorption spectrophotometer (Perkin Elmer model number 3100, USA), following an established protocol (Das et al., 1995).

3.4.5. Assays for Biochemical and Redox Markers in Liver

The levels of ROS, GSH, SOD, CAT, lipid peroxidation, and protein carbonylation in the livers of the Wistar rats that received various treatments were measured according to established protocols (Manna et al., 2022). The levels of SOD and CAT were expressed as the inhibition of NBT reduction per minute and H2O2 consumption per minute, respectively. The extent of lipid peroxidation and protein carbonylation were also estimated using established protocols (Manna et al., 2022).

3.4.6. Assay of Pro-inflammatory and Anti-inflammatory Cytokine Levels

The amounts of interleukin 1 beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) were measured in the sera and rat liver lysates using commercially available kits from Raybiotech, Georgia, USA. The protocols outlined in the purchased enzyme-linked immunoassay (ELISA) kits were diligently followed, and all reagents, samples, and standards were prepared in accordance with the instructions provided in the kit's manual. In brief, 100 µL of samples and standards were added to the wells, which had already been pre-coated with an antibody specifically targeting IL-1β, IL-6, and TNF-α. The mixture was then incubated for 2.5 hours at room temperature. The unbound substances were taken out, and then 100 µL of biotin-conjugated antibody, which is specific for IL-1β, IL-6, and TNF-α, was introduced into the well. Afterward, the mixtures were left to incubate at room temperature for a duration of 1 hour. After performing the washing steps (which involve removing the liquid from each well, filling them with 100 µL of Wash Buffer, and then removing the liquid again), we added 100 µl of prepared streptavidin solution to the wells and let them incubate for 45 minutes at room temperature. Next, we added 100 µL of 3,3',5,5' tetramethylbenzidine (TMB) substrate solution, followed by another incubation for 30 minutes at room temperature. This process results in the development of a color that is directly proportional to the amount of IL-1β, IL-6, and TNF-α bound

during the initial step. The stop solution was added to each well, then the plate was gently tapped to ensure thorough mixing, and finally, the intensity of the color was measured at 450 nm. To minimize the error, the assays for all standards and samples were conducted twice.

3.4.7. Histological studies

To conduct histological analyses, a portion of liver tissue from experimental animals was carefully washed with ice-cold phosphate buffer at a pH of 7.4. Subsequently, the tissue was fixed using a 10% formalin solution and then mounted in paraffin blocks for sectioning. In accordance with the established protocol, the sections were appropriately stained using hematoxylin and eosin (H&E) stains and imaged using a Leica DFC 450C microscope at a magnification of 100x (Dewanjee et al., 2013; Sahu et al., 2019).

3.4.8. Statistical Analysis

Experiments were performed in triplicate. The mean ± SD values were used to represent the data obtained from the experiment. The results underwent statistical analysis using one-way ANOVA followed by Dunnett's t-test with the assistance of GraphPad InStat (version 3.05), GraphPad Software, USA. Any p-values below 0.01-0.05 were deemed significant.

Chapter 4

Results and discussions

Contents

4. Results and discussions

4.1 Results

4.1.1 Effect of *L. plantarum* on $NaAsO_2$ intoxication

4.1.1.1 Impact on body weight, liver weight, liver, and urine arsenic concentration

No significant change in food and water intake was observed in the animals from either experimental group during the experiment. Rats treated with $NaAsO_2$ (10 mg/kg) experienced a significant decrease in body weight gain, but when *L. plantarum* (10^8 CFU/rat) was administered alongside $NaAsO_2$ (10 mg/kg), the body weight gain was restored to nearly normal levels. In contrast, the rats treated with *L. plantarum* had significantly higher weight gain compared to the control subjects. The experimental rats showed a significant difference in liver weight, with $NaAsO_2$ administration resulting in an increase in liver weight compared to the control. However, the administration of *L. plantarum* alone caused a significant decrease in liver weight compared to the control. Furthermore, the addition of *L. plantarum* to $NaAsO_2$-treated animals had a positive effect on the weight of the liver. Rats exposed to $NaAsO_2$ (10 mg/kg) had a significant increase in liver arsenic accumulation and poor urinary clearance of arsenic compared to normal rats. However, when *L. plantarum* was administered alongside $NaAsO_2$ (10 mg/kg), it significantly promoted arsenic clearance and resulted in significantly lower hepatic arsenic concentration (**Table 3**). There were no noticeable variations found in the levels of arsenic in the liver and urine of the groups treated solely with *L. plantarum*, as compared to the control group. The potential reason for the arsenic clearance role of *L. plantarum* from the body could possibly be attributed to the composition of its cell wall. Lactobacillus, such as *L. plantarum*, possesses a distinct cell wall comprised of peptidoglycan that contains various binding sites for toxic compounds. Therefore, the arsenic clearance role of *L. plantarum* might be attributed to its beneficial nature, which enhances the elimination of arsenic through urine and feces.

Table 3. The impact on body weight gain, liver weight, hepatic arsenic content, and urinary arsenic content in rats with and without ($NaAsO_2$) and with *L. plantarum* (*L. plantarum* + $NaAsO_2$), as well as *L. plantarum* alone.

Parameters	**Group I (Control)**	**Group II ($NaAsO_2$)**	**Group III (*L. plantarum*)**	**Group IV (*L. plantarum* + $NaAsO_2$)**
Body weight gain (gram)	26 ± 1.4	14 ± 1.3	29 ± 0.8	23 ± 1.4
Liver weight (gram)	4.62 ± 0.61	6.53 ± 0.85	4.51 ± 0.04	5.25 ± 0.97
Liver arsenic concentration (µg/g of tissue)	0.41 ± 0.09	389.54 ± 12.06	0.39 ± 0.12	148.56 ± 3.87
Urinary arsenic concentration (µg/ml)	0.245 ± 0.14	3.6 ± 0.64	0.21 ± 0.11	0.819 ± 0.07

4.1.1.2 Effects on blood parameters

Blood parameters provide important information about the pathological state of the body. The effects of *L. plantarum* on blood parameters in experimental rats are shown in **Figure 4**. Treatment with $NaAsO_2$ (10 mg/kg) resulted in a significant increase in ALP, ALT, AST, LDH, CK, and CRP levels in the rats' blood. However, when *L. plantarum* (10^8 CFU/rat) was given along with $NaAsO_2$ (10 mg/kg), there was a significant reduction in these elevated blood biochemical parameters. On its own, *L. plantarum* had little to no significant impact, suggesting that it improves the body's biochemical status (**Figure 4)**.

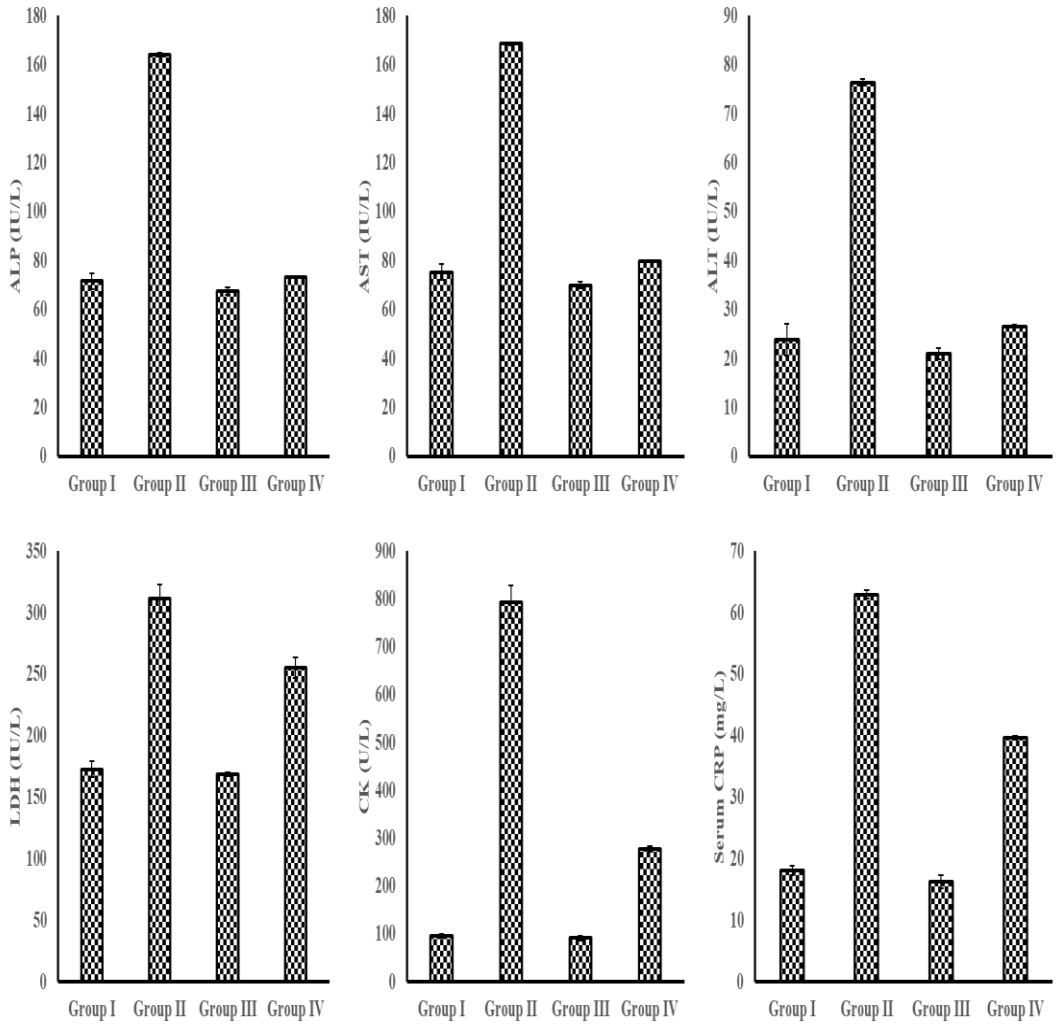

Figure 4. The effect of $NaAsO_2$ and *L. plantarum* supplementation on blood parameters of control and experimental rats.

4.1.1.3. Effects on serum lipid profile

The levels of serum total cholesterol, triglyceride, and LDL were found to be significantly higher in rats administered with $NaAsO_2$, while the level of HDL was significantly lower compared to control animals. However, when rats were co-treated with *L. plantarum* (10^8 CFU/rat) along with $NaAsO_2$, the HDL level significantly increased, and the levels of serum cholesterol, triglyceride, and LDL significantly decreased compared to rats treated with $NaAsO_2$ alone. In rats treated with *L. plantarum* alone, these levels were not significantly different from the control group, except for triglyceride level which was slightly higher (**Figure 5**).

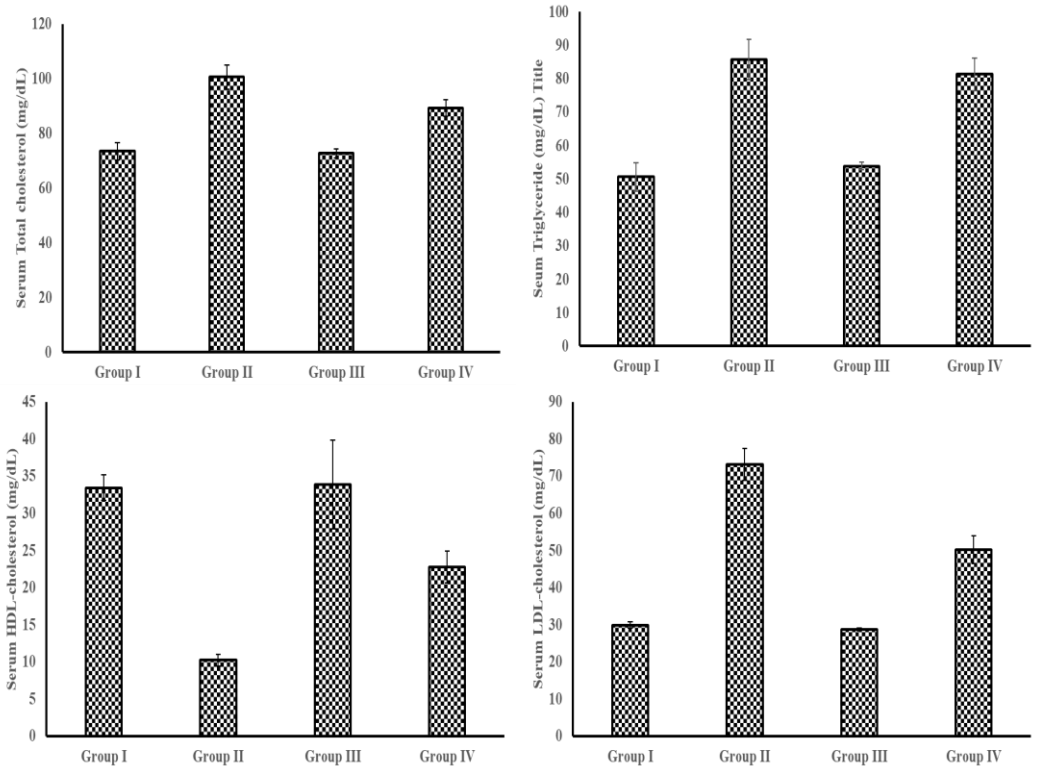

Figure 5. The effects of $NaAsO_2$ and *L. plantarum* supplementation on serum total cholesterol, HDL, triglycerides, and LDL levels.

4.1.1.4. Effects on redox status in hepatic tissue

The rats treated with $NaAsO_2$ (10 mg/kg) showed a significant increase in intracellular ROS levels within the liver tissue (Figure 4). However, when *L. plantarum* (10^8 CFU/rat) was administered simultaneously with $NaAsO_2$ (10 mg/kg), it effectively reduced intracellular ROS production in the liver (**Figure 4**). The liver of the rats exposed to $NaAsO_2$ (10 mg/kg) exhibited significant increases in lipid peroxidation and protein carbonylation. In contrast, treatment with *L. plantarum* (10^8 CFU/rat) significantly restored lipid peroxidation and protein carbonylation in the liver tissue of the experimental rats (**Figure 6**). $NaAsO_2$ (10 mg/kg) further enhanced oxidative stress by depleting GSH levels and endogenous antioxidant enzymes (SOD, CAT) in the liver tissue (Figure 6). However, simultaneous administration of *L. plantarum* (10^8 CFU/rat) with $NaAsO_2$ (10 mg/kg) effectively restored GSH levels and endogenous antioxidant enzymes to near-normal levels (**Figure 6**). No significant changes were found in the aforementioned parameters in the group treated solely with *L. plantarum* except in the ROS and TBARS level which were reduced compared to the control group (**Figure 6)**.

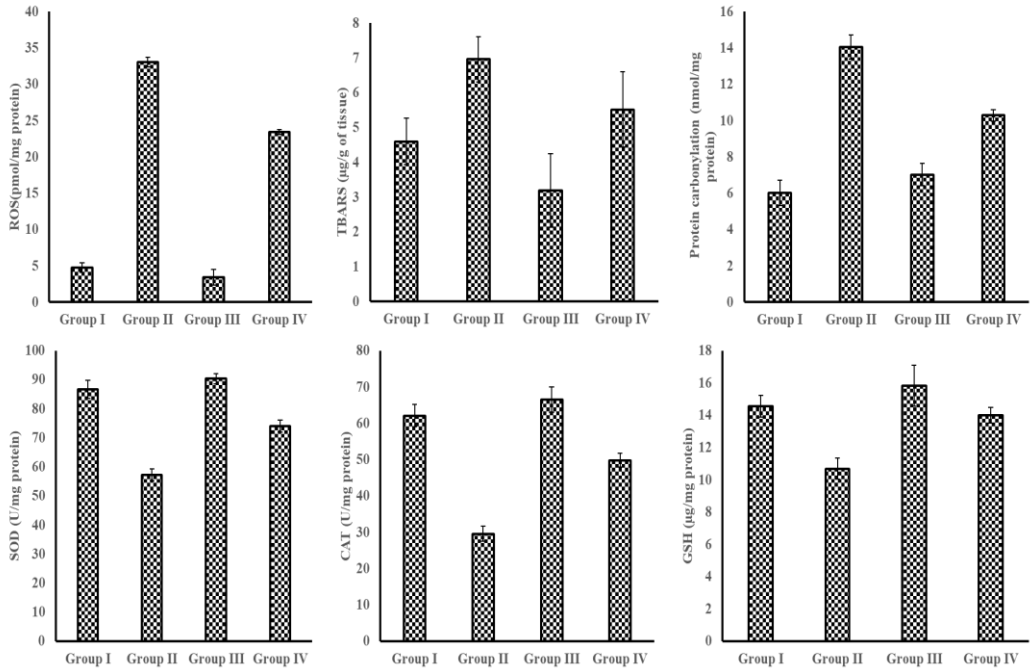

Figure 6. The effect on ROS accumulation, lipid peroxidation, protein carbonylation, and endogenous redox systems in the liver of control and experimental rats.

4.1.1.5 Effect on Pro-inflammatory and Anti-inflammatory Cytokine Levels

The administration of $NaAsO_2$ led to a substantial rise in the production of serum and hepatic IL-1β, IL-6, and TNF-α in comparison to the control animals (**Figure 7**). The administration of *L. plantarum* alone did not have a significant impact on the levels of IL-1β, IL-6, and TNF-α in the serum and hepatic tissue. In regards to the group treated with $NaAsO_2$ + *L. plantarum*, the levels of IL-1β, IL-6, and TNF-α in both serum and hepatic tissue were significantly reduced compared to rats treated with $NaAsO_2$ alone.

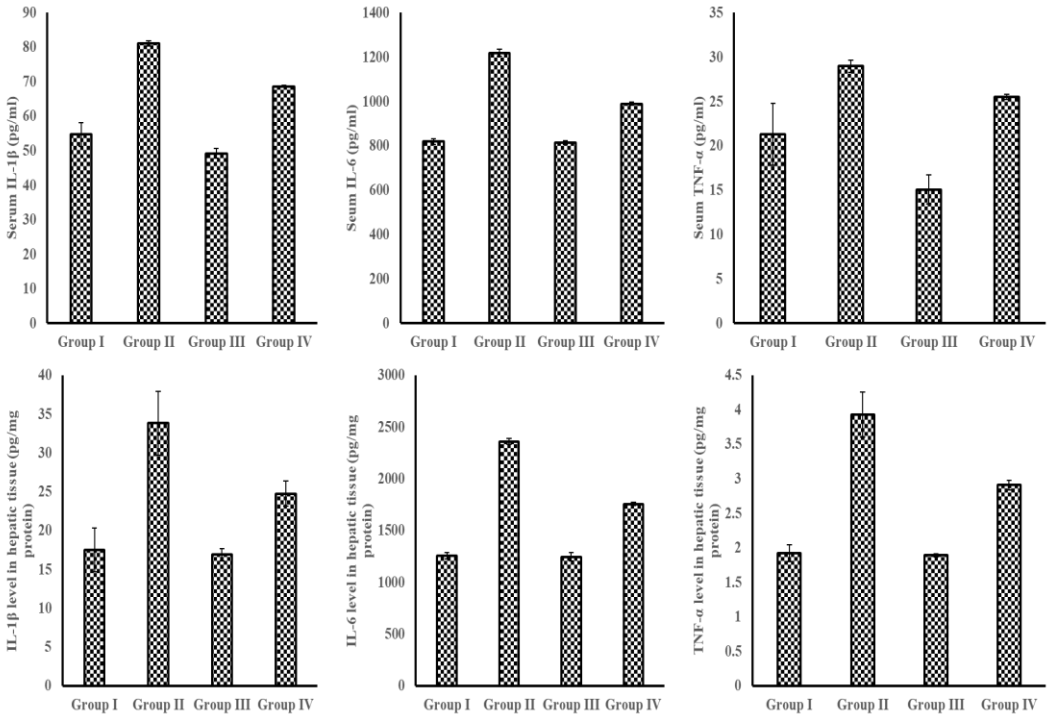

Figure 7. Changes in serum and hepatic pro-inflammatory and anti-inflammatory cytokine concentration of experimental rats.

4.1.1.6 Effects on the Histology of the Liver

According to **Figure 8**, the histology of the liver from the control group displayed well-organized structures with uniform cell size and normal lobular architecture. The cells had clear boundaries and centered nuclei. In contrast, the $NaAsO_2$ treated group exhibits disorganized histological structures with unclear cell boundaries, and the central vein shape was irregular and dilated. Additionally, this group showed signs of inflammatory infiltration and hepatocellular apoptosis. However, when *L. plantarum* was introduced, it mitigated these negative changes caused by arsenic (**Figure 8**). It reduced the apoptotic change caused by arsenic to a great level. The liver tissue regained its normal morphology and the hepatocytes appeared healthy, similar to the control group. However, in a few areas apoptotic changes were observed in Group 4 rat livers. The liver tissue regained its normal morphology and the hepatocytes appeared healthy, similar to the control group. Thus, *L. plantarum* may have a preventive effect on liver histopathological damage caused by arsenic consumption. The *L. plantarum* sole-treated group (Group 3) had excellent morphology compared to the other groups, indicating that

L. plantarum possesses hepatoprotective activity (**Figure 8**). Supplementation of this probiotic would be beneficial for both human and animal health.

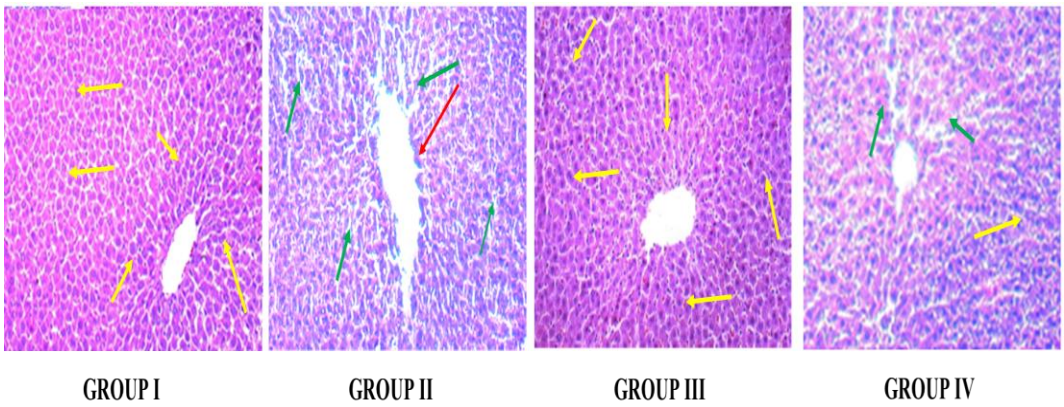

Figure 8. Hematoxylin-eosin (H&E) histo-pathological observations of liver in rats. The liver sections of a normal rat showed a normal portal vein and hepatocytes. In the liver section exposed to NaAsO2, the portal vein was dilated (indicated by the red arrow) and there were apoptotic changes (indicated by the green arrows) compared to the normal control liver section. However, when *L. plantarum* was co-administered, it reversed the pathological changes caused by NaAsO2 and the hepatocytes maintained their normal morphology (indicated by the yellow arrows) compared to the control group.

4.2. Discussions

The contamination of drinking water with arsenites is a serious problem in more than 70 countries worldwide (Das et al., 2018). Inorganic arsenicals have been found to cause detrimental effects on different organs by increasing the production of harmful substances within cells and reducing the body's natural defenses against oxidative stress (Das et al., 2018). Thus, the use of a probiotic could be a new and effective approach to mitigate the toxic effects of arsenic. *L. plantarum* is a probiotic that is frequently found in the mouth, gut, and fermented foods, and it is widely recognized as a beneficial bacteria for promoting gut health in both humans and animals.

The findings of this study demonstrate that *L. plantarum* offers numerous medicinal benefits by preventing oxidative injury caused by arsenic, either through its direct antioxidant effect or by stimulating antioxidant enzymes, thereby improving hepatocyte damage. The results indicate that *L. plantarum* can effectively protect against the accumulation of arsenic, cellular oxygen species,

inflammation, and apoptosis in hepatocytes induced by arsenic. The impact of arsenic on liver toxicity was assessed through histological studies and biochemical assays.

Despite rats having a higher metabolic turnover than humans, it is noteworthy that the rats exhibit similar metabolic signaling and genomic expression as human adults. Additionally, it is observed that the absorption, distribution, and excretion of trivalent arsenic follow comparable patterns in both rats and humans. Moreover, the metabolic pattern of trivalent arsenic, such as arsenite → MMA → DMA, is also similar in both humans and rats. Furthermore, it should be highlighted that the dysfunction in hepatic endothelial cells caused by arsenic follows comparable redox signaling in both rats and humans. Taking into account the shared associations in phenotypic anchors, including adsorption, arsenic burden in tissues, metabolism, excretion, and altered gene expression, between arsenic-exposed humans and rats, the rat model has been employed in this study.

Our findings indicate that animals treated with $NaAsO_2$ experienced a reduction in body weight gain, suggesting the toxic effect of arsenic. This toxicity is attributed to the ability of arsenic to disrupt the glucocorticoid hormonal system, which is crucial for regulating glucose, carbohydrates, lipids, and protein metabolism (Saeed et al., 2017). Also, the NaAsO2 treatment resulted in a notable increase in the liver's weight. When *L. plantarum* was administered along with $NaAsO_2$, it improved the negative effects caused by arsenic. However, when *L. plantarum* was given alone, it only had a slight positive impact on these toxic effects compared to the control group. Based on these effects, it can be inferred that *L. plantarum* has a favorable impact on body weight gain and hepatoprotection.

The liver has been found to concentrate arsenic after exposure and plays a crucial role in the metabolism of arsenic, potentially leading to liver damage. This highlights the liver as a susceptible organ for arsenic toxicity (Tyler and Allan, 2014). Our experiment showed that rats that were exposed to $NaAsO_2$ had more arsenic in their livers and had difficulty getting rid of it through urine compared to normal rats. The surface of *L. plantarum* is comprised of a thick layer of peptidoglycan, proteins, and polysaccharides (Chapot-Chartier and Kulakauskas, 2014). This means that probiotic bacteria have multiple potential ligands that aid in exporting metals out of the cell and subsequently reducing damage and

oxidative stress in the organism (Saeed et al., 2017). Many studies have explored the relationship between bacterial species and metals, as well as their ability to remove metals from contaminated areas, thus inhibiting the production of reactive oxygen species (ROS) and reducing oxidative stress, lipid peroxidation, and other harmful effects (Saeed et al., 2017). In the present study when the rats were treated with *L. plantarum* along with $NaAsO_2$, the arsenic was effectively eliminated and the concentration in the liver was significantly lower. The rats treated only with *L. plantarum* did not show any significant changes in arsenic levels compared to the control group. So based on these findings, we can conclude that *L. plantarum* might have a chelating effect on metaloids like arsenic. Therefore, it might be a good decision to consider *L. plantarum* as a potential therapeutic agent for xenobiotics-induced toxicities.

The levels of certain enzymes in the blood can indicate toxic effects in the body or specific organs. Arsenic undergoes metabolic changes in the liver by binding to proteins and causing damage to the liver cell membrane, leading to leakage of certain enzymes into the blood. These enzymes, including ALP, AST, and ALT are considered markers of liver damage. In this study, rats exposed to NaAsO2 showed significantly elevated levels of ALP, AST, and ALT in their blood, indicating liver damage also, elevated levels of LDH and CK indicate the alteration of membrane integrity in hepatocytes. However, co-treatment of *L. plantarum* along with $NaAsO_2$, caused the levels of these enzymes to get restored, suggesting a protective effect on the liver.

There was a notable rise in serum total cholesterol, LDL, and triglycerides in the rats treated with $NaAsO_2$, along with a considerable decrease in HDL cholesterol. Additionally, when *L. plantarum* was administered to the rats alongside $NaAsO_2$, there was a significant reduction in levels of serum total cholesterol, LDL, and triglycerides, while the serum HDL cholesterol showed a significant increase in comparison to the $NaAsO_2$-treated rats. The hyperlipidemic condition of the $NaAsO_2$ treated rats are a consequence of ROS-mediated oxidative damage, amelioration of which occurs following *L. plantarum* cotreatment which supports the antioxidant nature of this probiotic. The hyperlipidemic condition of the rats treated with $NaAsO_2$ is caused by oxidative damage mediated by reactive oxygen

species. However, the condition is improved when *L. plantarum* is co-administered, which suggests that this probiotic has antioxidant properties.

Oxidative stress is currently widely recognized and extensively studied as the main mechanism of arsenic toxicity (Flora, 2011; Sharma et al., 2017; Okereafor et al., 2020). The abnormal increase in hepatocyte apoptosis is an important pathological mechanism of arsenic-induced liver damage. Therefore, the inhibition of hepatocyte apoptosis is considered an important factor in assessing the effectiveness of hepatoprotective drugs (Kim et al., 2004; Ozaki, 2019).

The results of the histological analysis suggested that *L. plantarum* had a beneficial effect on liver health, as it reduced pathological changes such as inflammation and tissue damage. These findings indicate that *L. plantarum* can help alleviate the damage caused to liver cells. By evaluating serum biochemical parameters we concluded that arsenic reduced the viability of liver cells and increased the levels of hepatic marker enzymes in the blood. *L. plantarum* was found to improve cell viability and reduce these levels, although it did not significantly impact cell viability when used alone (Figure 8).

Oxidative stress occurs when there is an imbalance between the production and removal of ROS (Yan et al., 2019). In line with this idea, the accumulation of intracellular ROS in hepatic tissue due to arsenic exposure was observed through DCFH-DA fluorometry in vivo (Figure 6). These findings clearly demonstrate that the generation of ROS is significantly increased in hepatic tissue treated with arsenic. It has been shown that moderate levels of ROS can activate Nrf2, which plays a protective role by inducing antioxidant genes (Tebay et al., 2015). In turn, the activation of the Nrf2 defense system can alleviate oxidative stress damage by regulating ROS levels and the inflammatory process (Shang et al., 2017). GSH is known as one of the effective antioxidants in cells (Silva-Adaya et al., 2020). Arsenic has the ability to strongly bind with neighboring thiols or biological ligands that contain cysteine residues both in vitro and in vivo. This is important because the elimination of ROS requires substances that contain sulfur groups. In our study, we found that treatment with arsenic inhibited the antioxidant effect of GSH, leading to the accumulation of ROS. Interestingly, the use of *L. plantarum* treatment significantly reduced these oxidative stress parameters. Normally, under physiological conditions, Nrf2 remains at a low expression level in the cytoplasm

due to its interaction with the inhibitory protein Keap-1. This prevents Nrf2 from being transcribed. However, when stimulated by various inducers, such as ROS, Keap-1 dissociates from Nrf2 in the cytoplasm. The conformation of Keap-1 is altered by the presence of arsenic and excessive ROS production, specifically by modifying certain cysteine residues (Cys257, Cys273, Cys288, Cys293) (Li et al., 2020). This alteration ultimately leads to the prevention of Nrf2 degradation from the Keap1/Nrf2 complex (Saito et al., 2015). The increase in the activities of SOD and CAT, which play a crucial role in cellular defense, as well as the rise in GSH levels and the activities of GSH-dependent enzymes, has been observed in several studies (Perez et al., 2006; Battogtokh et al., 2018). Our experiments found that the activities of antioxidant defense systems like SOD, CAT, and GSH in liver tissue exposed to arsenic were noticeably decreased. The level of lipid peroxidation indirectly indicates the extent of damage to hepatocytes (Moniruzzaman et al., 2018). Moreover, TBARS levels significantly increased after arsenic poisoning, suggesting an elevated production of peroxides in response to toxicity. However, co-treatment with *L. plantarum* was found to enhance the activity of SOD, CAT, and GSH while reducing levels of TBARS and ROS in liver tissue, although it was still slightly lower than the control. Additionally, it can be concluded that *L. plantarum* facilitated the movement of Nrf2 into the nucleus in the liver and caure Nrf2/ARE mediated antioxidant response, leading to a decrease in arsenic-induced oxidative stress and subsequent liver toxicity.

Furthermore, it was observed that $NaAsO_2$ resulted in a significant increase in the levels of cytokines (specifically IL-1β, IL-6, and TNF-α). This suggests that the production of inflammatory mediators may play a role in the development of arsenic-induced tissue injury in response to local inflammation. Additionally, ROS are also involved in promoting inflammatory processes through the activation of transcription factors like NF-κB and AP-1, which in turn stimulate the production of cytokines (Mittal et al., 2014). In rats treated with $NaAsO_2$ and *L. plantarum*, the addition of *L. plantarum* resulted in the reduction of cytokine levels in the hepatic tissue and sera of rats. Additionally, *L. plantarum* prevents NF-κB activation, which is crucial for the expression of inflammatory cytokines, without negatively impacting the viability of surrounding cells (Hegazy and El-Bedewy,

2010). As a probiotic, *L. plantarum* is able to suppress ROS production and cytokine levels (IL-1β, IL-6, and TNF-α) by activating the Nrf2 signaling pathway and downregulating the NF-κB mediated inflammatory response in hepatic tissue affected by arsenic-induced toxicity.

However, treatment with *L. plantarum* alone did not yield significant changes in any of our experimental assays compared to the control group, except for body weight gain, liver weight, ROS and TBARS levels in hepatic tissue, and serum triglyceride levels. This finding is in line with several other studies, which have reported that probiotics like *L. plantarum* do not cause any negative effects on healthy rats. These results indicate that the use of probiotics in a specific dosage has been proven to be safe for the body (Saeed et al., 2017). Additionally, it can be inferred that *L. plantarum* is highly effective as a hepatoprotective agent, even when administered at extremely low dosages. According to the findings from the literature review, the dosage (10^8 CFU/rat) utilized in our rat model is considered to be exceptionally low (Chien et al 2022; Wen et al., 2023).

In summary, our study has shown that *L. plantarum* has the potential to alleviate oxidative stress and liver injury caused by arsenic by activating the Nrf2 pathway. This is significant because oxidative damage is a major factor in various liver diseases. Therefore, we believe that *L. plantarum* could be a beneficial treatment for liver disorders by reducing oxidative stress through the activation of the Nrf2 pathway. Additionally, it is hypothesized that the anti-inflammatory response exhibited by this probiotic is primarily attributed to the downregulation of the NF-κB signaling pathway and activation of the Nrf2 defense system. This sequential modulation can effectively alleviate the damage caused by oxidative stress by regulating ROS levels and mitigating the inflammatory process. Furthermore, our findings suggest the potential role of the Nrf2 pathway in prolonged oxidative stress. A schematic representation of probable protective mechanism of *L. plantarum* against $NaAsO_2$-mediated hepatic injury has been represented in **Figure 9**.

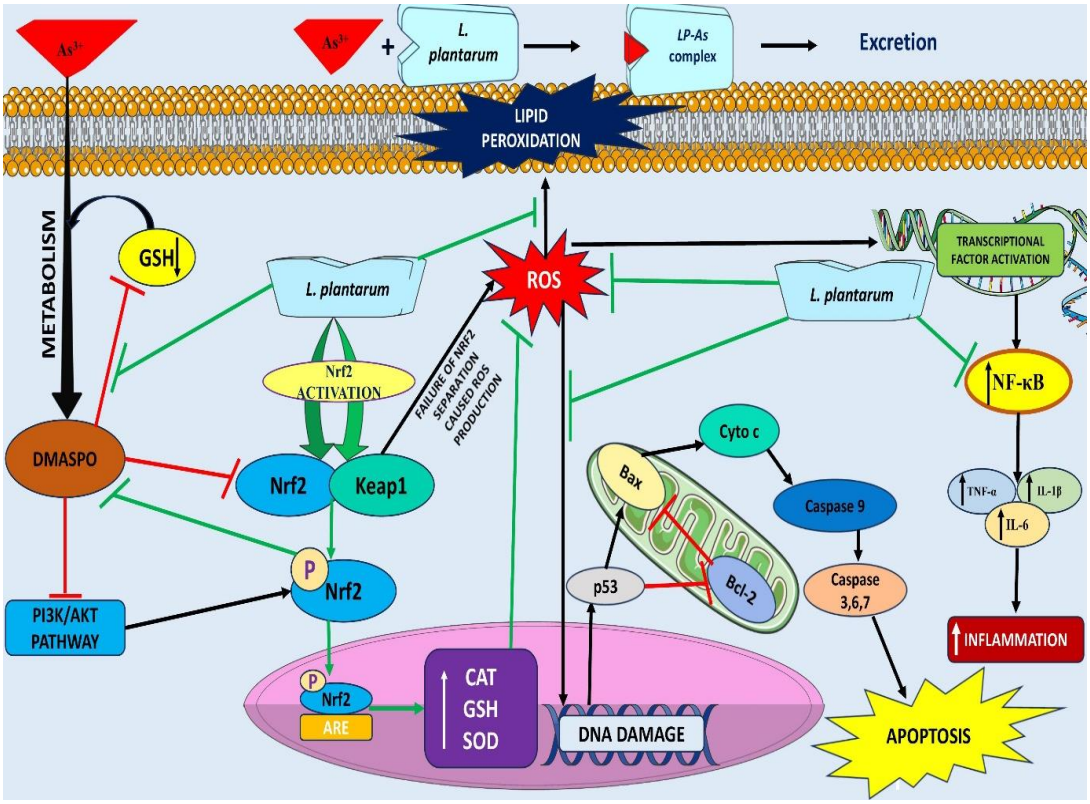

Figure 9. Probable protective mechanism of *L. plantarum* against $NaAsO_2$ mediated hepatic injury. The green arrows show the protective actions of *L. plantarum* in arsenic-mediated hepatic injury. Black arrows represent downstream reaction. Flat-headed arrows indicate inhibition.

Chapter 5

Conclusion

Contents

5. Conclusion

This research contributes to a better understanding of the mechanism of *L. plantarum* and provides additional evidence for its potential as a treatment for liver diseases. Additionally from our results, we concluded that *L. plantarum* also possesses anti-inflammatory and antioxidant activity, which enlighte new opportunities for treatment of inflammatory and oxidative stress-related disorders. Future studies should focus on validating the role of both the Nrf2 pathway and NF-κB signaling pathway by examining the effects of *L. plantarum* on liver injury in animals lacking the Nrf2 gene or using a blockade of the Nrf2 receptor model. Additionally, further investigation through immunoblotting analysis and in vitro cell experiments would be valuable in assessing the dissociation of the Keap1/Nrf2 complex, the translocation of Nrf2, and the accumulation of Nrf2 in the nucleus.

Chapter 6

References

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6. References

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