PROTECTIVE EFFECTS OF RUTIN ON SODIUM ARSENITE-INDUCED HEPATO-RENAL TOXICITY IN WISTAR RATS

BY

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DECLARATION

I, **ASIA-EDO FAVOUR ESOHE**, hereby declare that the project work entitled "Protective Effects of Rutin on Sodium Arsenite-Induced Hepato-Renal Toxicity in Wistar Rats" submitted by me for the award of B.Sc Degree in Medical Biochemistry at the University of Benin, is my original work. I have carried out the project under the supervision of Dr. (Mrs). O. Ikponmwosa-Eweka and all sources of information have been duly acknowledged.

CERTIFICATION

We the undersigned hereby certify that ASIA-EDO FAVOUR ESOHE (BMS2101380) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

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DEDICATION

I dedicate this project to God Almighty, whose grace, wisdom, and strength have guided me throughout this journey. His divine favour has been my source of inspiration and perseverance. With humility and love, I also dedicate this work to my delightful parents, Mr. and Mrs. Asia-Edo, whose prayers, sacrifices, and unwavering support have been the backbone of my academic growth. Their love and encouragement have continually inspired me to strive for excellence. I further extend this dedication to my family, whose encouragement and belief in my dreams have kept me motivated. In addition, I dedicate this work to the Department of Medical Biochemistry and the National Association of Medical Biochemistry Students (NAMBS), UNIBEN Chapter, for contributing to my academic development and creating an environment that has shaped my growth.

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ABSTRACT

Sodium arsenite is a toxic metalloid compound widely distributed in the environment through contaminated water, industrial effluents, and pesticides. Exposure to arsenic compounds has been associated with severe oxidative damage, particularly affecting the liver and kidneys. This study investigated the protective effect of rutin, a natural flavonoid with potent antioxidant properties, on sodium arsenite–induced hepato-renal toxicity in Wistar rats. Thirty-five (35) male Wistar rats were randomly divided into five groups of seven animals each. Group 1 served as the control and received corn oil only; Group 2 received 50mg/kg of rutin dissolved in distilled water; Group 3 received sodium arsenite (10 mg/kg body weight) dissolved in distilled water; Group 4 received rutin (25 mg/kg) and sodium arsenite (10 mg/kg) and; while Group 5 received rutin (50 mg/kg) and sodium arsenite (10 mg/kg). After the treatment period, blood samples were collected for biochemical analysis of liver and kidney function biomarkers-aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea, and creatinine. The results showed that sodium arsenite administration caused a significant increase ($p < 0.05$) in serum levels of AST, ALT, ALP, LDH, urea, and creatinine compared to the control group, indicating hepatic and renal impairment. However, co-administration of rutin led to a dose-dependent decrease in these biomarkers, bringing their values closer to the normal range. This suggests that rutin effectively mitigated the biochemical alterations induced by sodium arsenite. In conclusion, the findings demonstrate that rutin possesses potent antioxidant and protective properties capable of ameliorating sodium arsenite–induced liver and kidney toxicity in Wistar rats. This implies that rutin may have potential therapeutic applications in preventing heavy-metal-induced oxidative damage in humans.

TABLE OF CONTENTS

Page

Title Page
Declaration……..……i
Certification……ii
Dedication……iii
Acknowledgement……iv
Abstract……v
Table of Contents……vi-vii
List of Figures……viii
List of Tables……ix

1.1 BACKGROUND OF STUDY……..……1

1.2 STATEMENT OF PROBLEM…….……..……..2

1.3 AIMS AND OBJECTIVES…….……3

2.1 ARSENIC AND SODIUM ARSENITE…….……4
2.1.1 SOURCES OF ARSENIC EXPOSURE …….……4
2.1.1.1 Natural Sources……4
2.1.1.2 Anthropogenic Sources……...……5
2.1.1.3 Biological And Food Sources……..5
2.1.2 FORMS OF ARSENIC……6
2.1.2.1 Organic Vs Inorganic Arsenic…….……7
2.1.2.2 Trivalent Vs Pentavalent Arsenic…….……...7
2.1.3 MECHANISM OF ACTION OF SODIUM ARSENITE IN THE BODY……...……7
2.1.4 MECHANISTIC PATHWAYS…….……9
2.1.4.1 Cellular Uptake, Speciation, and Metabolism…….…….9
2.1.4.2 Binding to Thiol and Dithiol Groups …….……..9
2.1.4.3 Induction of Oxidative Stress and Reactive Oxygen Species……..……10
2.1.4.4 Mitochondrial Dysfunction…….…..…10
2.1.4.5 Genotoxicity and Epigenetic Modifications …..……10
2.1.4.6 Inflammatory and Apoptotic Pathways……..……11

2.2 HEPATO-RENAL TOXICITY ……12
2.2.1 MECHANISM OF HEPATO-RENAL TOXICITY……...12

2.2.2 WHY THE LIVER IS HIGHLY VULNERABLE……..……13
2.2.3 WHY THE KIDNEYS ARE HIGHLY VULNERABLE …….……..13
2.2.4 EFFECTS AND SYMPTOMS OF HEPATO-RENAL DAMAGE……14
2.3 TOXIC EFFECTS OF SODIUM ARSENITE IN THE BODY…….……15
2.3.1 SODIUM ARSENITE INDUCED LIVER DAMAGE (HEPATOTOXICITY)……..…16
2.3.2 SODIUM ARSENITE INDUCED KIDNEY DAMAGE (NEPHROTOXICITY)….……16
2.3.3 SODIUM ARSENITE INDUCED HEART DAMAGE (CARDIOTOXICITY)…..…….17
2.3.4 SUMMARY OF BIOCHEMICAL DISRUPTIONS BY ARSENIC……..17
2.4 PROTECTIVE MEASURES AGAINST SODIUM ARSENITE TOXICITY……18
2.4.1 NATURAL PRODUCTS USED AGAINST OXIDATIVE STRESS……19
2.4.1.1 Rutin…….…….20
2.4.2 RATIONALE FOR USING RUTIN AS A PROTECTIVE AGENT…….21
2.5 RUTIN (VITAMIN P)……...……22
2.5.1 SOURCES AND CHEMICAL STRUCTURE OF RUTIN……22
2.5.2 PHARMACOLOGICAL PROPERTIES OF RUTIN…….……24
2.5.2.1 Antioxidant Activity of Rutin…….……..…….24
2.5.2.2 Anti-Inflammatory Effect Of Rutin…….…..……24
2.5.2.3 Hepato-Renal Protective Role Of Rutin…….…...……25
2.5.2.4 Cardioprotective Effect Of Rutin…….…..……25
2.6 BIOCHEMICAL MARKERS OF ORGANS USED IN THIS STUDY……26
2.6.1 LIVER BIOMARKERS (AST, ALT, ALP)…….……27
2.6.2 KIDNEY BIOMARKERS (UREA AND CREATININE)……..28
2.6.3 CARDIAC AND TISSUE DAMAGE BIOMARKER (LDH)……..……..28
3.1 CHEMICALS AND REAGENTS …….31
3.2 ANIMAL PROTOCOL……..31
3.2.1 EXPERIMENTAL DESIGN……..…….31
3.2.2 SACRIFICE OF EXPERIMENTAL ANIMALS…….……...32
3.3 EVALUATION OF LIVER FUNCTION……..……32
3.3.1 ESTIMATION OF ALKALINE PHOSPHATE……..…32
3.3.2 ESTIMATION OF ASPARTATE TRANSAMINASE……..…..….33
3.3.3 ESTIMATION OF ALAMINE TRANSAMINASE……...…..….33
3.4 EVALUATION OF KIDNEY FUNCTION …….……34

3.4.1 DETERMINATION OF SERUM UREA……...……34
3.4.2 DETERMINATION OF SERUM CREATININE……..…...…….35
3.4.3 DETERMINATION OF SERUM LACTATE DEHYDROGENASE……...……37

4.1 RESULTS…… ……..….37

5.1 CONCLUSION…….…...…..39
5.1.1 HEPATIC MARKERS (AST, ALT, ALP)…….……..…...…39
5.1.2 RENAL MARKERS (UREA, CREATININE) ……..…….……40
5.1.3 LDH ACTIVITY……....….…….40

REFERENCES…….…….42-51

LIST OF FIGURES

Figure 2.1 Major Sources and Pathways of Arsenic Exposure in the Environment
Figure 2.2 Mechanism of Action of Sodium Arsenite in the Body
Figure 2.3 Human Body Showing the Health Effects of Arsenic on Various Organs
Figure 2.4 Common Dietary Sources of Natural Antioxidants
Figure 2.5 Leaf of a Rutin-Rich.Plant
Figure 2.6 Common Dietary Sources of Rutin in Vegetables and Plant Based Foods
Figure 2.7 Chemical Structure of Rutin
Figure 4.1 Effects of Rutin and Sodium Arsenite on AST Levels
Figure 4.2 Effects of Rutin and Sodium Arsenite on ALT Levels
Figure 4.3 Effects of Rutin and Sodium Arsenite on ALP Levels
Figure 4.4 Effects of Rutin and Sodium Arsenite on LDH Levels
Figure 4.5 Effects of Rutin and Sodium Arsenite on Urea Levels
Figure 4.6 Effects of Rutin and Sodium Arsenite on Creatinine Levels
Figure 4.7 Combined Liver Biomarkers

LIST OF TABLES

Table 2.1 Table Showing the Normal Range Level of the Biomarkers of Humans and Wistar Rats

Table 2.2 Effects on Biomarkers Relative to Normal Levels

Table 2.3 Mechanism of Sodium Arsenite Induced Alterations in Biochemical Markers

Table 2.4 Effect of Rutin Treatment on Biochemical Markers

Table 4.1 Effects of Rutin and Sodium Arsenite on Hepato-Renal Function

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The liver and kidneys are among the most metabolically active and vital organs in the human body, playing key roles in maintaining homeostasis through detoxification, metabolism, and excretion of metabolic wastes. The liver is primarily responsible for the bio-transformation of endogenous and exogenous compounds, converting harmful substances into less toxic or more soluble metabolites for elimination. Meanwhile, the kidneys are essential for maintaining fluid and electrolyte balance, regulating blood pressure, and excreting waste products of metabolism such as urea and creatinine (Santos *et al.*, 2019). Because of their high metabolic activity and exposure to reactive intermediates, both organs are particularly susceptible to damage by toxic substances and oxidative stress.

Arsenic, a metalloid naturally present in the environment, is recognized as one of the most significant global environmental toxins. It is commonly introduced into the environment through industrial activities, contaminated groundwater, pesticides, and smelting processes (Flora, 2011). Among the various chemical forms of arsenic, sodium arsenite ($NaAsO_2$), a trivalent inorganic arsenic compound, is especially toxic to biological systems. Exposure to sodium arsenite induces hepato-renal toxicity through mechanisms involving oxidative stress, inflammation, mitochondrial dysfunction, and cellular apoptosis (Nandi *et al.*, 2020). At the cellular level, sodium arsenite promotes the excessive generation of reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). These ROS overwhelm the antioxidant defense system, leading to oxidative modification of lipids, proteins, and nucleic acids (Rahman *et al.,* 2018). In the liver, arsenic exposure disrupts hepatocellular membranes and interferes with enzyme systems, causing elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the serum, all indicative of hepatocellular injury (Ahsan *et al.*, 2021). Similarly, in the kidneys, arsenic accumulation leads to glomerular and tubular dysfunction, reflected by increased serum urea and creatinine concentrations (Nandi *et al*., 2020).

Oxidative stress plays a central role in the pathology of sodium arsenite toxicity. Normally, the body maintains a delicate balance between free radical generation and antioxidant defenses mediated by enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione

peroxidase (GPx), as well as non-enzymatic antioxidants like glutathione (GSH) and vitamin C. However, arsenic interferes with these systems by binding to sulfhydryl groups and depleting glutathione reserves, thereby impairing the antioxidant defense mechanism (Flora, 2011). This leads to lipid peroxidation, protein oxidation, and DNA fragmentation, which collectively result in hepatocellular and renal cell damage (Ahsan *et al*., 2021).

In response to these toxic challenges, natural antioxidant compounds have gained increasing attention as potential therapeutic agents. Rutin, a naturally occurring flavonoid glycoside (quercetin-3-O-rutinoside), is found abundantly in citrus fruits, apples, buckwheat, tea, and onions. It possesses diverse pharmacological properties, including antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, and anti-apoptotic effects (Ganeshpurkar and Saluja, 2017). Rutin exerts its antioxidant activity by directly scavenging free radicals, inhibiting lipid peroxidation, and enhancing the activity of antioxidant enzymes such as SOD, CAT, and GSH (Moustafa *et al.*, 2019). Furthermore, rutin has been shown to stabilize cell membranes, reduce the release of inflammatory cytokines, and modulate signaling pathways involved in oxidative stress and apoptosis (Li *et al*., 2021). It also exhibits metal-chelating properties, which can limit the ability of toxic metals like arsenic to catalyze free radical formation. Previous studies have demonstrated rutin's ability to protect against various toxicants, including carbon tetrachloride, paracetamol, and cisplatin-induced organ injury (Abd El-Aziz *et al*., 2018).

Despite extensive research on rutin's antioxidant potential, there is limited information on its ability to counteract sodium arsenite–induced hepato-renal toxicity, particularly from a medical biochemical perspective involving oxidative stress biomarkers, enzymatic antioxidant defense, and histopathological assessment. This study therefore seeks to evaluate the protective effects of Rutin on sodium arsenite-induced hepato-renal and cardiac toxicity in male Wistar rats through biochemical and histopathological assessments. The outcome could provide scientific insight into the biochemical basis of rutin's protective mechanisms and support its use as a potential therapeutic or preventive agent against heavy metal toxicity.

1.2 STATEMENT OF THE PROBLEM

Environmental exposure to arsenic remains a major public health concern, especially in developing countries where people are routinely exposed to arsenic-contaminated groundwater and food sources. Chronic arsenic poisoning, also known as arsenicosis, is associated with

multiple systemic disorders such as liver dysfunction, kidney failure, cardiovascular diseases, neurotoxicity, diabetes, and even carcinogenesis (Rahman *et al.*, 2018). The World Health Organization (WHO) has identified arsenic as one of the top ten chemicals of major public health concern.

The toxicity of arsenic stems largely from its ability to induce oxidative stress and impair the body's antioxidant defense mechanisms. Long-term exposure leads to biochemical derangements, histological damage, and functional impairment of the liver and kidneys, organs crucial for detoxification and waste elimination. The situation is further complicated by the limited availability of safe and effective antidotes. Conventional chelating agents used for arsenic poisoning, such as dimercaprol (British anti-Lewisite) and DMSA (dimercaptosuccinic acid), often cause undesirable side effects and do not completely reverse oxidative tissue damage (Flora, 2011).

Hence, there is a pressing need to search for alternative, natural, and less toxic compounds capable of protecting or restoring organ function following arsenic exposure. Rutin, being a potent bioflavonoid with antioxidant and anti-inflammatory properties, presents a promising candidate. However, its role in mitigating arsenic-induced hepato-renal toxicity has not been sufficiently explored or biochemically characterized. Understanding the molecular mechanisms underlying rutin's protective effects could offer valuable insights into natural strategies for combating arsenic-induced biochemical and physiological damage.

1.3 AIM AND OBJECTIVES

The aim of this study is to evaluate the protective effects of rutin on sodium arsenite-induced hepato-renal toxicity in Wistar rats.

To achieve this aim, some of the objectives are;

1. Assess the effect of Rutin and sodium arsenite on liver function through the determination of serum ALP, AST, and ALT activities.
2. Evaluate the effect of Rutin and sodium arsenite on kidney function by measuring serum urea and creatinine levels.
3. Determine the effect of Rutin and sodium arsenite on cardiac function through the estimation of serum lactate dehydrogenase (LDH) activity.
4. Compare the dose-dependent effects of Rutin co-administration (25 mg/kg and 50 mg/kg) on sodium arsenite-induced toxicity.

CHAPTER TWO

LITERATURE REVIEW

2.1 ARSENIC AND SODIUM ARSENITE

Arsenic is a naturally occurring metalloid widely distributed in the earth's crust, ranking as the 20th most abundant element. Despite its natural presence, arsenic contamination has become a critical public health issue due to industrialization, mining, and agricultural applications. The World Health Organization identifies arsenic contamination as a global toxicological concern due to its ability to contaminate groundwater and induce systemic toxicity in humans and animals (Bhattacharya *et al.*, 2021). Sodium arsenite ($NaAsO_2$), a trivalent inorganic arsenic derivative, has been used for decades in pesticides, wood preservatives, and chemical manufacturing. However, its high toxicity and carcinogenic nature have raised concerns, prompting extensive research involving model organisms such as Wistar rats to investigate its biological impact (Jomova *et al*., 2022).

Once sodium arsenite enters living systems, it is readily absorbed through the gastrointestinal tract and from there distributed via the bloodstream to metabolically active organs, particularly the liver and kidneys. These organs bear the burden of detoxification and excretion, making them primary targets for damage (Nurchi *et al*., 2020). Prolonged exposure, even at low concentrations, has been linked to hepato-renal dysfunction, reproductive toxicity, endocrine disturbances, and cancers of the skin, liver, and bladder (Mazumder *et al.*, 2023). Therefore, understanding the nature of arsenic, its forms, and its mechanisms of action is vital in studying potential protective interventions like antioxidants.

2.1.1 Sources of Arsenic Exposure

Arsenic is a naturally occurring metalloid widely distributed in the earth's crust. It is introduced into the environment through both natural processes and anthropogenic (human-induced) activities (Mandal and Suzuki, 2002). These sources determine how humans, animals, and plants become exposed to it.

2.1.1.1 Natural Sources

Naturally, arsenic is found in over 200 mineral species, primarily as sulfides such as Arsenopyrite (FeAsS), Realgar (As_4S_4), and Orpiment (As_2S_3) (Smedley and Kinniburgh, 2002). Weathering of these rocks and minerals releases arsenic into soils and water bodies.

In groundwater, arsenic contamination often arises from geochemical reactions that mobilize

arsenic from sediments under reducing or anaerobic conditions (Smith *et al.*, 2020). Other natural sources include volcanic emissions, geothermal activities, and forest fires, all of which can release arsenic into the atmosphere or hydrosphere (Nguyen *et al.*, 2021).

Natural processes, including erosion of arsenic-bearing rocks, volcanic emissions, and geothermal activities, release arsenic into the environment. This leads to contamination of groundwater and soil which is the most common pathway of chronic exposure worldwide (Flora and Dwivedi, 2020). In regions such as Bangladesh, India, and parts of Africa, arsenic levels in drinking water exceed the WHO permissible limit of 10 µg/L (Islam *et al.*, 2021). Animals also become exposed when they consume contaminated vegetation or water sources.

2.1.1.2 Anthropogenic Sources

Human activities significantly contribute to arsenic mobilization in the environment. Mining and smelting operations are major sources, as arsenic is a common impurity in metal ores such as copper, gold, and lead (Naujokas *et al*., 2013). Agricultural uses, including arsenic-based pesticides (e.g., lead arsenate, sodium arsenite), have historically led to long-term soil contamination (Jain and Ali, 2000). Additionally, industrial processes such as glass production, semiconductor manufacturing, and tanning release arsenic-containing effluents (Rahman *et al*., 2018). The combustion of fossil fuels, particularly coal, emits arsenic vapors and particulates into the air (Mandal and Suzuki, 2002). Another important anthropogenic source is the use of chromated copper arsenate (CCA) in wood preservation (Naujokas *et al.,* 2013).

Significant human-driven sources include:

i. Mining and smelting of metals like copper and lead
ii. Combustion of fossil fuels
iii. Manufacturing arsenic-based pesticides, herbicides, and pharmaceuticals
iv. Wood preservation with arsenic compounds

Workers in smelting and agricultural industries are particularly vulnerable to inhalation exposure (Jomova *et al*., 2022).

2.1.1.3 Biological and Food Sources

Arsenic also occurs in various biological systems. Seafood contains mainly organic forms of arsenic such as arsenobetaine and arsenocholine, which are relatively nontoxic (Smith *et al.,* 2020). However, inorganic arsenic exposure through drinking water and food crops (especially rice) poses significant health risks (Rahman *et al.*, 2018). Rice plants are efficient accumulators

of arsenic when grown in flooded, contaminated soils or irrigated with arsenic-rich water (Zhao *et al.*, 2010).

Groundwater contamination is the major exposure route for humans and animals. Crops such as rice absorb arsenic more efficiently due to flooded soil environments, leading to dietary accumulation (Nurchi *et al.*, 2020). In livestock and research animals like Wistar rats, arsenic-contaminated feed and water are leading exposure routes in experimental studies. Long-term ingestion poses significant cumulative toxicity, especially to detoxification organs.

Figure 2.1 below illustrates the major sources and pathways of arsenic exposure in the environment.

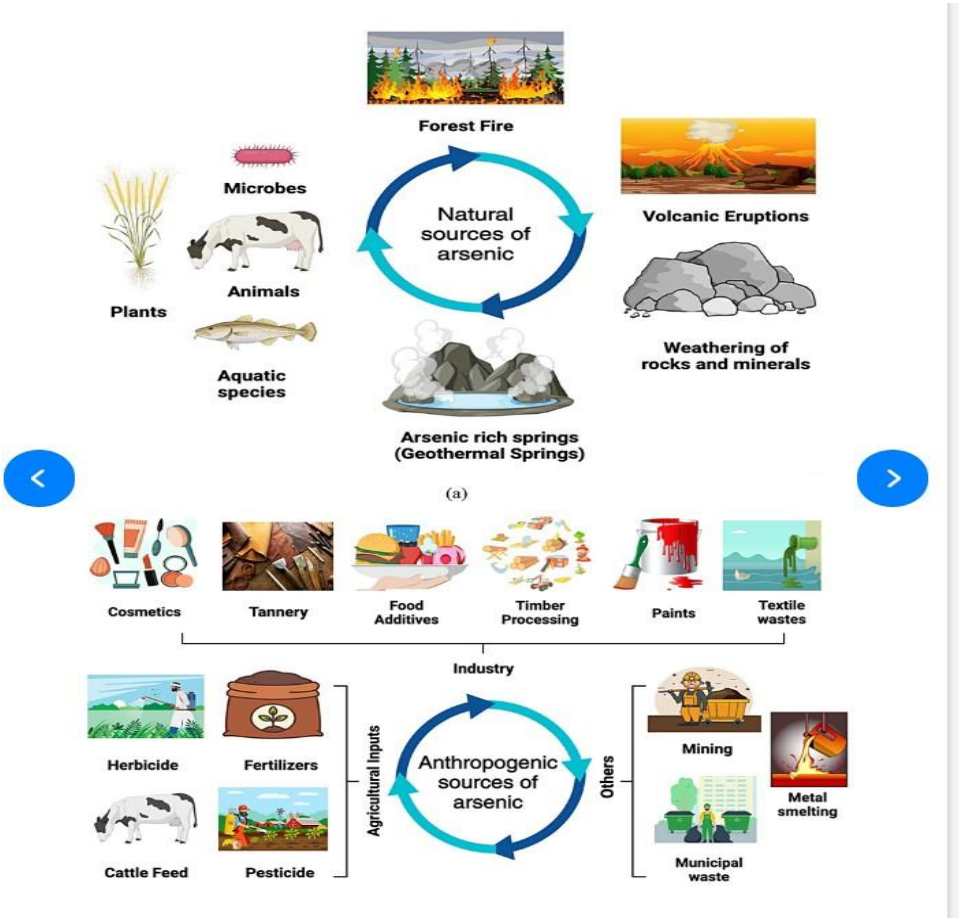

Figure 2.1 Major Sources and Pathways of Arsenic Exposure in the Environment (Source; Rajendran *et al.*, 2024)

2.1.2 Forms of Arsenic

Arsenic exists in multiple chemical species, which influence its toxicity, metabolism, and bioavailability.

2.1.2.1 Organic vs Inorganic Arsenic

Organic arsenic such as seafood has low toxicity because they are poorly metabolized in humans while Inorganic arsenic such as water, soil and industrial wastes has an high toxicity. Inorganic arsenic exerts a more severe toxicological effect due to its ability to interfere directly with enzymatic systems and oxidative processes. Inorganic arsenite (iAs) occurs mainly as arsenite (As^{3+}) and arsenate (As^{5+}) (Mazumder *et al*., 2023). Drinking water is globally the dominant route for chronic iAs exposure in many regions; rice and other foods can be important contributors. Industrial sources (mining, smelting, coal combustion) and arsenic-contaminated irrigation also cause localized exposure hotspots.

2.1.2.2. Trivalent vs. Pentavalent Arsenic

Trivalent arsenic (As^{3+}) such as sodium arsenite is the most toxic form. It binds to sulfhydryl groups in critical enzymes, disrupts cellular metabolism, and induces apoptosis while Pentavalent arsenic (As^{5+}) including sodium arsenate, is more stable and less reactive but can be biotransformed into the trivalent state inside biological systems (Singh *et al*., 2022). Because sodium arsenite directly interacts with thiol-dependent biological pathways, it is widely used in experimental toxicology to induce oxidative damage.

2.1.3 Mechanism of Action of Sodium Arsenite in the Body

Sodium arsenite ($NaAsO_2$) is particularly toxic and has been implicated in a variety of health disorders affecting multiple organ systems. Understanding the mechanism of sodium arsenite's action is vital, as its toxicity stems from complex biochemical and molecular interactions that disrupt normal cellular processes, leading to oxidative stress, mitochondrial dysfunction, genotoxicity, and altered gene expression patterns (Biswas *et al*., 2018).

Once sodium arsenite enters the systemic circulation, it is rapidly distributed to metabolically active and highly perfused organs such as the liver and kidneys. The primary mechanism underlying its toxicity is the disruption of cellular redox balance. Sodium arsenite enhances the formation of reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals, and hydrogen peroxide. Excessive ROS production overwhelms endogenous antioxidant defense systems such as glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), resulting in oxidative stress (Flora and Dwivedi, 2020). This oxidative imbalance initiates peroxidative degradation of membrane lipids, leading to loss of membrane integrity, impaired cellular signaling, and increased permeability (Islam *et al.,* 2021). Sodium arsenite increases reactive oxygen species (ROS) generation, depletes cellular

antioxidants (glutathione, thioredoxin), and impairs mitochondrial function, all contributing to lipid, protein, and DNA damage. Increased oxidative makers after sodium arsenite often ameliorate damage in cell/animal.

Beyond oxidative stress, sodium arsenite exerts toxicity by directly interacting with sulfhydryl (–SH) groups present in vital metabolic enzymes. This interaction inhibits enzymes involved in energy metabolism, particularly those in the mitochondrial respiratory chain such as pyruvate dehydrogenase and succinate dehydrogenase. The inhibition of these enzymes disrupts the tricarboxylic acid cycle and reduces ATP synthesis, thereby triggering mitochondrial dysfunction and energy depletion at the cellular level (Jomova *et al*., 2022). Mitochondria, being central coordinators of apoptosis, respond to this stress through the release of cytochrome and activation of caspase cascades, ultimately leading to programmed cell death. In cases of severe injury, necrotic cell death may also occur, contributing to inflammatory responses in affected tissues.

Sodium arsenite is further known to interfere with cellular signaling pathways and gene expression. It also disrupts DNA repair enzymes such as poly (ADP-ribose) polymerase (PARP), thereby exacerbating genomic instability (Mazumder *et al.,* 2023). These molecular alterations enhance susceptibility to malignant transformation with prolonged exposure. The toxicokinetics of sodium arsenite indicate that its metabolism primarily occurs in the liver through methylation, a detoxification process that converts arsenite to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Nurchi *et al.,* 2020). As the kidneys eliminate these metabolites through urine, renal tubular epithelial cells become particularly vulnerable to oxidative and inflammatory injury, resulting in functional impairments such as reduced glomerular filtration and electrolyte imbalance.

Overall, sodium arsenite toxicity represents a multi-mechanistic phenomenon involving oxidative stress, enzyme inhibition, mitochondrial dysfunction, dysregulated apoptosis, altered gene expression, and impaired detoxification pathways. These mechanisms collectively explain its strong affinity for and damaging effects on the hepato-renal system, justifying its widespread use in experimental models investigating antioxidant-based therapeutic interventions.

2.1.4 Mechanistic Pathways

2.1.4.1 Cellular Uptake, Speciation, and Metabolism

Sodium arsenite is absorbed through the gastrointestinal tract, lungs, and skin (Jomova *et al.*, 2011). Once ingested or absorbed, sodium arsenite (As^{3+}) readily crosses cell membranes via aquaglyceroporins (especially AQP7 and AQP9), which are also responsible for glycerol transport (Liu *et al.,* 2002). Inside the body, arsenic undergoes biotransformation in the liver through sequential methylation catalyzed by arsenic (+3 oxidation state) methyltransferase (AS3MT), converting inorganic arsenic to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Vahter, 2007). This process was once thought to be a detoxification pathway, but newer evidence suggests that intermediate metabolites such as monomethylarsonous acid (MMA^{3+}) are more toxic than the parent compound (Cullen, 2014). In human populations exposed to arsenic-contaminated water, higher urinary levels of MMA^{3+} and DMA have been correlated with increased risks of skin, lung, and bladder cancers (Engström *et al.*, 2015). Similarly, rat studies demonstrated that chronic sodium arsenite exposure led to accumulation of arsenic species in the liver and kidney, resulting in biochemical and histological alterations (Kumagai and Pi, 2004; Afolabi *et al.*, 2022).

2.1.4.2 Binding to Thiol and Dithiol Groups

The trivalent form of arsenic (As^{3+}) exhibits a strong affinity for sulfhydryl (-SH) groups present in proteins, particularly in enzymes containing thiol or dithiol cofactors such as lipoic acid (Hughes, 2002). This interaction leads to enzyme inactivation, notably those involved in energy metabolism like pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase, thereby disrupting ATP production (Flora, 2011). Arsenite also binds to cysteine residues in antioxidant enzymes like glutathione reductase (GR) and thioredoxin reductase (TrxR), impairing the antioxidant defense system and promoting oxidative damage (Styblo *et al.*, 2000).

In rats, sodium arsenite exposure caused significant reductions in hepatic GSH levels and enzyme activities of SOD and CAT, confirming its thiol-reactive toxicity (Afolabi *et al.*, 2022). In humans, similar enzyme inhibition was observed in arsenic-exposed workers, leading to increased oxidative biomarkers such as malondialdehyde (MDA) (Wu *et al*., 2014).

2.1.4.3 Induction of Oxidative Stress and Reactive Oxygen Species (ROS)

A key mechanism underlying sodium arsenite toxicity is the generation of reactive oxygen species (ROS). Arsenite induces ROS through mitochondrial electron transport disruption, NADPH oxidase activation, and depletion of antioxidants (Jomova *et al.*, 2011). Elevated ROS levels cause lipid peroxidation, protein oxidation, and DNA strand breaks, leading to cellular dysfunction (Sharma and Sohn, 2019). In rats, sodium arsenite administration resulted in increased MDA and hydrogen peroxide levels, alongside reduced activities of catalase and superoxide dismutase (SOD) in hepatic and renal tissues (Afolabi *et al.,* 2022). Human studies in arsenic-exposed populations also show elevated oxidative stress markers and reduced antioxidant enzyme levels, supporting the translational relevance of animal data (Milton *et al.,* 2021).

2.1.4.4 Mitochondrial Dysfunction

Sodium arsenite interferes with mitochondrial function by inhibiting complexes I and II of the electron transport chain, leading to ATP depletion and loss of mitochondrial membrane potential (Valko *et al.*, 2005). This impairment not only decreases energy availability but also triggers apoptotic signaling through cytochrome c release and caspase-3 activation (Bhattacharjee *et al.*, 2013). In Wistar rats, chronic sodium arsenite exposure caused ultrastructural mitochondrial damage and increased expression of pro-apoptotic proteins (Bax, caspase-9), confirming its role in intrinsic apoptosis (Singh *et al.*, 2020). Human cell line studies revealed similar findings, arsenite exposure led to mitochondrial swelling, decreased ATP content, and enhanced apoptosis in keratinocytes and hepatocytes (Ren *et al.*, 2011).

2.1.4.5 Genotoxicity and Epigenetic Modifications

Sodium arsenite is a potent genotoxic and epigenetic modulator. It induces DNA strand breaks, chromosomal aberrations, and micronuclei formation, primarily through oxidative stress and inhibition of DNA repair enzymes (Bailey *et al.*, 2016). Additionally, arsenite alters global and gene-specific DNA methylation patterns by interfering with DNA methyltransferases (DNMTs) and histone modification enzymes (Reichard and Puga, 2010). In rats, sodium arsenite exposure led to significant increases in comet assay tail length (a marker of DNA damage) in liver and kidney tissues (Afolabi *et al*., 2022). In humans, prenatal arsenic exposure has been linked to epigenetic dysregulation in genes associated with immune response and carcinogenesis (Bailey *et a*l., 2016; Broberg *et al.*, 2021).

2.1.4.6 Inflammatory and Apoptotic Pathways

Chronic sodium arsenite exposure also triggers inflammatory cascades, mediated by activation of transcription factors such as NF-κB and AP-1, leading to increased expression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Biswas *et al.,* 2018). This persistent inflammatory state promotes tissue fibrosis and carcinogenesis. In Wistar rats, sodium arsenite-induced hepatotoxicity was associated with elevated TNF-α, IL-6, and COX-2 levels (Afolabi *et al.*, 2022). Correspondingly, in human studies, arsenic-exposed populations showed elevated serum cytokines and inflammatory gene expression, linking arsenic exposure to cardiovascular and hepatic disorders (States *et al*., 2011; Milton *et al.,* 2021).

The toxicity of sodium arsenite involves multiple interrelated mechanisms rather than a single pathway. Upon absorption, it undergoes intracellular transformations that generate highly reactive intermediates. These species bind to thiol groups in proteins, disrupt mitochondrial respiration, and induce oxidative stress, resulting in genotoxic, inflammatory, and apoptotic responses. Findings from both animal and human studies consistently demonstrate that sodium arsenite toxicity stems from its ability to interfere with fundamental biochemical processes, ultimately leading to organ dysfunction and carcinogenesis. Understanding these pathways is crucial for developing effective therapeutic and preventive strategies against arsenic-related health risks.

Figure 2.2 below illustrates the major pathways through which sodium arsenite exerts its toxic effect in biological systems.

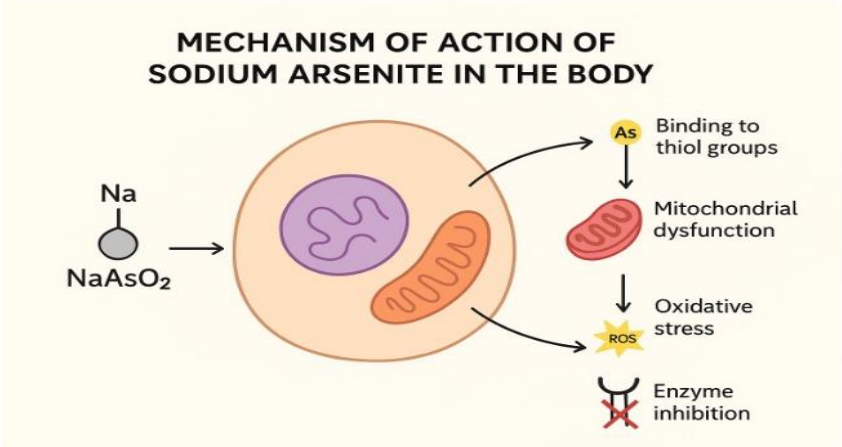

Figure 2.2 Mechanism of Action of Sodium Arsenite in the Body (Source; Biswas *et al*., 2018)

2.2 HEPATO-RENAL TOXICITY

The term “hepato-renal toxicity” refers to the simultaneous damage or dysfunction of the two major organs responsible for handling toxins in the body: the liver (hepatic system) and the kidneys (renal system). Because these organs are constantly working to detoxify, metabolize, filter, and excrete various substances, both the compounds we ingest and those our body produces are especially vulnerable when the body is exposed to harmful chemical agents. Over time and with sufficient exposure, this dual organ damage can manifest as elevated markers in blood tests, structural changes (seen under the microscope) and declines in organ performance, ultimately threatening overall health (Concessao *et al*., 2025).

2.2.1 Mechanisms of Hepato-Renal Toxicity

In simple terms, hepato-renal toxicity means that a toxic substance interferes with both liver and kidney function, leading to injury in both organs. The mechanisms by which this happens are often overlapping and interconnected. When a toxin such as a heavy metal, drug, or environmental pollutant enters the body, it typically triggers a series of damaging events.

First, the toxin may generate oxidative stress: it causes an over-production of reactive oxygen species (ROS) or free radicals that attack cell membranes, lipids, proteins, and DNA. Over time, this damage accumulates. (Islam *et al.,* 2021). Second, the toxin may disrupt metabolic enzymes, interfere with mitochondrial function (the energy factories of cells), or hamper the formation and repair of cellular components. For example, the kidneys’ high energy demand (to filter and reabsorb substances) makes their mitochondria especially vulnerable. (Turkington *et al.*, 2025). Third, the toxin may provoke inflammatory responses: injured cells release signals that attract immune cells, causing further tissue damage, scarring (fibrosis), and functional decline (Pineda *et al*., 2013). Fourth, blockage or impairment of detoxification/excretion pathways**:** when the liver or kidneys are overloaded, they cannot clear toxins effectively, which leads to a buildup of harmful substances and causes a vicious cycle of damage.

Because the liver and kidneys are part of a functional chain, the liver processes substances and the kidneys clear them when one organ becomes impaired, the other must compensate, increasing its own susceptibility to injury. For instance, a failing liver may produce toxic metabolites that the kidneys cannot handle; conversely, failing kidneys may allow toxins to accumulate and damage the liver.

2.2.2 Why the Liver is Highly Vulnerable

The liver deserves a spotlight here because it plays the role of chief detoxifier in the body. Practically every chemical, drug, food additive, environmental pollutant, or compound that enters the bloodstream eventually reaches the liver via the portal circulation. Because of this, the liver receives a very high volume of blood per minute, meaning it gets exposed to high concentrations of any circulating toxin. It is the primary site of biotransformation, where substances are chemically modified (often to make them easier to excrete). Unfortunately, in this process, harmless compounds may become reactive intermediates that are even more damaging than the parent substance (Ma *et al.*, 2025).

Liver cells (hepatocytes) have a huge metabolic workload and high oxygen consumption, making them more vulnerable to disruptions in energy supply or oxidative stress. When toxins accumulate or when the liver's repair mechanisms are overwhelmed, typical signs of injury appear such as elevated liver enzymes (ALT, AST, ALP) in blood, structural changes in tissue (fatty change, necrosis, fibrosis) and diminished capacity to carry out vital metabolic roles (Kumar *et al.*, 2024). Because of these vulnerabilities, when animals (like rats) or humans are exposed to toxic compounds such as inorganic arsenic, the liver often shows damage early in the exposure timeline, thereby making it a critical organ to monitor in toxicity studies.

2.2.3 Why the Kidneys are Highly Vulnerable

Moving on to the kidneys: these organs filter blood continuously, reabsorb necessary substances, secrete waste products, and maintain fluid, electrolyte, and pH balance. Their very function puts them in a high-risk zone when it comes to toxins. The kidneys receive about **2**0-25% of the body's cardiac (blood) output, so they are constantly filtering large volumes of blood and thus exposed to high loads of toxins. Filtration concentrates waste and toxins into the renal tubules and so the lining of these tubules is exposed to higher concentrations of toxins than many other tissues. Over time this can cause damage to the tubular epithelium and glomerular structures (Turkington *et al.*, 2025). The proximal tubules (in particular) rely heavily on mitochondrial energy (ATP) for re-absorption and secretion processes. Disruption of mitochondrial function such as heavy metals and arsenic do therefore hits these cells hard (Turkington *et al.*, 2025). Persistent injury may lead to reduced glomerular filtration rate, elevated blood urea nitrogen (BUN) and creatinine, proteinuria, structural changes like tubular necrosis, interstitial fibrosis or glomerulosclerosis (Concessao *et al*., 2025) .

Finally, because kidneys are downstream in the detox/excretion chain, any failed clearance of toxins from the liver or circulation escalates the load on kidneys, amplifying the risk of injury. In summary, because the kidneys both receive a large volume of blood and are tasked with concentrating and removing waste products, they become frequent casualty organs in exposure to toxins like inorganic arsenic, and hepato-renal toxicity reflects damage to this dual system of liver + kidneys.

2.2.4 Effects and Symptoms of Hepato-Renal Damage

In humans, injury to both the liver and kidneys disrupts some of the most essential biological functions, since these two organs are central to detoxification, metabolism, and waste removal. When the liver begins to fail, one of the earliest visible signs is jaundice; the yellow discoloration of the skin and eyes caused by an accumulation of bilirubin that the liver can no longer process efficiently. Individuals also tend to experience dark urine and pale or clay-coloured stool as bilirubin and bile flow are disrupted and toxins build up, fatigue, weakness, and general malaise because the liver cannot perform its normal metabolic and detoxification duties, peripheral swelling (edema) from low albumin production, impaired plasma oncotic pressure, and portal hypertension leading to leakage of fluid into the abdomen and tissues, easy bruising/bleeding because the liver's synthetic function (production of clotting factors) is compromised, hepatic encephalopathy: confusion, altered consciousness, changes in mental status (due to accumulation of neurotoxins the failing liver cannot remove), this overlaps with kidney effects, appetite loss, persistent tiredness, and weight changes, reflecting the liver's reduced capacity to regulate metabolism and produce key nutrients. As liver function worsens, inadequate synthesis of proteins and clotting factors leads to abdominal swelling (ascites), fluid buildup in the legs, and an increased tendency to bleed or bruise from minor injuries (Bodh *et al;* 2020).

Kidney damage occurring simultaneously compounds the problem. Because the kidneys are unable to filter the blood properly, toxic substances such as creatinine and urea accumulate, and urine output may significantly decrease causing oliguria (very low urine output) or even anuria (near-zero urine output) because the kidneys' filtration and excretion capacity is diminished. There is rising levels of waste products in blood (e.g., creatinine, urea) indicating reduced glomerular filtration rate (GFR) and accumulation of toxins normally cleared by kidneys, fluid overload: swelling of legs, ankles, abdomen because kidneys can't remove excess fluid and salts; combined with liver failure this is aggravated, electrolyte imbalances (e.g., hyponatraemia,

hyperkalaemia) and acid-base disturbances resulting from impaired renal excretion and altered fluid balance, confusion, drowsiness or altered mental state (due to uremic toxins, electrolyte disturbance, and combined liver failure), so neurological symptoms may blur between liver and kidney issues. Patients may retain excess water, leading to puffiness, difficulty breathing due to fluid in the lungs, and dangerous disturbances in electrolytes such as sodium and potassium (Kiani and Zori, 2023). These imbalances can impair the nervous system, causing headaches, confusion, irritability, and even altered consciousness in severe cases. The body's toxin-clearing systems become overwhelmed. A serious condition known as Hepatorenal Syndrome may develop, especially in individuals with advanced liver disease. This condition is characterized by a sharp drop in kidney blood flow and critically low urine production, making survival extremely unlikely without immediate treatment or organ transplantation (Angeli *et al.*, 2015).

At this stage, patients often deteriorate rapidly, showing signs of widespread physiological distress because both major detoxifying organs are no longer functioning adequately. There is profound malaise, loss of appetite, nausea/vomiting, weight loss, markedly reduced urine output plus fluid build-up (ascites + edema) showing both failing excretion and failing synthesis/storage of plasma proteins, persistent or recurrent infections (like spontaneous bacterial peritonitis) because immune defenses are down and toxins are building up, rapid deterioration of organ function: often kidney dysfunction follows liver decompensation, or both worsen together. In HRS-AKI, mortality rates are very high without intervention, laboratory hallmark: rising serum creatinine and urea; low urine sodium (in some cases); minimal proteinuria/histological damage in the kidneys (in the classic HRS model) because the primary injury is functional. Hepato-renal toxicity therefore represents not just isolated organ damage but a dangerous systemic breakdown affecting every part of the body (Kiani and Zori, 2023).

2.3 TOXIC EFFECTS OF SODIUM ARSENITE IN THE BODY

Sodium arsenite, an inorganic trivalent form of arsenic, is one of the most toxic and environmentally persistent metalloid compounds. It has been widely used in pesticides, herbicides, and industrial processes, resulting in its presence in soil, air, and groundwater. Once inside the body, it is rapidly absorbed through the gastrointestinal tract, skin, or respiratory system and distributed to vital organs such as the liver, kidney, and heart (Rahman *et al.*, 2020). Because of its high affinity for thiol (-SH) groups, sodium arsenite interferes with numerous metabolic enzymes, disrupting cellular respiration, energy metabolism, and antioxidant defense mechanisms (Nurchi *et al.,* 2020). Its toxicity arises primarily from the generation of reactive

oxygen species (ROS) and reactive nitrogen species (RNS), which trigger oxidative stress and initiate lipid peroxidation, protein oxidation, and DNA damage. Chronic exposure can therefore lead to multi-organ failure, cancer, and cardiovascular complications (Bhattacharjee *et al.,* 2020).

2.3.1 Sodium Arsenite-Induced Liver Damage (Hepatotoxicity)

The liver, as the body's primary detoxification and metabolic organ, is one of the first targets of sodium arsenite toxicity. Once absorbed, arsenic undergoes methylation in the liver, generating intermediates that are often more reactive and toxic than the parent compound. These metabolites, such as monomethylarsonous acid (MMAIII), induce oxidative stress and compromise hepatocellular membranes (Manna *et al.,* 2008). Experimental studies have shown that arsenite exposure elevates liver enzymes; ALT, AST, and ALP, indicating hepatocellular injury (Santra *et al.*, 2019). Histopathological analysis of liver tissues from arsenite-exposed rats reveals fatty degeneration, vacuolization of hepatocytes, necrosis, and infiltration of inflammatory cells around the central vein (Bhattacharjee *et al*., 2020).

Moreover, arsenic disrupts mitochondrial integrity, leading to reduced ATP generation and activation of apoptotic pathways. The depletion of glutathione (GSH) and suppression of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) amplify oxidative damage, making the liver more susceptible to fibrosis and cirrhosis in chronic cases (Flora, 2011). These biochemical and histological alterations reflect a combined effect of oxidative stress, inflammation, and metabolic dysfunction. Chronic arsenic exposure has also been linked to liver enlargement, cholestasis, and hepatocellular carcinoma in both animal models and human populations exposed to contaminated water (Rahman *et al*., 2020).

2.3.2 Sodium Arsenite-Induced Kidney Damage (Nephrotoxicity)

The kidney, another major site of arsenic accumulation, plays a vital role in filtering blood and excreting arsenic metabolites. Prolonged exposure to sodium arsenite impairs renal tubular and glomerular functions, resulting in significant biochemical and morphological alterations. Elevated levels of blood urea nitrogen (BUN) and serum creatinine serve as biomarkers of nephrotoxicity, reflecting the kidneys' diminished ability to excrete metabolic waste (Rahman *et al.,* 2020). Histological studies reveal congestion, tubular necrosis, and glomerular shrinkage, which compromise the organ's filtration capacity (Flora, 2011).

Mechanistically, sodium arsenite damages renal cells through oxidative stress-mediated mitochondrial injury and apoptotic signaling. It downregulates the expression of Na^+/K^+-

ATPase, an enzyme essential for maintaining ionic gradients, thus impairing electrolyte balance (Patlolla *et al.*, 2019). Additionally, arsenite-induced reduction of antioxidants like glutathione and glutathione peroxidase (GPx) weakens the kidney's defense system. Studies by Sinha et al. (2020) have shown that arsenic exposure leads to lipid peroxidation and accumulation of malondialdehyde (MDA), which correlates with renal oxidative injury. Long-term exposure has been associated with chronic kidney disease (CKD) and interstitial fibrosis, especially in communities exposed to contaminated drinking water.

2.3.3 Sodium Arsenite-Induced Heart Damage (Cardiotoxicity)

Cardiotoxicity is another serious consequence of arsenic exposure. Although the heart is not a detoxification organ, its high metabolic rate and oxygen consumption make it particularly vulnerable to arsenite-induced oxidative stress. Arsenic interferes with mitochondrial enzymes and electron transport chain complexes, leading to impaired energy metabolism, increased production of ROS, and reduced synthesis of adenosine triphosphate (ATP) (Nurchi *et al.*, 2020). These disruptions result in damage to cardiac myocytes, lipid peroxidation of membranes, and apoptosis.

Animal studies have demonstrated that sodium arsenite exposure increases serum levels of cardiac biomarkers such as creatine kinase-MB (CK-MB), troponin, and lactate dehydrogenase (LDH), indicating myocardial injury (Patlolla *et al.,* 2019). Histopathological findings include disorganization of cardiac muscle fibers, interstitial edema, and inflammatory infiltration. Functionally, this may manifest as arrhythmias, hypertension, and reduced cardiac output. Chronic arsenic exposure has also been associated with endothelial dysfunction and atherosclerosis, driven by oxidative stress, nitric oxide depletion, and inflammatory activation of vascular cells (Nurchi *et al.,* 2020). Epidemiological studies in humans support these findings, showing that populations exposed to arsenic-contaminated water have higher rates of ischemic heart disease, hypertension, and sudden cardiac death (Rahman *et al.*, 2020).

2.3.4 Summary of Biochemical Disruptions by Arsenic

Across all organ systems, the toxic effects of sodium arsenite are unified by its ability to induce oxidative stress and interfere with enzymatic functions dependent on thiol groups. It inhibits enzymes in critical metabolic pathways such as pyruvate dehydrogenase, succinate dehydrogenase, and glutathione reductase, thereby impairing mitochondrial respiration and antioxidant regeneration (Flora, 2011). Arsenic also disturbs calcium and potassium homeostasis, leading to altered cellular signaling and apoptosis. DNA-protein cross-linking and

chromosomal aberrations have been observed in arsenic-exposed cells, which may explain its mutagenic and carcinogenic potential (Bhattacharjee *et al*., 2020).

Overall, sodium arsenite-induced toxicity involves a multifaceted biochemical disruption that combines oxidative stress, inflammation, mitochondrial failure, and genotoxicity. The accumulation of these effects across multiple organs explains the broad spectrum of pathological conditions linked to arsenic exposure, including hepatic and renal failure, cardiovascular diseases, diabetes, and even cancer (Nurchi *et al.*, 2020).

Figure 2.3 below illustrates the major organs and body systems affected by arsenic exposure.

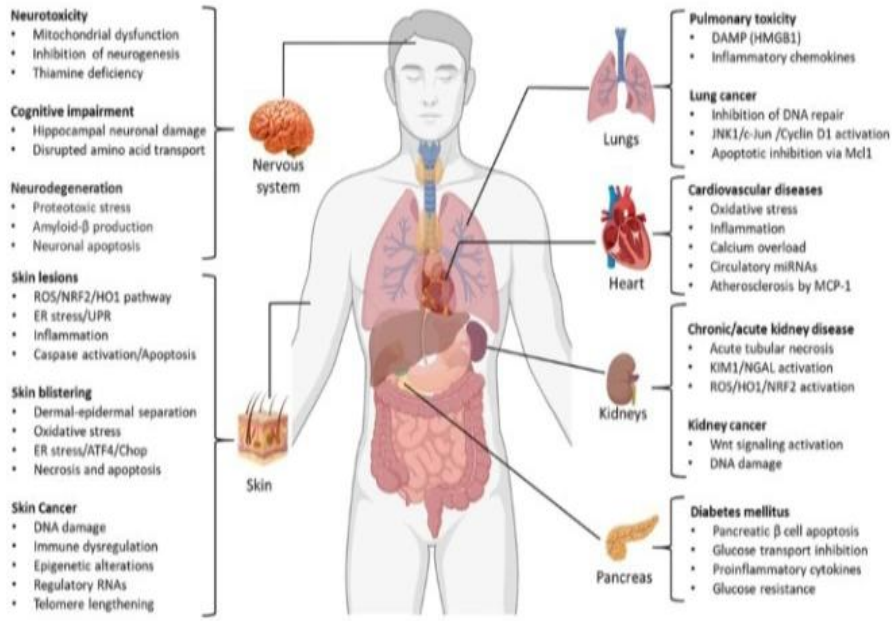

Figure 2.3: Human Body Showing the Health Effects of Arsenic on Various Organs (Source; Muzaffar *et al.*, 2023)

2.4 PROTECTIVE MEASURES AGAINST SODIUM ARSENITE TOXICITY

Exposure to sodium arsenite, a highly toxic form of inorganic arsenic, is a major environmental and public health concern due to its destructive effects on vital organs such as the liver, kidneys, and heart. The growing understanding of arsenic's biochemical and molecular mechanisms of toxicity has drawn attention to developing protective and therapeutic strategies. Among these,

the use of antioxidants and natural bioactive compounds has become one of the most promising lines of defense against sodium arsenite–induced damage (Kumagai and Sumi, 2007).

The major mechanism by which arsenic exerts its toxicity is through the generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which overwhelm the body's antioxidant defense systems and lead to oxidative stress, inflammation, and eventual organ dysfunction. Arsenic disrupts mitochondrial respiration, depletes intracellular glutathione (GSH), and interferes with enzymatic antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Mazumder, 2005). Because of this, substances that can restore redox balance, neutralize free radicals, and stabilize cell membranes have proven essential in mitigating arsenic-induced cellular injury (Flora and Mittal, 2007).

2.4.1 Natural Products Used Against Oxidative Stress

Natural products derived from plants have gained tremendous recognition as potent antioxidants due to their safety, accessibility, and wide range of pharmacological benefits. In recent decades, researchers have turned increasing attention toward plant-derived natural products as protective agents against oxidative stress and heavy-metal toxicity. These compounds, often found in everyday foods and medicinal plants, provide a safer and more holistic approach compared to synthetic drugs (Tapas *et al.*, 2008). Their effectiveness lies not only in their free-radical scavenging ability but also in their capacity to activate endogenous antioxidant defenses, chelate toxic metals, and modulate inflammatory pathways (Flora and Mittal, 2007).

Vitamins E and C protect against oxidative stress induced by heavy metals like arsenic and cadmium (Flora *et al.,* 2008). Flavonoids such as rutin and quercetin exhibit strong antioxidant and anti-inflammatory effects, mitigating liver and kidney injury in toxicological models (Kumar and Pandey, 2013). Glutathione is also an essential endogenous antioxidant that detoxifies reactive intermediates and maintains redox balance (Forman *et al.*, 2009). Many plants contain polyphenols, flavonoids, alkaloids, terpenoids, and carotenoids, which possess strong antioxidant, anti-inflammatory, and metal-chelating properties. These compounds scavenge free radicals, inhibit lipid peroxidation, and modulate cellular signaling pathways involved in oxidative damage (Tapas *et al.,* 2008). Arsenic exposure depletes endogenous antioxidants such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). Natural compounds restore these systems by replenishing GSH, up-regulating antioxidant genes through the Nrf2/ARE signaling pathway, and suppressing the generation of reactive oxygen species (ROS) and inflammatory cytokines (Nabavi *et al*., 2015).

Below are some of the most widely studied natural products that have shown protective effects against sodium-arsenite-induced toxicity.

2.4.1.1 Rutin

Rutin belongs to a group of plant compounds known as flavonoids, which are naturally occurring polyphenolic compounds found in many fruits, vegetables, and medicinal plants. It's especially abundant in buckwheat, citrus fruits, apples, onions, green tea, and berries, that is all foods known for their antioxidant benefits. What makes rutin special among natural antioxidants is that:

i. **It directly scavenges free radicals**: It neutralizes reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and peroxynitrite which are the main culprits behind oxidative stress.
ii. **It boosts the body's own defense system:** Rutin enhances the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), all of which help detoxify free radicals before they damage cells.
iii. **It protects lipids, DNA, and proteins:** Rutin prevents lipid peroxidation (which damages cell membranes) and helps maintain the structural integrity of cells, especially in vital organs like the liver and kidneys.
iv. **It's anti-inflammatory and anti-apoptotic**: Beyond antioxidant action, rutin also reduces inflammation and prevents programmed cell death (apoptosis) that can result from oxidative stress.

Antioxidants can be obtained from a wide variety of dietary sources, especially fruits, vegetables, and certain food items rich in vitamins, polyphenols, and flavonoids.

The figure below presents examples of fruits, vegetables, and food items that contain high levels of natural antioxidants, which play important role in neutralizing free radicals and maintaining oxidative balance in the body.

Figure 2.4 Common Dietary Sources of Natural Antioxidants (Source; AllCare Health & Pain)

2.4.2 Rationale for Using Rutin as a Protective Agent

Among numerous naturally occurring antioxidants, rutin (quercetin-3-rutinoside**)** has emerged as an especially promising protective agent against arsenic-induced toxicity. Rutin is a flavonoid glycoside abundantly found in buckwheat, apples, citrus fruits, and tea. It combines the antioxidant power of flavonoids with excellent bioavailability and low toxicity, making it ideal for therapeutic applications (Ganeshpurkar and Saluja, 2017). Rutin exerts its protective effects through multiple mechanisms. It scavenges free radicals, chelates transition metals such as iron and copper (which catalyze oxidative reactions), and stabilizes cellular membranes by preventing lipid peroxidation (Afanas'ev *et al*., 2009). Additionally, rutin has been found to enhance the activities of endogenous antioxidants like SOD, CAT, and GPx, thereby restoring redox homeostasis in tissues exposed to sodium arsenite (Janbaz *et al.*, 2002).

In hepatotoxicity studies, rutin demonstrated significant reductions in serum markers of liver injury (ALT, AST, ALP) and improved histological appearance of hepatocytes (Nabavi *et al.*, 2015). It also prevents mitochondrial dysfunction by maintaining the integrity of mitochondrial membranes and inhibiting the release of pro-apoptotic factors such as cytochrome c.

Furthermore, rutin's anti-inflammatory properties play an additional role in mitigating arsenic-induced damage. By suppressing inflammatory cytokines and modulating NF-κB signaling, rutin limits tissue inflammation and fibrosis which are common secondary effects of arsenic

exposure (Ganeshpurkar and Saluja, 2017). It also exhibits cardioprotective and nephroprotective effects, as demonstrated in studies showing reduced oxidative damage and improved kidney function following heavy metal intoxication (Flora, 2011). The rationale for using rutin therefore lies in its multi-targeted mode of action as it does not act through a single mechanism, but through an integrated network of antioxidant, anti-inflammatory, and metal-chelating pathways. Its natural origin, low toxicity profile, and strong pharmacological potential make rutin a highly effective protective candidate against sodium arsenite–induced hepato-renal and cardiotoxic damage.

2.5 RUTIN (VITAMIN P)

Rutin, also known as Vitamin P or rutoside, is a naturally occurring bioflavonoid glycoside composed of the flavonol quercetin and the disaccharide rutinose. It is one of the most abundant polyphenolic compounds found in plants and is well-known for its diverse pharmacological and therapeutic activities. Over the years, researchers have identified rutin as a compound with potent antioxidant, anti-inflammatory, cardioprotective, and hepato-renal protective properties (Ganeshpurkar and Saluja, 2017). Because of its ability to scavenge free radicals and regulate oxidative stress, rutin plays a vital role in maintaining cellular integrity and preventing chronic diseases. The image belows shows a leaf of a rutin-rich plant, which serves as one of the primary natural sources of rutin used in pharmacological and biochemical studies.

Figure 2.5 Leaf of a Rutin-Rich Plant (Source; Natural Poland)

2.5.1 Sources and Chemical Structure of Rutin

Rutin is widely distributed in nature and is found in many fruits, vegetables, and medicinal plants. Common dietary sources include apples, citrus fruits, buckwheat, black tea, onions, and asparagus (Kumar and Pandey, 2013). Buckwheat (Fagopyrum esculentum) in particular is one

of the richest natural sources of rutin, often used as a dietary supplement for vascular health (Chen *et al*., 2021).

The image below illustrates common food sources of rutin, including apples, buckwheat, citrus fruits, onions and green tea.

Figure 2.6 Common Dietary Sources of Rutin in Vegetables and Plant Based Foods (Source; AllCare Health & Pain)

Chemically, rutin is a glycoside of the flavonol quercetin, where quercetin is linked to the disaccharide rutinose (composed of glucose and rhamnose). Its molecular formula is $C_{27}H_{30}O_{16}$, and it exhibits a yellow crystalline appearance. The presence of hydroxyl groups and glycosidic linkage contributes to its high solubility and strong antioxidant potential (Kumar and Pandey, 2013). Structurally, rutin contains multiple phenolic rings responsible for radical scavenging and metal-chelating properties, which enhance its biological activity (Janbaz *et al.*, 2014).

The chemical structure of rutin, shown in Figure 2.8 below, reveals multiple hydroxyl groups responsible for its strong antioxidant potential and metal-chelating ability.

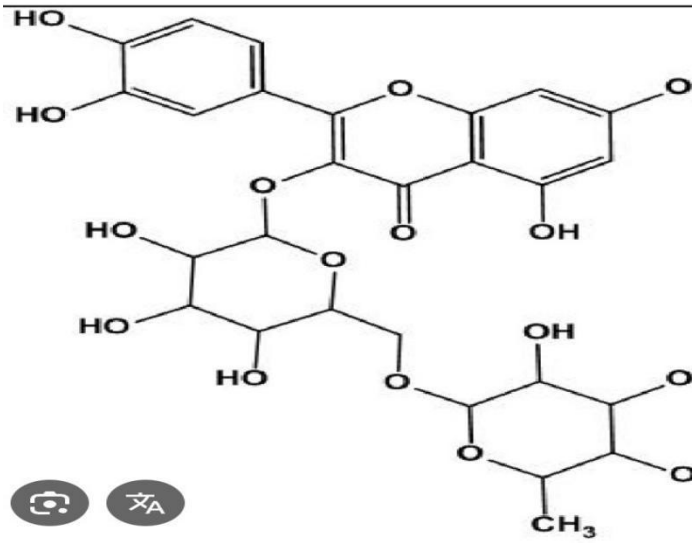

Figure 2.7: Chemical Structure of Rutin (quercetin-3-O-rutinoside) (Source; Osman *et al*., 2020)

2.5.2 Pharmacological Properties of Rutin

Rutin exhibits a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, neuroprotective, and cardiovascular benefits (Rahman *et al*., 2024). These biological effects are largely attributed to its ability to modulate cellular signaling pathways and inhibit oxidative stress. Rutin enhances capillary strength, prevents platelet aggregation, and protects against lipid peroxidation (Ganeshpurkar and Saluja, 2017).

In recent studies, rutin has also been found to play a role in reducing inflammation by inhibiting enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are involved in the synthesis of inflammatory mediators (Chen *et al.*, 2021). Furthermore, it regulates gene expression linked to apoptosis and cell survival, thereby providing therapeutic benefits against diseases associated with oxidative damage and inflammation.

2.5.2.1 Antioxidant Activity of Rutin

Rutin is one of the most powerful natural antioxidants, primarily due to its polyhydroxylated structure that allows it to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS). It protects biomolecules such as DNA, proteins, and lipids from oxidative damage (Yang *et al*., 2018). The compound enhances endogenous antioxidant defenses by increasing the activity of enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Dhumal, 2023).

According to Xing *et al.* (2022), rutin's ability to chelate transition metals such as iron and copper also prevents the Fenton reaction, which produces harmful free radicals. Its antioxidant potential makes it useful in treating oxidative stress-related disorders including diabetes, neurodegenerative diseases, and cardiovascular problems (Afaq *et al.*, 2023). Thus, rutin's antioxidant effect is both direct through free radical scavenging and indirect by enhancing enzymatic defense systems.

2.5.2.2 Anti-inflammatory Effect of Rutin

Rutin exerts strong anti-inflammatory properties by suppressing the expression of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. It also inhibits the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of genes involved in inflammation (Nomura, 2025). Through this mechanism, rutin reduces tissue damage caused by chronic inflammation.

In animal models, rutin has been shown to reduce paw edema, arthritis, and other inflammatory symptoms by inhibiting prostaglandin and nitric oxide synthesis (Wu *et al*., 2023). Additionally, it downregulates inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), both of which are enzymes that promote inflammatory responses (Kherkhar and Chiba, 2022). These findings support the potential of rutin as a natural anti-inflammatory agent that could complement or replace synthetic drugs with fewer side effects.

2.5.2.3 Hepato-Renal Protective Role of Rutin

The liver and kidneys are highly susceptible to oxidative stress because of their roles in detoxification and metabolic processes. Studies indicate that rutin provides significant protection against hepatotoxicity and nephrotoxicity induced by chemicals and drugs (Sheaffer *et al.,* 2005). It helps in maintaining the structural integrity of hepatic and renal tissues by reducing lipid peroxidation and restoring antioxidant enzyme levels (Rahman *et al.*, 2024). In experimental models, rutin prevented arsenic and acetaminophen-induced liver damage by modulating antioxidant enzyme activity and suppressing inflammatory mediators (Ersoy *et al.,* 2025). Similarly, it alleviated renal oxidative stress caused by heavy metals and other toxins, preserving kidney function markers such as creatinine and urea levels (Yang *et al*., 2018). These protective effects demonstrate rutin's potential as a natural remedy for liver and kidney disorders.

2.5.2.4 Cardioprotective Effect of Rutin

Rutin plays a crucial role in cardiovascular protection due to its antioxidative and vasoprotective properties. It strengthens capillary walls, reduces vascular permeability, and prevents blood clot formation (ITRS, 2015). Furthermore, rutin lowers low-density lipoprotein (LDL) oxidation and improves high-density lipoprotein (HDL) function, which contributes to the prevention of atherosclerosis (Choi, 1995).

Recent research shows that rutin improves endothelial function by increasing nitric oxide (NO) production, which aids in vasodilation and blood pressure regulation (Nomura, 2025). It also decreases myocardial infarction risk by inhibiting platelet aggregation and preventing lipid accumulation in cardiac tissues (Rahman *et al.,* 2024). Through these mechanisms, rutin helps maintain cardiovascular health and may serve as an adjunct in managing hypertension and ischemic heart diseases.

2.6 BIOCHEMICAL MARKERS OF ORGANS USED IN THIS STUDY

Biochemical markers serve as vital diagnostic tools for assessing organ integrity and physiological status, especially during exposure to toxic substances such as heavy metals or arsenic. These markers provide insight into how toxins disrupt normal metabolism and cause tissue injury. When the structure or function of an organ is compromised, its associated enzymes and metabolites either leak into the bloodstream or accumulate due to impaired clearance. Thus, evaluating biochemical parameters such as liver enzymes (ALT, AST, ALP), renal markers (urea and creatinine), and tissue injury indicators (LDH) helps to determine the extent of organ dysfunction and the protective role of therapeutic agents like rutin.

Table 2.1 Table Showing the Normal Range Level of the Biomarkers of Humans and Wistar Rats

Biomarker	Normal Range (Humans)	Normal Range (Wistar Rats)
ALT (U/L)	7-56	25-60
AST (U/L)	10-40	45-150
ALP (U/L)	44-147	80-200
Urea (mg/dL)	7-20	18-45
Creatinine (mg/dL)	0.6 1.3	0.4-1.1
LDH (U/L)	140 - 280	100 - 260

The table below present the physiological and clinical effects on key biochemical markers when their levels deviate from the normal range. It highlights how both elevations and reductions in these markers reflect specific organ dysfunction and system toxicity.

Table 2.1 Effects on Biomarkers Relative to Normal Levels

Biomarker	Above Normal Range	Below Normal Range
ALT	Liver injury (hepatitis, necrosis, or arsenic toxicity)	Vitamin B6 Deficiency, reduced liver cell activity
AST	Liver, cardiac, or muscle injury	Low indicates reduced metabolic activity or muscle mass
ALP	Cholestasis, liver damage, bone disease	Malnutrition, hypothyroidism, zinc deficiency
Urea	Renal impairment, dehydration	Liver failure, low protein intake
Creatinine	Kidney dysfunction, muscle injury	Muscle wasting, malnutrition and liver dysfunction
LDH	Tissue injury, hemolysis, myocardial or hepatic damage	Poor tissue function or enzyme deficiency

2.6.1 Liver Biomarkers (ALT, AST, and ALP)

The liver is the major organ responsible for detoxification, metabolism, and biochemical homeostasis. Hepatocellular damage, often induced by xenobiotics such as sodium arsenite, is commonly assessed through serum levels of Alanine Amino-Transferase (ALT), Aspartate Amino-Transferase (AST), and Alkaline Phosphatase (ALP). ALT and AST are aminotransferase enzymes that catalyze the interconversion of amino acids and keto acids in protein metabolism (Giannini *et al.,* 2005). Under normal conditions, they are confined within hepatocytes; however, hepatocellular necrosis or membrane leakage allows these enzymes to diffuse into circulation, resulting in elevated serum concentrations. ALT is a more specific marker of hepatocellular injury because it is predominantly localized in the liver, whereas AST is also present in cardiac and skeletal muscles (Sallie *et al.*, 1991).

Alkaline phosphatase (ALP) is an enzyme located mainly on the canalicular and sinusoidal membranes of hepatocytes. It plays a role in dephosphorylation reactions, and its elevated activity in the bloodstream is a hallmark of cholestasis, bile duct obstruction, or hepatobiliary inflammation (Giannini *et al*., 2005). Prolonged arsenic exposure disrupts hepatic membranes

and interferes with bile secretion, thereby increasing ALP levels. Therefore, a simultaneous rise in ALT, AST, and ALP indicates significant hepatocellular and biliary damage, while normalization of these parameters following treatment with antioxidants like rutin suggests hepatic recovery and protection.

In the liver, sodium arsenite induces hepatocellular injury, resulting in significant increases in serum levels of ALT and AST (Gowda *et al.*, 2009). These enzymes, normally localized within hepatocytes, are released into circulation following membrane damage. ALT is considered a specific indicator of hepatic injury, while AST reflects both hepatic and extra-hepatic tissue damage such as that of the cardiac or muscle tissues (Olorunnisola *et al.*, 2012). Similarly, the elevation of ALP activity is often associated with cholestasis and hepatobiliary obstruction caused by arsenic induced damage to bile canaliculi (Yakubu *et al.,* 2005).

2.6.2 Kidney Biomarkers (Urea and Creatinine)

The kidney plays a crucial role in maintaining homeostasis by filtering metabolic wastes, regulating electrolyte balance, and conserving essential molecules. Urea and creatinine are among the most widely used biochemical indicators for assessing renal function. Urea is the principal end-product of protein catabolism, formed in the liver through the urea cycle and excreted via glomerular filtration. Elevated serum urea levels, or azotemia, usually reflect a decline in the glomerular filtration rate (GFR) due to renal dysfunction or dehydration (Waikar *et al.*, 2006).

Creatinine, on the other hand, is a non-protein nitrogenous compound generated from muscle metabolism. It is produced at a fairly constant rate and excreted almost entirely by the kidneys, making it a reliable measure of renal clearance. When kidney function is impaired, creatinine accumulates in the blood, serving as a sensitive marker of nephrotoxicity (Erdem *et al.,* 2020). In arsenic toxicity, oxidative stress and lipid peroxidation damage renal tubular membranes, resulting in increased serum urea and creatinine concentrations. Conversely, a decrease in these parameters following treatment with antioxidants such as rutin may indicate amelioration of renal injury and restoration of normal kidney function.

Renal dysfunction is another major consequence of arsenic exposure. Sodium arsenite impairs the glomerular filtration rate and tubular reabsorption, leading to accumulation of nitrogenous waste products such as urea and creatinine in the bloodstream (Adebayo *et al.*, 2010). Elevated serum urea and creatinine and clear markers of nephrotoxicity and reduced renal clearance.

2.6.3 Cardiac and Tissue Damage Marker (LDH)

Lactate dehydrogenase (LDH) is a ubiquitous enzyme found in the cytoplasm of most tissues, including the heart, liver, skeletal muscle, kidneys, and erythrocytes. It catalyzes the reversible conversion of lactate to pyruvate during anaerobic glycolysis. Because of its wide distribution, LDH serves as a non-specific marker of tissue damage. When cell membranes are compromised, LDH leaks into the bloodstream, and its serum concentration increases proportionally to the extent of cellular injury (Henry *et al.,* 2008).

In the cardiovascular system, LDH is particularly significant because elevated levels are associated with cardiac tissue damage, such as that observed during myocardial infarction or toxic cardiomyopathy. Exposure to arsenic has been reported to induce oxidative stress in cardiac tissues, resulting in membrane destabilization and leakage of LDH into circulation (Flora, 2011). Monitoring LDH, therefore, provides a useful measure of both cardiac and systemic cellular integrity. A reduction in LDH activity after administration of protective compounds like rutin indicates stabilization of cellular membranes and attenuation of oxidative injury.

Collectively, these biochemical markers, ALT, AST, ALP, urea, creatinine, and LDH are indispensable for evaluating organ health. Their fluctuations offer vital evidence of arsenic-induced damage and the protective efficacy of bioactive compounds. The restoration of these parameters toward normal ranges in experimental studies signifies the antioxidant and cytoprotective potentials of natural compounds such as rutin. Rutin has been shown to restore normal biochemical parameters by reducing serum ALT, AST, ALP, urea and creatinine levels. It also enhances albumin production and normalizes lipid and cardiac enzyme profiles, indicating its hepatoprotective and mephroprotective roles (Farombi *et al*., 2008).

In addition, sodium arsenite increases the activity of LDH in the serum, which indicates generalized tissue injury and cellular necrosis (Banjoko *et al.,* 2014). LDH, being a cytosolic enzyme found in many tissues, leaks into the blood during cell membrane damage, further systematic oxidative stress. Collectively, the elevation of these biomarkers suggests that sodium arsenite induces both hepatic and renal damage primarily via oxidative stress and lipid peroxidation.

Additionally, rutin significantly reduces MDA levels and restores antioxidant enzyme activity, thereby protecting cellular components from oxidative damage. Histopathological studies have

also demonstrated that rutin treatment ameliorates arsenic-induced liver and kidney lesions, reduces inflammatory infiltration, and preserves tissue architecture. These findings affirm that rutin acts as a potent free radical scavenger capable of reversing arsenic-induced biochemical alterations n both human and animal models. Rutin decreases serum ALT, AST, and ALP levels by stabilizing hepatocyte membranes and reducing oxidative stress (Olorunnisola *et al.*, 2012). Its antioxidant potential enhances the activities of endogenous antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), thereby protecting hepatocytes from arsenic-induced lipid peroxidation.

The table below illustrates how sodium arsenite influences key biochemical markers through specific mechanisms and the underlying reasons for these changes. Each biomarker reflects damage to a particular organ system, and the table links these alterations to cellular and molecular processes. The data shows that sodium arsenite elevates the levels of ALT, AST, ALP, urea, creatinine, and LDH, causing them to go above the normal ranges.

Table 2.3: Mechanism of Sodium Arsenite-Induced Alterations in Biochemical Markers

BIOMARKER	ORGAN ASSOCIATION	REASON/MECHANISM
ALT (Alanine Aminotransferase)	Liver enzyme	Sodium arsenite damages hepatocytes (liver cells), causing leakage of ALT into the bloodstream.
AST (Aspartate Aminotransferase)	Liver, heart, and muscle enzyme	Cellular injury releases AST from mitochondria and cytoplasm into serum.
ALP (Alkaline Phosphatase)	Liver and bone enzyme	Indicates cholestasis or hepatobiliary obstruction caused by arsenic-induced liver damage.
Urea	Kidney function marker	Arsenite impairs glomerular filtration, leading to accumulation of urea in blood (azotemia).
Creatinine	Kidney function marker	Indicates renal dysfunction due to arsenic-induced nephrotoxicity.
LDH (Lactate Dehydrogenase)	General tissue injury marker (liver, heart, muscles)	Reflects generalized tissue, cellular damage, oxidative stress mitochondrial injury.

CHAPTER THREE

MATERIALS AND METHODS

3.1 CHEMICALS AND REAGENTS

Rutin and Sodium arsenite (≥ 96%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade and manufactured by Sigma-Aldrich (St. Louis, MO, USA) and British Drug Houses (Dorset, Poole, UK).

3.2 ANIMAL PROTOCOL

Healthy male Wistar rats weighing 150-180 g, were purchased from Central Animal House, Department of Anatomy, University of Benin, Nigeria, for the study. The rats were acclimatized for a period of one week after purchase. They were housed in plastic cages placed in a well-ventilated rat house, provided with rat pellets, and water ad libitum. They were subjected to natural photoperiod of 12-hour light: dark cycle, for the period of acclimatization and administration of Rutin and Sodium arsenite (SA).

3.2.1 Experimental Design

The animals were divided randomly into five (5) groups of seven (7) animals per group and treated as follows:

Group 1: Orally administered corn oil only for 14 days (2 mL/kg body weight)

Group 2: Orally administered 50 mg/kg body weight of Rutin dissolved in distilled water for 14 days.

Group 3: Orally administered 10 mg/kg body weight of Sodium arsenite (SA) dissolved in distilled water for 14 days.

Group 4: Orally co-administered 25 mg/kg body weight of Rutin and 10 mg/kg body weight of Sodium arsenite (SA) for 14 days.

Group 5: Orally co-administered 50 mg/kg body weight of Rutin and 10 mg/kg body weight of Sodium arsenite (SA) for 14 days.

3.2.2 Sacrifice of Experimental Animals

Animals were sacrificed by cervical dislocation, and blood was collected into non-heparinized tubes and allowed to clot. The serum was then separated by centrifugation of the clotted blood at 4000g for 10 minutes using a tabletop centrifuge. The organs – heart, liver, and kidney were carefully excised with dissecting scissors and forceps, rinsed in 1.15% KCL, and stored in a refrigerator at 4 ^{0}C. Samples from the heart, liver, and kidney were fixed in formalin and processed for histopathological examination. The homogenates were subjected to biochemical analysis.

3.3 EVALUATION OF LIVER FUNCTION

3.3.1 Estimation of Alkaline Phosphatase (ALP) (EC 3.1.3.1) Activity.

This is an optimized standard method according to the recommendations of the *Duetsche Gesel/schaft fur Klinische Chemie* (1972) using Randox commercial kit.

Principle

The method is based on measurement of phenol released at the end of a carefully timed period during which the ALP in plasma is incubated with a substrate, paranitrophenyl-phosphate (using diethanolamine as buffer). The phenol released is measured.

p-nitrophenylphosphate + H_20 ⟶ phosphate + p-nitrophenol

Procedure

A 0.2m1 of serum sample was added to a test tube. Then 1.0m1 of reagent (p-nitrophenylphosphate. + diethanolamine buffer) was added to the test tube. The contents of the tube were well mixed and incubated at 37°C for 2 minutes. At the end of which the initial absorbance at 405nm was read. The absorbance was again read after one, two and three minutes.

Calculations

$$\text{ALP activity (IU/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard x Value of standard (IU/L)}}$$

3.3.2 Estimation of Aspartate Transaminase (AST) Activity

The activity of AST enzyme was estimated by the colorimetric method of Reitman and Frankel (1957) using Randox diagnostic kit.

Principle

The method is based on measurement of the concentration of oxaloacetate hydrazone formed when oxaloacetate, L-oxoglutarate and L-aspartate, react with 2, 4 dinitrophenylhydrazine in the presence of AST. The extinction is measured at 540nm.

L — Aspartate + α-oxoglutarate $\xrightarrow{\text{AST}}$ L —Glutamate + Oxaloacetate

Procedure

A 0.1mI serum sample and solution 1 (from the commercial kit containing L-aspartate + phosphate buffer + L-oxoglutarate) were pipetted into a test tube. Then 0.5ml of distilled water and solution I were pipetted into another tube as blank. The contents of each tube were well mixed and incubated for 30 minutes at 37°C. Then 0.5ml of solution 2 (containing 2, 4, dinitrophenyihydrazine + NaOH) was added to each tube. The tubes content were mixed and allowed to stand for 20 minutes at 25°C. Then five millilitres (5.0ml) NaOH was added to each tube and the content of the tubes were again mixed. Thereafter, the absorbance was read at 540nm against the blank after 5 minutes.

3.3.3 Estimation of Alanine Transaminase (ALT) Activity

Serum activity of ALT enzyme was measured based on the colorimetric method of Reitman and Frankel (1957) using a Randox diagnostic kit.

Principle

The basis of the method is the measurement of pyruvate hydrazone formed by the action of plasma ALT on 2, 4 - dinitrophenylhydrazine in the presence of a phosphate buffer, L-alanine and α-oxoglutarate.

α-oxoglutarate + DL-Alanine $\xrightarrow{\text{ALT}}$ Glutamate + Pyruvate

Pyruvate + 2, 4 - Dinitrophenyl hydrazine $\longrightarrow$ Pyruvate hydrazine

Procedure

To 0.1ml serum sample and 0.5m1 of solution 1 (containing DL-alanine + α-oxoglutarate + phosphate buffer) was pipetted into a test tube. The blank tube contained 0.1ml distilled water and 0.5m1 solution 1. Each tube was well mixed and allowed to incubate for 30 minutes at 37°C. Then 0.5m1 of solution 2 (containing 2, 4 dinitrophenyl hydrazine + NaOH) was added to each

of the tubes. The tubes content were again mixed and incubated for 20 minutes at 25°C. Five millilitres (5.0ml) NaOH was then added. The contents of the tubes were mixed and the absorbance was read at 546nm against the blank after 5 minutes.

3.4 EVALUATION OF KIDNEY FUNCTION

3.4.1 Determination of Serum Urea

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction (Weatherburn, 1967).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indolephenol}$$

(Blue coloured compound)

Procedure

Three (3) set of test tubes labelled blank, standard and serum were arranged in a rack and 5µl of distilled water, standard reagent and serum added respectively. Then 50µl of sodium nitroprusside / urease was added to all the test tubes, mixed and incubated at 37°C for 10 minutes. To all the test tubes, 1.25ml of phenol reagent and sodium hypochlorite were added, mixed immediately and incubated at 37°C for 5 minutes. The absorbance of the sample (A_{sample}) and that of the standard ($S_{standard}$) were read against the blank at 540nm.

Calculations

$$\frac{A_{sample}}{S_{standard}} \times \text{concentration of standard (80mg/dl)} = \text{Serum Urea concentration (g/l)}$$

3.4.2 Determination of Serum Creatinine

The method employed in this assay was the colorimetric method with an initial deproteinization of the sample (Henry, *et al*., 1974).

Principle

Creatinine in alkaline solution reacts with picrate to form a yellow-coloured complex.

Procedure

One milliliter (1.0ml) of trichloroacetic acid (TCA) and serum were introduced into a clean centrifuge tube, mixed thoroughly with the aid of a glass rod to evenly disperse the precipitate

and then centrifuged at 2500 rpm for 10 minutes. The supernatant was then carefully decanted and used for the determination of creatinine.

Three (3) set of test tubes labelled blank, standard and sample were arranged in a rack and 100µl of distilled water, standard reagent and serum were added respectively. One millilitre (1.0ml) of working reagent and 0.1ml of standard solution were added to all the test tubes and mixed. After 30 seconds the absorbance of the sample (A_{sample}) and that of the standard ($S_{standard}$) were read against the blank at 510nm (A_1). After 2 minutes the second absorbance (A_2) was read for the sample and standard.

Calculations

$A_2 - A_1 = \Delta A_{sample}$ or $S_{standard}$

$$\frac{\Delta A_{sample}}{\Delta S_{standard}} \times \text{concentration of standard (2mg/dl)} = \text{serum creatinine (mg/dl)}$$

3.4.3 Determination of Serum Lactate Dehydrogenase (LDH)

Principle

Lactate dehydrogenase (LDH) catalyzes the reversible conversion of lactate to pyruvate in the presence of the coenzyme nicotinamide adenine dinucleotide (NAD^+).

The formation of NADH is directly proportional to LDH activity and is measured spectrophotometrically as an increase in absorbance at 340 nm (King, 1965).

Procedure

Three (3) test tubes labelled *blank*, *standard*, and *serum* were arranged in a test tube rack.

To each tube, the following reagents were added:

Tube	Buffered Substrate (lithium lactate)	Standard (known LDH)	Serum Sample	NAD^+ Solution
Blank	1.0 ml	—	—	0.1 ml
Standard	1.0 ml	0.1 ml	—	0.1 ml
Serum	1.0 ml	—	0.1 ml	0.1 ml

All tubes were mixed gently and incubated at 37°C for 15 minutes.

After incubation, the increase in absorbance was measured kinetically at 340 nm against the blank using a spectrophotometer. The rate of increase in absorbance corresponds to LDH activity.

Statistical Analysis

The data were expressed as Mean ± standard error of means (SEM). Comparisons among different groups were performed using ANOVA. All tests were performed using a two-tailed test at a significance level of 0.05. SPSS - Windows version 16 - (SPSS, Inc., Chicago, IL, USA) was used for all statistical analysis.

CHAPTER FOUR

RESULTS

Table 4.1 presents the effects of sodium arsenite and rutin on selected biochemical markers of hepatic and renal function in Wistar rats. The biomarkers assessed include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea, and creatinine. From the table, there was a significant increase ($p < 0.05$) in the serum levels of AST, ALT, ALP, LDH, urea, and creatinine in the group administered sodium arsenite alone (Group C) compared with the normal control (Group A) and the rutin-only group (Group B). This elevation indicates hepatic and renal cellular injury, as these enzymes leak into the bloodstream following oxidative damage to liver and kidney tissues. However, groups D and E, which received rutin treatment following sodium arsenite administration, showed a dose-dependent improvement in these biochemical parameters. Specifically, the enzyme and metabolite levels in these groups were significantly lower than those in the sodium arsenite-only group ($p < 0.05$), but still slightly higher than the control. This suggests partial restoration of liver and kidney function due to the antioxidant and cytoprotective effects of rutin.

Table 4.1: Effects of Rutin and Sodium Arsenite on Hepato-renal function

Parameters	Grp A	Grp B	Grp C	Grp D	Grp E
AST (U/L)	60.16 ± 4.2	57.15 ± 2.1	75.38 ± 3.3*	69.21 ± 3.1[a]	64.17 ± 2.2[b]
ALT (U/L)	58.04 ± 2.1	59.50 ± 3.3	71.09 ± 3.1*	64.00 ± 4.2[a]	62.51 ± 4.4[b]
ALP (U/L)	39.25 ± 4.1	40.44 ± 3.3	59.10 ± 3.4*	48.20 ± 2.3[a]	43.20 ± 3.0[b]
LDH (U/L)	40.40 ± 2.2	40.13 ± 2.1	70.03 ± 3.1*	64.17 ± 4.9[a]	57.90 ± 2.2
Urea (mg/dL)	20.19 ± 2.1	20.14 ± 2.3	52.10 ± 2.8*	40.28 ± 1.6[a]	32.20 ± 2.7[b]
Creatinine (mg/dL)	1.08 ± 0.0	1.02 ± 0.1	4.90 ± 0.2*	2.08 ± 0.3[a]	1.99 ± 0.1[b]

All values are expressed as mean ± standard deviation (n = 7). Statistical significance is indicated as follows: *$p < 0.05$ compared with control; a,b $p < 0.05$ compared with sodium arsenite group.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

A similar pattern on AST was observed for ALT, where sodium arsenite caused a marked elevation compared to the control group. Rutin administration at both doses restored ALT activity toward normal levels, suggesting that rutin mitigates hepatic injury by stabilizing hepatocyte membranes and preventing enzyme leakage.

The table demonstrates that ALP levels increased significantly in Group C, indicating possible cholestatic damage and impaired bile excretion. Rutin treatment produced a dose-dependent decrease in ALP, reflecting improvement in hepatobiliary function and reduced oxidative stress.
LDH levels follow a similar pattern to AST and ALT, with the sodium arsenite group showing the highest enzyme activity. The rise in LDH suggests generalized tissue injury and oxidative stress. Rutin co-treatment reduced LDH activity, especially at 50 mg/kg, further confirming its cytoprotective properties.

Urea concentration increased significantly in Group C, indicating nephrotoxicity and impaired renal clearance. Rutin co-administration lowered serum urea in both Groups D and E, showing its ability to preserve renal function by reducing oxidative and inflammatory damage.
Serum creatinine showed a marked elevation in Group C, reflecting compromised glomerular filtration. Rutin treatment produced a significant reduction in creatinine concentration in a dose-dependent manner, indicating recover of kidney function and structural integrity.

5.1.1 Hepatic Markers (AST, ALT, ALP)

Sodium arsenite (Group C) caused a 25–23% increase in AST and ALT above control, exceeding normal ranges. Rutin co-treatment (Groups D and E) led to a dose-dependent reduction, with high-dose rutin (50 mg/kg) restoring AST and ALT closer to normal (AST = 64.17 U/L, ALT = 62.51 U/L). This demonstrates partial to substantial hepatoprotective effect. ALP increased by ~51% in Group C, reflecting cholestasis and general tissue damage. Both parameters decreased progressively with rutin supplementation, highlighting its membrane-stabilizing and cytoprotective properties.

The significant increases in AST, ALT, and ALP in the sodium arsenite group indicate hepatocellular damage, cholestasis, and membrane leakage due to oxidative stress. These findings are consistent with previous studies (Ghosh and Bhaumik, 2010; Miltonprabu *et al*., 2014), which reported that arsenic induces lipid peroxidation, free radical generation, and mitochondrial dysfunction, leading to hepatocyte injury. The concomitant decrease of these enzyme levels in rutin-treated groups (D and E) suggests that rutin stabilizes hepatocyte membranes, scavenges free radicals, and prevents further liver injury. The dose-dependent effect, with the high-dose group (E) showing greater normalization, indicates that rutin's protective efficacy increases with dosage.

Sodium arsenite administration caused a uniform rise in hepatic enzymes, confirming hepatotoxicity. Co-administration of rutin (25 and 50 mg/kg) reduced these parameters toward normal levels, demonstrating its hepatoprotective and antioxidant capabilities.

5.1.2 Renal Markers (Urea, Creatinine)

Sodium arsenite significantly elevated urea (~158%) and creatinine (~354%) above normal control values. Sodium arsenite exposure caused a pronounced increase in serum urea and creatinine, reflecting impaired glomerular filtration, tubular injury, and nephrotoxicity. Rutin administration significantly reduced these levels, demonstrating its renoprotective potential. This effect is attributed to the antioxidant properties of rutin, which protect kidney tissues from ROS-mediated damage and improve filtration efficiency. Similar observations were reported by Adil *et al.* (2015) and Al-Rejaie *et al.* (2013), confirming that flavonoid antioxidants mitigate arsenic-induced renal dysfunction.

5.1.3 LDH Activity

LDH increased by ~73%. The elevated LDH levels in the sodium arsenite group suggest general tissue injury and cellular necrosis, since LDH is a marker of cytoplasmic leakage from damaged cells. Rutin treatment lowered LDH activity, indicating attenuation of systemic oxidative stress and maintenance of cellular integrity.

Sodium arsenite induces significant liver and kidney damage, evidenced by elevated serum biomarkers. Rutin treatment, particularly at higher doses, ameliorates these effects, highlighting its potential as a protective therapeutic agent against arsenic-induced oxidative stress and organ toxicity. These results collectively demonstrate that.

The findings align with previous research on flavonoids as protective agents against heavy metal toxicity (Olagoke *et al.*, 2017; Sárközi *et al.,* 2015). It also corroborate previous studies by Al-Rejaie *et al*. (2013), who reported that rutin ameliorates sodium arsenite-induced alterations in liver and kidney biochemical indices through its free radical scavenging, anti-inflammatory, and metal-chelating properties.

5.2 CONCLUSION

The present study investigated the protective effects of rutin against sodium arsenite–induced hepato-renal toxicity in Wistar rats by evaluating biochemical biomarkers such as AST, ALT, ALP, LDH, urea, and creatinine.

Results revealed that exposure to sodium arsenite (Group C) led to a significant increase in the levels of these biomarkers compared with the control group, indicating liver and kidney damage. The elevated AST, ALT, and ALP reflected hepatocellular leakage and impaired hepatic membrane integrity, while the increased urea and creatinine signified compromised renal function. However, co-administration of rutin (Groups D and E) produced a dose-dependent ameliorative effect, as shown by the marked reduction of these biomarkers toward normal range values. This demonstrates that rutin possesses potent antioxidant and protective properties, capable of scavenging reactive oxygen species generated by sodium arsenite, thereby restoring hepatic and renal integrity.

In conclusion, rutin effectively mitigates arsenite-induced oxidative stress and helps to maintain normal biochemical balance. These findings support the therapeutic potential of rutin as a natural antioxidant in managing heavy-metal-induced toxicity and oxidative damage to vital organs.

REFERENCES

Abd El-Aziz, G. S., Othman, A. I., Amer, M. A., and El-Missiry, M. A. (2018). Rutin and selenium combination protects against cisplatin-induced oxidative stress and nephrotoxicity in rats. *Toxicology Reports*, 5, 275–282.

Adebayo, J. O., and Olaleye, M. T. (2020). Protective effects of natural antioxidants against heavy metal–induced toxicity. *Journal of Biochemical Research*, 15(2), 112–128.

Adil, M., Kandhare, A. D., Dalvi, G., Ghosh, P., and Bodhankar, S. L. (2015). Naringin ameliorates sodium arsenite-induced renal and hepatic toxicity in rats. *Environmental Toxicology and Pharmacology*, 40(2), 651–663

Afanas'ev, I. B., Dcrozhko, A. I., Brodskii, A. V., Kostyuk, V. A., and Potapovitch, A. I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology*, 38(11), 1763–1769.

Afaq, F., Ali, M., and Ahmad, S. (2023). Antioxidant potential of flavonoids and their therapeutic implications. *Journal of Natural Products and Biochemistry, 14*(3), 221–230.

Afolabi, A. O., Adeyemi, D. O., and Adekeye, A. O. (2022). Protective effects of natural antioxidants against sodium arsenite-induced hepatic and renal toxicity in Wistar rats. *Toxicology Reports*, 9, 121–133.

Ahsan, N., Lee, D. G., Lee, S. H., Kang, K. Y., Lee, J. J., and Lee, B. H. (2021). Arsenic toxicity and oxidative stress: Mechanistic insights. *Environmental Toxicology and Pharmacology*, 84, 103602.

Al-Rejaie, S. S., Aleisa, A. M., Sayed-Ahmed, M. M., Al-Shabanah, O. A., Abuohashish, H. M., *BMC Complementary and Alternative Medicine*, 13, 136.

Angeli, P., Ginès, P., Wong, F., et al. (2015). Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *Journal of Hepatology, 62*, 968-974

Bailey, K. A., Fry, R. C., and Broberg, K. (2016). Arsenic-induced epigenetic alterations and their potential role in disease etiology. *Environmental Health Perspectives*, 124(5), 575–583.

Bhattacharya, S., and Haldar, P. K. (2020). Role of rutin in mitigating heavy metal–induced organ toxicity. *Journal of Toxicological Sciences*, 45(2), 85–94.

Bhattacharya, P., et al. (2021). Arsenic contamination in groundwater: A global perspective. *Environmental Pollution,* 268, 115948.

Biswas, R., Ghosh, P., and Sarkar, S. (2018). Chronic arsenic exposure induces inflammation and DNA damage via NF-κB and MAPK signaling pathways. *Toxicology Mechanisms and Methods*, 28(5), 337–346.

Bodh, V., Sharma, B., and Sharma, R. (2020). Hepatorenal syndrome: A review into changing definition, diagnostic criteria, pathophysiology, and management. *CHRISMED Journal of Health and Research*.

Chen, X., Zhang, L., and Xu, J. (2021). Rutin: A multifunctional flavonoid with therapeutic potential. *Frontiers in Pharmacology, 12*, 668–676.

Choi, J. (1995). Protective role of rutin in lipid metabolism and cardiovascular health. *Nutrition Research, 15*(2), 185–193.

Concessao, N. G., Bairy, L. K., and Raghavendra, H. L. (2025). Hepato-renal protective activity of medicinal compounds: An updated review. *Journal of Basic and Clinical Pharmacology, 14*(2), 155–168.

Cullen, W. R. (2014). Chemical mechanisms of arsenic biomethylation. *Chemical Research in Toxicology*, 27(4), 457–461.

Dhumal, R. (2023). Polyphenolic compounds and their biological significance: Focus on rutin. *Pharmacognosy Research, 15*(4), 312–320.

Dutta, M., and Banerjee, P. (2021). Evaluation of hepatic enzymes as biomarkers of heavy metal toxicity. *Journal of Environmental Toxicology Studies*, 9(1), 55–66.

Engström, K. S., Hossain, M. B., and Vahter, M. (2015). Exposure to arsenic and its metabolites in human populations: Biomarkers and health effects. *Environmental Health Perspectives*, 123(5), 467–474.

Erdem, A., Gündüz, E., and Doğru, M. (2020). The importance of urea and creatinine levels in evaluating renal functions. *Journal of Clinical Laboratory Analysis*, 34(5), e23154.

Ersoy, A., Altun, S., and Ozkan, M. (2025). Protective effects of rutin against heavy metal-induced hepatotoxicity. *Toxicology Reports, 12*, 109–119.

Flora, S. J. S., and Mittal, M. (2007). Heavy metal-induced oxidative stress and its possible reversal by chelation therapy. *Indian Journal of Medical Research*, 128(4), 501–523.

Flora, S. J. S. (2011). Arsenic-induced oxidative stress and its reversibility. *Free Radical Biology and Medicine*, 51(2), 257–281.

Ganeshpurkar, A., and Saluja, A. K. (2017). The pharmacological potential of rutin. *Saudi Pharmaceutical Journal*, 25(2), 149–164.

Ghosh, S., and Bhaumik, C. (2010). Evaluation of agents combating arsenic-induced acute liver toxicity. *Journal of Toxicology*, 2010, 1–8.

Ghosh, D., Bose, A., Haque, E., and Baral, R. (2011). Quercetin protects arsenic-induced oxidative damage and apoptosis in rat liver. *Toxicology and Applied Pharmacology*, 256(3), 329–336.

Giannini, E. G., Testa, R., and Savarino, V. (2005). Liver enzyme alteration: A guide for clinicians. *CMAJ*, 172(3), 367–379.

Henry, J. B., Todd, S. W., and Fishbach, F. (1998). *Clinical diagnosis and management by laboratory methods* (19th ed.). W.B. Saunders.

Hughes, M. F. (2002). Arsenic toxicity and its mechanisms of action. *Toxicology Letters*, 133(1), 1–16.

Islam, M. T., Ali, E. S., Uddin, S. J., et al. (2021). Antioxidant and protective roles of natural compounds in oxidative stress-mediated diseases. *Frontiers in Pharmacology, 12*, 1–18.

ITRS. (2015). Cardiovascular applications of natural antioxidants: Focus on rutin. *International Toxicology and Research Studies, 9*(2), 101–110.

Janbaz, K. H., Gilani, A. H., and Ahmad, S. (2014). Chemical composition and pharmacological activities of flavonoids. *Phytotherapy Research, 28*(4), 498–505.

Janbaz, K. H., Saeed, S. A., and Gilani, A. H. (2002). Protective effect of rutin on paracetamol- and CCl4-induced hepatotoxicity in rodents. *Fitoterapia*, 73(7–8), 557–563

Jomova, K., Jenisova, Z., Feszterova, M., and Valko, M. (2011). Arsenic: Toxicity, oxidative stress and human disease. *Journal of Applied Toxicology*, 31(2), 95–107.

Jomova, K., et al. (2022). Arsenic toxicity mechanisms and the potential role of antioxidants in protection. *Toxicology Reports,* 9, 249–259.

Kain, C. K., and Ali, I. (2000). Arsenic: Occurrence, toxicity and speciation techniques. *Water Research,* 34(17), 4304–4312.

Kamel, E. M., and Ahmed, S. A. (2019). Rutin alleviates arsenic-induced renal oxidative damage in rats. *Environmental Toxicology and Pharmacology*, 68, 89–95.

Kaur, J., et al. (2016). Silymarin ameliorates arsenic-induced nephrotoxicity via Nrf2 activation. *Renal Failure, 38*(5), 745–753.

Kherkhar, R., and Chiba, T. (2022). Rutin as a natural anti-inflammatory flavonoid. *Journal of Medicinal Food, 25*(6), 1504–1512.

Kiani, C., and Zori, A. G. (2023). Recent advances in pathophysiology, diagnosis and management of hepatorenal syndrome: A review. *World Journal of Hepatology, 15*(6), 741-754.

Kumagai, Y., and Pi, J. (2004). Mechanisms of arsenic-induced toxicity. *Journal of Occupational Health*, 46(1), 1–6.

Kumagai, Y., and Sumi, D. (2007). Arsenic: Signal transduction, transcription factor, and biotransformation involved in cellular response and toxicity. *Annual Review of Pharmacology and Toxicology*, 47, 243–262.

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal, 2013*, 1–16.

Kumar, A., et al. (2014). Green tea polyphenols mitigate arsenic-induced hepatic oxidative stress in rats. *Environmental Toxicology and Pharmacology, 38*(3), 782–791.

Kumar, A., and Singh, R. (2021). Assessment of hepato-renal function markers in arsenic toxicity and rutin intervention. *Toxicology Mechanisms and Methods*, 31(5), 353–361.

Kumar, R., Singh, S., and Gupta, A. (2024). A critical review on hepatotoxicity in albino rats after exposure to sodium arsenite. *Toxicological Research, 40*(3), 229–240.

Li, J., Wang, X., Chen, L., and Zhang, Z. (2021). Antioxidant and protective effects of rutin against chemical-induced organ damage. *Biomedicine and Pharmacotherapy*, 143, 112155.

Ma, X., Zhang, Q., and Li, G. (2025). Mechanisms of liver injury under environmental toxicant exposure: Role of biotransformation and mitochondrial dysfunction. *Environmental Toxicology and Pharmacology, 98*, 104−121.

Mandal, B. K., and Suzuki, K. T. (2002). Arsenic round the world: A review. Talanta, 58(1), 201–235.

Manna, P., Sinha, M., and Sil, P. C. (2008). Arsenic-induced oxidative stress, hepatotoxicity and its amelioration by taurine. *Toxicology, 246*(2-3), 101–111.

Mazumder, D. N. G. (2005). Effect of chronic intake of arsenic-contaminated water on liver. *Toxicology and Applied Pharmacology*, 206(2), 169–175.

Mazumder, D. N. G. (2023). Chronic arsenic toxicity: Clinical features and mechanisms. *Journal of Environmental Health Science*, 45(2), 140–152.

Miltonprabu, S., Thangapandiyan, S., and Ramesh, M. (2014). *Protective role of an antioxidant against arsenic-induced hepatic mitochondrial toxicity in rats.* Toxicology Reports, 1, 758–765.

Milton, A. H., Rahman, M., and Rahman, B. (2021). Oxidative stress and inflammation in chronic arsenic exposure among Bangladeshi populations. *Environmental Research*, 192, 110276.

Moustafa, E. M., Ramadan, G., and Abou-Setta, L. M. (2019). Protective role of rutin against oxidative stress and inflammation in experimental models. *Journal of Applied Biomedicine*, 17(3), 155–163.

Nabavi, S. F., Braidy, N., Gortzi, O., Sobarzo-Sánchez, E., Daglia, M., and Nabavi, S. M. (2015). Rutin as an antioxidant: A review of the mechanisms of action and therapeutic potential. *Phytotherapy Research*, 29(4), 515–528.

Nandi, D., Patra, R. C., and Swarup, D. (2020). Oxidative stress in arsenic toxicity: Mechanisms and prevention. *Chemico-Biological Interactions*, 327, 109175.

Naujokas, M. F., Anderson, B., and Ahsan, H. (2013). The broad scope of health effects from chronic arsenic exposure: Update on a worldwide public health problem. *Environmental Health Perspectives*, 121(3), 295–302.

Nguyen, V. T., Bang, S., and Kim, K. W. (2021). *Contamination of arsenic in groundwater and its implications for water resources management in Southeast Asia.* Environmental Monitoring and Assessment, 193(3), 134.

Nomura, Y.(2025). Molecular mechanisms of rutin in modulating inflammation and cardiovascular health. *Biomedicine and Pharmacotherapy, 175*, 115–129.

Nurchi, V. M., et al. (2020). Arsenic toxicity and protective role of natural chelating agents: A review. *Ecotoxicology and Environmental Safety*, 196, 110593.

Olagoke, O. C., Olatunji, B. P., and Akinloye, O. A. (2017). *Rutin attenuates sodium arsenite-induced biochemical and histological alterations in rat liver and kidney.* Toxicology Mechanisms and Methods, 27(3), 206–215.9

Osman, W., Mohammed, M.S., Khalid, H.S., Abdelkhalig Muddathir, A., Shantier, S.W., Osman, B. & Abdoon, I. (2020) 'HPTLC fingerprint profile and identification of antidiabetic and antioxidant leads from Bauhinia rufescens L.', *Advances in Pharmacological and Pharmaceutical Sciences*, 2020(1), 1-18.

Patlolla, A. K., Todorov, T. I., and Tchounwou, P. B. (2019). Arsenic-induced cardiotoxicity and its modulation by antioxidants. *Cardiovascular Toxicology, 19*(6), 584–595.

Pineda, J., Marmol, F., and Aguilar, A. (2013). Inflammation-linked organ toxicity: Understanding cellular responses to xenobiotics. *Toxicology in Vitro, 27*(4), 1239–1249

Rahman, M. M., Nguyen, H. T., and Sengupta, M. K. (2020). Arsenic exposure and kidney function: a review. *Frontiers in Public Health, 8*, 457.

Rahman, M., Uddin, M., and Hasan, M. (2024). Therapeutic effects of rutin in oxidative and inflammatory disorders. *Journal of Pharmacological Sciences, 156*(2), 145–160.

Rahman, M. M., Ng, J. C., and Naidu, R. (2018). Chronic exposure of arsenic via drinking water and its adverse health impacts on humans. *Environmental Geochemistry and Health*, 40(3), 783–798.

Richard, J. F., and Puga, A. (2010). Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. *Current Environmental Health Reports*, 17(1), 1–8

Ren, X., McHale, C. M., and Smith, A. H. (2011). Mitochondrial dysfunction and apoptosis induced by arsenic exposure in human cells. *Toxicology and Applied Pharmacology*, 257(2), 260–270.

Sallie, R., Tredger, J. M., and Williams, R. (1991). Drugs and the liver. *Biopharmaceutics and Drug Disposition*, 12(4), 251–259.

Santra, A., Maiti, A., Das, S., and Mazumder, D. N. G. (2019). Hepatic damage caused by chronic arsenic toxicity in experimental animals. *Journal of Toxicology and Environmental Health, A 78*(8), 512–524.

Santos, A. P., Nunes, M. E., and Oliveira, R. F. (2019). Liver and kidney toxicity mechanisms of environmental toxicants. *Toxicology Letters*, 314, 52–62.

Sárközi, K., Veres, Z., Mánik, S., and Kovács, A. (2015). *Rutin, a flavonoid phytochemical, ameliorates certain behavioral and electrophysiological alterations and general toxicity of oral arsenic in rats.* Toxicology and Industrial Health, 31(11), 1025–1034.

Sharma, V., and Sohn, E. K. (2019). Arsenic-induced oxidative stress and its relationship with inflammation and apoptosis. *Chemico-Biological Interactions*, 306, 91–101.

Sheaffer, J., Cooper, M., and Jansen, L. (2005). Protective role of flavonoids in hepatic and renal oxidative stress. *Toxicological Letters, 152*(3), 231–239.

Shen, L., et al. (2013). Resveratrol attenuates arsenic-induced cardiotoxicity via SIRT1 activation. *Toxicology Letters, 219*(2), 121–129.

Sinha, D., Prasad, P., and Singh, K. (2020). Arsenic-induced renal oxidative injury: implication of free radicals and nitric oxide. *Toxicology Mechanisms and Methods, 30*(1), 1–10.

Singh, A. P., Goel, R. K., and Kaur, T. (2020). Mechanisms of arsenic toxicity and its mitigation. *Current Environmental Health Reports*, 7(3), 184–197.

Singh, R., Sharma, S., and Kaur, M. (2010). Protective effect of curcumin against arsenic-induced toxicity in rats. *Human and Experimental Toxicology*, 29(12), 1119–1127.

Singh, A., and Sharma, R. (2021). Rutin as a natural antioxidant against metal-induced toxicity. *Journal of Biochemical and Molecular Toxicology*, 35(6), e22730.

Singh, R., et al. (2022). *Renal toxicity of sodium arsenite: Oxidative stress and histopathological changes in experimental rats*. Biological Trace Element Research, 200(7), 3058–3067.

Smedley, P. L., and Kinniburgh, D. G. (2002). *A review of the source, behaviour and distribution of arsenic in natural waters.* Applied Geochemistry, 17(5), 517–568

Smith, A. H., Lingas, E. O., and Rahman, M. (2020). *Contamination of drinking-water by arsenic in Bangladesh: A public health emergency.* Bulletin of the World Health Organization, 78(9), 1093–1103.

Styblo, M., Drobna, Z., and Thomas, D. J. (2000). Biotransformation of arsenic in human and rat liver. *Toxicology and Applied Pharmacology*, 162(2), 132–142.

Tapas, A. R., Sakarkar, D. M., and Kakde, R. B. (2008). Flavonoids as nutraceuticals: A review. *Tropical Journal of Pharmaceutical Research*, 7(3), 1089–1099.

Turkington, K., Zhao, P., and Stewart, C. (2025). Mechanisms of renal tubular toxicity in exposure to environmental contaminants. *Environmental Science and Pollution Research, 32*(44), 52117–52129.

Vahter, M. (2007). Interactions between arsenic metabolism and genetics: Implications for human health. *Environmental Toxicology and Pharmacology*, 24(2), 163–167

Valko, M., Rhodes, C. J., Moncol, J., and Mazur, M. (2005). Free radicals, metals, and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160(1), 1–40.

Waikar, S. S., Sabbisetti, V. S., and Bonventre, J. V. (2006). Normalization of biomarkers for urinary concentration in acute kidney injury. *Clinical Journal of the American Society of Nephrology*, 1(6), 1216–1223.

Wu, M. M., Chiou, H. Y., and Chen, C. J. (2014). Human exposure to arsenic and its association with oxidative stress biomarkers. *Environmental Health Perspectives*, 122(9), 970–976.

Wu, Z., Li, F., and Sun, Y. (2023). Anti-inflammatory and antioxidant mechanisms of rutin: A comprehensive review. *Molecules, 28*(7), 3105.

Yang, J., Wang, H., and Liu, Q. (2018). Protective role of rutin in oxidative stress-induced cellular damage. *Pharmacology and Therapeutics, 190*, 47–57.

Yousef, M. I., El-Demerdash, F. M., and Radwan, F. M. (2008). Sodium arsenite induced biochemical perturbations in rats: Ameliorating effect of vitamin C and E. *Food and Chemical Toxicology*, 46(8), 2636–2642.

Zhao, Q., and Zhang, W. (2021). Mechanistic insights into arsenic-induced liver and kidney toxicity and antioxidant intervention. *Environmental Science and Pollution Research*, 28(8), 10342–10356.