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ANTI-INFLAMMATORY EFFECTS OF RUTIN ON SODIUM ARSENITE-INDUCED TOXICITY IN WISTAR RAT

BY
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IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc. HONS.) IN MEDICAL BIOCHEMISTRY

SUPERVISED BY
DR. (MRS). O. IKPONMWOSA-EWEKA
NOVEMBER, 2025

CERTIFICATION

We the undersigned hereby certify that OKORO, Victor Osagie (BMS1801234) carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of the Bachelor of Science Degree (B.Sc. Hons.) in Medical Biochemistry.

Signed

_____

Dr. (Mrs.) A. B. SUPERVISOR (Project Supervisor)

Date

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Prof. C. D. OMONKHUA (Head of Department)

Date

_____

Prof. E. F. EXTERNAL (External Examiner)

Date

DEDICATION

This project is dedicated to my loving parents, Mr. and Mrs. Edosomwan whose unwavering support, encouragement, and sacrifices have been the bedrock of my academic journey.
To my siblings, for their constant motivation and belief in me, and to God Almighty, for His infinite grace, wisdom, and strength.

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Finally, I am eternally indebted to my family for their unending love, prayers, and financial support. Their belief in my potential has been my greatest source of inspiration.

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ABSTRACT

Arsenic contamination of the environment poses a significant global health threat, with chronic exposure leading to severe toxicity in vital organs, primarily mediated by oxidative stress and inflammation. This study was designed to investigate the anti-inflammatory and protective effects of Rutin, a dietary flavonoid, against sodium arsenite (SA)-induced hepato- and nephrotoxicity in Wistar rats. A total of thirty-five male Wistar rats were randomly divided into five groups (n=7): Group 1 (Control), Group 2 (Rutin 50 mg/kg), Group 3 (SA 10 mg/kg), Group 4 (SA 10 mg/kg + Rutin 25 mg/kg), and Group 5 (SA 10 mg/kg + Rutin 50 mg/kg). Treatments were administered orally for 14 consecutive days. Following the treatment period, animals were sacrificed, and liver and kidney tissues were harvested for biochemical analysis. Key markers of oxidative stress and inflammation, including Reactive Oxygen and Nitrogen Species (RONS), Myeloperoxidase (MPO) activity, and Nitrite (NO_2^-) levels, were assayed in the tissue homogenates. The results revealed that administration of sodium arsenite alone (Group 3) caused a highly significant ($p < 0.05$) increase in the levels of RONS, MPO activity, and Nitrite in both the liver and kidney when compared to the control group, indicating severe oxidative damage and inflammatory infiltration. Conversely, co-administration of Rutin at both 25 mg/kg and 50 mg/kg (Groups 4 and 5) significantly and dose-dependently attenuated these SA-induced increases. The higher dose of Rutin (50 mg/kg) demonstrated a more pronounced protective effect, restoring the biochemical parameters towards control levels. This study concludes that Rutin possesses potent antioxidant and anti-inflammatory properties that effectively ameliorate sodium arsenite-induced hepatic and renal toxicity in Wistar rats, suggesting its potential as a therapeutic agent against arsenic-induced organ damage

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

The contamination of the environment with heavy metals and metalloids represents a formidable and escalating challenge to global public health (Jaishankar et al., 2014; Tchounwou et al., 2012). These persistent toxins accumulate in ecosystems and enter the human food chain, posing a significant threat to human health and the environment. Among these toxicants, arsenic stands out as one of the most significant and pervasive threats, primarily due to its widespread presence in drinking water, soil, and food sources across numerous regions of the world (Chung et al., 2014). The World Health Organization (WHO) has identified arsenic as a major public health concern, with an estimated 140 million people in at least 50 countries exposed to concentrations in drinking water far exceeding the recommended safe limit of 10 µg/L (WHO, 2019).
Chronic exposure to arsenic, even at low levels, is unequivocally linked to a broad spectrum of debilitating health conditions. It is classified as a Group 1 human carcinogen and is well-established to cause various forms of cancer, particularly of the skin, bladder, and lung (States et al., 2009). Beyond its carcinogenic effects, chronic arsenic exposure is also strongly associated with cardiovascular diseases, diabetes, and severe, progressive damage to vital organs such as the liver and kidneys, which are the primary sites for its metabolism and excretion (Navas-Acien et al., 2005; Rahman et al., 1998).
The profound toxicity of arsenic is rooted in its complex interactions at the cellular and molecular levels. Upon absorption, it disrupts fundamental biochemical processes, most notably by inducing a state of severe oxidative stress (Shi et al., 2004). This occurs through the overwhelming generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which damages cellular macromolecules like lipids, proteins, and DNA, and triggers a cascade of inflammatory responses (Ren et al., 2020). This dual assault of oxidative damage and inflammation is central to the pathophysiology of arsenic-induced organ injury.
In response to the limitations and potential side effects of conventional treatments for heavy metal poisoning, such as chelation therapy (Flora and Pachauri, 2010) , the scientific community has increasingly turned its attention to the field of phytotherapy. This involves the use of natural, plant-derived compounds, or phytochemicals, as therapeutic agents. Of particular interest are flavonoids, a large class of polyphenolic compounds that are abundant in the human diet and are renowned for their potent biological activities (Karak, 2019). These compounds are valued for their capacity to counteract the damaging effects of xenobiotics through various mechanisms, chief among them being their antioxidant and anti-inflammatory properties (Panche et al., 2016).
Within this promising class of compounds, Rutin (quercetin-3-O-rutinoside) has emerged as a particularly compelling candidate for investigation. Rutin is a prominent dietary flavonoid glycoside found in a wide variety of plants, including buckwheat, citrus fruits, apples, and teas (Prasad and Prasad, 2019). A substantial body of scientific literature has documented its extensive pharmacological benefits, which include potent antioxidant, anti-inflammatory, vasoprotective, hepatoprotective, and neuroprotective effects (Al-Dhabi et al., 2015). Its ability to scavenge free radicals, enhance endogenous antioxidant defenses, and modulate key inflammatory pathways makes it a theoretically ideal agent to combat the specific mechanisms of arsenic-induced toxicity (Gullon et al., 2017). This study, therefore, is situated at the intersection

of environmental toxicology and pharmacognosy, seeking to explore the therapeutic potential of a natural compound against a prevalent environmental toxicant.

1.2 Statement of the Problem

Arsenic exists in the environment in several forms, with the inorganic trivalent state, sodium arsenite (SA), being particularly toxic (Hughes, 2002). The primary mechanism through which SA exerts its deleterious effects is the induction of overwhelming oxidative and nitrosative stress within cells. This process begins with the metabolic activation of arsenic, which leads to the massive generation of reactive oxygen and nitrogen species (RONS) (Ren et al., 2020). These highly reactive molecules attack and damage vital cellular components, leading to lipid peroxidation of membranes, denaturation of proteins, and damage to DNA (Shi et al., 2004). This initial wave of oxidative damage does not occur in isolation; it acts as a powerful trigger for a secondary, and equally destructive, inflammatory cascade. The cellular stress signals the activation of pro-inflammatory pathways, such as the Nuclear Factor-kappa B (NF-κB) pathway, leading to the recruitment of immune cells like neutrophils to the affected tissues (Wei et al., 2016). This infiltration, while intended as a protective response, often exacerbates the injury by releasing more RONS and cytotoxic enzymes, thus perpetuating a vicious cycle of damage.
The liver and kidneys, as the principal organs involved in the metabolism and clearance of arsenic, bear the brunt of this toxicological assault, resulting in progressive organ dysfunction, clinically defined as hepatotoxicity and nephrotoxicity (Tchounwou et al., 2019). Although chelation therapy is the standard medical intervention for acute arsenic poisoning, its efficacy in chronic exposure is limited, and it is often accompanied by significant adverse effects, including potential redistribution of the toxicant and its own inherent toxicity (Flora and Pachauri, 2010). Consequently, there is a critical and unmet need for the development of safer, more effective, and accessible therapeutic or protective strategies to mitigate the widespread health consequences of chronic arsenic exposure. The exploration of natural compounds that can specifically target the root causes of arsenic toxicity—oxidative stress and inflammation—represents a vital area of research to address this public health problem.

1.3 Justification of the Study

The rationale for investigating Rutin as a potential protective agent against sodium arsenite-induced toxicity is firmly grounded in its well-established biochemical properties, which align remarkably well with the known mechanisms of arsenic damage. The primary pathological drivers of arsenic toxicity are oxidative stress and inflammation, and Rutin is documented to possess potent activities that directly counteract both processes (Ganeshpurkar and Saluja, 2017).
Firstly, Rutin's powerful antioxidant capacity is central to its protective potential. Its molecular structure, rich in phenolic hydroxyl groups, enables it to effectively scavenge a wide array of free radicals, thereby neutralizing the initial oxidative burst induced by arsenic metabolism (Koval'skii et al., 2019). Beyond direct scavenging, Rutin has been shown to bolster the body's intrinsic antioxidant defense system by upregulating the expression and activity of crucial enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Prasad and Prasad, 2019). This dual antioxidant action—both direct and indirect—makes it a robust candidate for mitigating arsenic-induced oxidative damage.
Secondly, Rutin exhibits significant anti-inflammatory effects. It can modulate key signaling pathways, such as the nuclear factor-kappa B (NF-κB) pathway, which is a central regulator of

the inflammatory response (Sharma et al., 2021; Liu et al., 2022). By inhibiting this pathway, Rutin can suppress the production of pro-inflammatory cytokines and chemokines, thereby reducing the recruitment of neutrophils and other immune cells to the site of injury (Ganeshpurkar and Saluja, 2017). This action is critical for breaking the self-perpetuating cycle where inflammation leads to further oxidative stress.

Previous research has already provided a strong foundation for this hypothesis. Studies on quercetin, the aglycone (non-sugar part) of Rutin, have demonstrated its ability to protect against heavy metal toxicity (Mishra and Flora, 2008). More directly, several investigations have reported the promising effects of Rutin itself in ameliorating general toxicity and neurotoxicity induced by arsenic, attributing these effects to its antioxidant and potential chelating activities (Sárközi et al., 2015). This study is therefore justified as it seeks to build upon this existing knowledge by focusing specifically on the hepato- and nephroprotective effects of Rutin. By employing specific and sensitive biomarkers—namely Myeloperoxidase (MPO) to quantify neutrophil infiltration, Nitrite to measure nitrosative stress, and total Reactive Oxygen and Nitrogen Species (RONS) to assess the overall oxidative burden—this research aims to provide direct biochemical evidence of Rutin's anti-inflammatory and antioxidant efficacy in the primary target organs of arsenic toxicity.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the Study

The primary aim of this study is to investigate the anti-inflammatory effects of Rutin on sodium arsenite-induced hepato- and nephrotoxicity in male Wistar rats.

1.4.2 Specific Objectives

The specific objectives designed to achieve this aim are as follows:

- To determine the effect of oral administration of sodium arsenite on the levels of Reactive Oxygen and Nitrogen Species (RONS) in the liver and kidney of Wistar rats.
- To assess the impact of sodium arsenite on the activity of Myeloperoxidase (MPO), a marker of neutrophil infiltration, in the liver and kidney.
- To measure the levels of Nitrite, a stable metabolite of nitric oxide, in the liver and kidney following sodium arsenite administration.
- To evaluate the potential of Rutin, at two different doses (25 mg/kg and 50 mg/kg), to ameliorate the changes in RONS, MPO, and Nitrite levels induced by sodium arsenite.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sodium Arsenite: A Potent Environmental Toxicant

2.1.1 Sources and Environmental Exposure

Arsenic is a naturally occurring metalloid that is ubiquitously distributed in the Earth's crust (Chung et al., 2014). Its presence in the environment is a result of both natural processes, such as volcanic emissions and the weathering of arsenic-containing minerals, and anthropogenic activities, including mining, smelting, agricultural use of pesticides, and industrial waste disposal (Abernathy et al., 1999). The most significant route of human exposure globally is through the consumption of contaminated groundwater, a problem that affects millions of people in countries such as Bangladesh, India, Taiwan, and parts of the United States (Chung et al., 2014; WHO, 2019).

Arsenic exists in the environment in two main forms: organic and inorganic. Organic arsenic compounds, which are found in some foods like seafood, are generally considered less toxic. In contrast, inorganic arsenic compounds are highly toxic and are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) (States et al., 2009). Inorganic arsenic typically exists in two primary oxidation states: pentavalent arsenate (As^{5+}) and trivalent arsenite (As^{3+}). While both are toxic, the trivalent arsenite form is recognized as being significantly more toxic and reactive than the pentavalent arsenate form, primarily due to its higher affinity for sulfhydryl groups on proteins and its greater ease of cellular uptake (Hughes, 2002; Tchounwou et al., 2019). Sodium arsenite ($NaAsO_2$), the compound used in this study, is a water-soluble salt of arsenous acid and serves as a representative and potent trivalent arsenical.

2.1.2 Metabolism and Toxicokinetics

The toxicokinetics of arsenic begin with its efficient absorption from the gastrointestinal tract, typically around 80-90% for soluble inorganic forms (ATSDR, 2007). Following absorption, arsenic is distributed via the bloodstream to various tissues, with initial accumulation in the liver, kidneys, spleen, and lungs, followed by slower accumulation in tissues rich in sulfhydryl groups, such as skin, hair, and nails (Vahter, 2002).

The liver is the central organ for arsenic metabolism. A key metabolic step is the reduction of any ingested pentavalent arsenate (As^{5+}) to the more toxic trivalent arsenite (As^{3+}) by enzymes like arsenate reductase (Kenyon et al., 2021). Subsequently, arsenite undergoes a series of oxidative methylation reactions, a process catalyzed by arsenic (+3 oxidation state) methyltransferase (As3mt), using S-adenosylmethionine (SAM) as the methyl donor. This pathway converts inorganic arsenic into monomethylarsonic acid (MMA) and then dimethylarsinic acid (DMA) (Thomas, 2007).

For a long time, this methylation process was considered a detoxification pathway, as the methylated metabolites are more readily excreted by the kidneys. However, this view has been revised. It is now understood that the intermediate metabolites formed during this process, particularly the trivalent forms—monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III})—are highly reactive and even more cytotoxic and genotoxic than the parent

inorganic arsenite (Styblo et al., 2002; Thomas et al., 2001). Therefore, the metabolic pathway of arsenic is, in fact, a process of "bioactivation" that generates some of its most toxic species. The final methylated products, along with any remaining inorganic arsenic, are primarily eliminated from the body via the kidneys through urinary excretion, making the renal system a major target for arsenic-induced damage (Kenyon et al., 2021).

2.1.3 Molecular Mechanisms of Toxicity

The multifaceted toxicity of sodium arsenite stems from its ability to disrupt cellular function through several interconnected molecular mechanisms. These mechanisms collectively lead to cellular dysfunction, death, and the organ-level pathology observed in arsenicosis. This process can be understood as a three-tiered cascade: an initial chemical insult, followed by biochemical amplification, which triggers a systemic biological overreaction.

2.1.3.1 Enzyme Inhibition via Sulfhydryl Binding

The classical mechanism of trivalent arsenic toxicity is its high affinity for sulfhydryl (-SH) groups present in proteins and enzymes (Shen et al., 2013). Arsenite forms stable covalent bonds with vicinal sulfhydryl groups, particularly those in the active sites of critical enzymes. A prime example is the inhibition of the pyruvate dehydrogenase (PDH) complex, a key enzyme in cellular respiration that links glycolysis to the citric acid cycle. Arsenite binds to the dithiol groups of lipoic acid, a cofactor for PDH, thereby inactivating the enzyme and severely impairing mitochondrial energy production (ATP synthesis) (Bergquist et al., 2009). This disruption of cellular bioenergetics contributes significantly to arsenic's cytotoxicity. Over 200 other enzymes are thought to be susceptible to inhibition by arsenic through this mechanism, leading to widespread metabolic chaos (Webb, 1966).

2.1.3.2 Induction of Oxidative and Nitrosative Stress

A central and overarching mechanism of arsenic toxicity is the induction of profound oxidative and nitrosative stress (Ren et al., 2020). The metabolism of arsenic, particularly the redox cycling between its trivalent and pentavalent states, generates a massive flux of reactive oxygen species (ROS), including the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (•OH) (Shi et al., 2004). This surge in ROS overwhelms the cell's endogenous antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), and the glutathione (GSH) system.

The resulting state of oxidative stress leads to widespread cellular damage. ROS attack polyunsaturated fatty acids in cell membranes, initiating a chain reaction of lipid peroxidation that compromises membrane integrity and function. Proteins are oxidized, leading to their denaturation and loss of function, and DNA is damaged, causing strand breaks and base modifications that can lead to mutations and carcinogenesis (Jomova et al., 2011).

Simultaneously, arsenic exposure can lead to nitrosative stress through the overproduction of nitric oxide (NO) by the inducible nitric oxide synthase (iNOS) enzyme. The excess NO can react with superoxide anions to form peroxynitrite ($ONOO^-$), a potent and highly damaging reactive nitrogen species (RNS) that can nitrate proteins and further exacerbate oxidative damage

(Gurr et al., 2003). The collective assault by this broad spectrum of Reactive Oxygen and Nitrogen Species (RONS) is a primary driver of arsenic-induced cell death and tissue injury.

2.1.3.3 Triggering of the Inflammatory Cascade

The oxidative stress induced by arsenic is not merely a damaging agent but also a potent signaling event that triggers a robust inflammatory response. ROS can activate key redox-sensitive transcription factors, most notably Nuclear Factor-kappa B (NF-κB), which is a master regulator of inflammation (Wei et al., 2016). Upon activation, NF-κB translocates to the nucleus and promotes the transcription of a wide array of pro-inflammatory genes (Ghosh and Karin, 2002).

This leads to the increased production and secretion of inflammatory mediators, including cytokines like Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α), and chemokines such as Monocyte Chemoattractant Protein-1 (MCP-1) (Lee et al., 2005). These signaling molecules orchestrate the inflammatory response, primarily by recruiting immune cells, such as neutrophils and macrophages, from the bloodstream into the affected tissue. While this infiltration is part of the body's defense mechanism, in the context of arsenic toxicity, it becomes a major contributor to the pathology. The recruited neutrophils release their own arsenal of cytotoxic agents, including proteolytic enzymes and a further burst of ROS generated by enzymes like NADPH oxidase and myeloperoxidase (MPO), creating a self-amplifying cycle of inflammation and oxidative damage that perpetuates tissue injury (Loria et al., 2008).

This intricate interplay—whereby arsenic's direct chemical toxicity leads to oxidative stress, which in turn ignites an inflammatory cascade that generates further oxidative stress—forms a vicious cycle. This cycle is fundamental to understanding the progressive and severe nature of arsenic-induced organ damage.

2.1.4 Target Organ Toxicity: Hepatotoxicity and Nephrotoxicity

The liver and kidneys are the primary targets for arsenic-induced toxicity, a consequence of their central roles in its metabolism and excretion, which exposes them to high concentrations of the metalloid and its reactive metabolites (Tchounwou et al., 2019).

2.1.4.1 Hepatotoxicity

The liver, as the main site of arsenic methylation, is particularly vulnerable to injury. Arsenic-induced hepatotoxicity manifests through a spectrum of clinical and pathological changes. Biochemically, it is characterized by a significant elevation in the serum levels of liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are released from damaged hepatocytes (Mazumder, 2005). Histopathological examinations of the liver in arsenic-exposed animals reveal a range of degenerative changes, including hepatocyte swelling, cytoplasmic degeneration, necrosis (cell death), inflammation, and fibrosis (the excessive deposition of collagen), which can ultimately progress to cirrhosis (Santra et al., 2000).

The underlying mechanisms involve the generation of intense oxidative stress within hepatocytes, leading to lipid peroxidation and mitochondrial damage. This cellular stress activates apoptotic pathways, mediated by caspases and the JNK signaling pathway, leading to

programmed cell death of hepatocytes (Liu et al., 2005).

2.1.4.2 Nephrotoxicity

The kidneys are responsible for filtering and excreting arsenic and its metabolites from the body, a process that exposes renal tubular epithelial cells to high concentrations of these toxicants. Arsenic-induced nephrotoxicity is characterized by impaired renal function, evidenced by elevated levels of serum creatinine and blood urea nitrogen (BUN), which are waste products that accumulate when the kidneys fail to filter them effectively (Zalups and Ahmad, 2003). Histological studies show damage primarily to the renal tubules, including tubular degeneration, necrosis, and interstitial inflammation (Fowler et al., 2004). The mechanisms mirror those in the liver, with oxidative stress playing a central role in damaging renal cells and triggering inflammatory and apoptotic processes. Chronic exposure to arsenic is a recognized risk factor for the development and progression of chronic kidney disease (CKD) (Chen et al., 1992).

2.2 Rutin: A Multifunctional Flavonoid Glycoside

2.2.1 Chemical Structure and Sources

Rutin, also known as rutoside or vitamin P, is a prominent member of the flavonoid family, specifically classified as a flavonol glycoside. Its chemical structure consists of the flavonol aglycone, quercetin, linked to the disaccharide rutinose (a combination of rhamnose and glucose) at the C3 position (Prasad and Prasad, 2019). The full chemical name is 3′,4′,5,7-tetrahydroxy-flavone-3-rutinoside, with the molecular formula $C_{27}H_{30}O_{16}$. This complex polyphenolic structure, with its multiple hydroxyl groups, is the basis for its potent biological activities.

Rutin is widely distributed throughout the plant kingdom and is a common component of the human diet. Some of the richest dietary sources include buckwheat (Fagopyrum esculentum), asparagus, unpeeled apples, figs, citrus fruits (especially the peel and pulp), and various teas, including black, green, and elderflower tea (Prasad and Prasad, 2019; Wikipedia, 2024). Its presence in such a wide array of commonly consumed foods underscores its importance as a dietary antioxidant. Physicochemically, Rutin is a yellowish crystalline powder. A key characteristic that influences its therapeutic application is its poor solubility in water and lipids, particularly in acidic and neutral environments, although its solubility increases in alkaline conditions (Semwal et al., 2021). This low solubility can limit its bioavailability upon oral administration, which is an important consideration in pharmacological studies.

2.2.2 Pharmacological Properties and Bioactivities

Rutin has been the subject of extensive scientific investigation, which has revealed a remarkable spectrum of pharmacological properties and health benefits. It is recognized as a multifunctional bioactive compound with therapeutic potential against a wide range of chronic and degenerative diseases (Ganeshpurkar and Saluja, 2017). Its bioactivities are diverse and include well-documented antioxidant, anti-inflammatory, vasoprotective (strengthening blood vessels), antidiabetic, cardiovascular-protective, hepatoprotective, neuroprotective, antimicrobial, and anticancer effects (Al-Dhabi et al., 2015).

In the context of diabetes, Rutin has been shown to improve glycemic control by enhancing insulin signaling and glucose uptake (Hsu et al., 2014). Its cardiovascular benefits are linked to its ability to improve endothelial function, lower blood pressure, and reduce cholesterol levels (Ugusman et al., 2014). In neurodegenerative diseases like Alzheimer's, Rutin's antioxidant and anti-inflammatory actions help protect neurons from damage (Xu et al., 2014). This broad range of activities stems from its ability to interact with and modulate multiple molecular pathways within the body, making it a compound of significant therapeutic interest.

2.2.3 Mechanisms of Antioxidant Action

The antioxidant properties of Rutin are arguably its most prominent and well-studied feature. It combats oxidative stress through a multi-pronged approach that involves both direct interaction with reactive species and indirect enhancement of the body's own defense mechanisms (Prasad and Prasad, 2019).

2.2.3.1 Direct Radical Scavenging

The polyphenolic structure of Rutin is ideally suited for neutralizing free radicals. The multiple hydroxyl (-OH) groups on its flavonoid rings can readily donate a hydrogen atom to unstable reactive oxygen and nitrogen species, such as the superoxide radical (O_2^{•−}), hydroxyl radical (•OH), and peroxyl radicals (Yang et al., 2008). This process stabilizes the free radicals, effectively terminating the damaging chain reactions they propagate, particularly the lipid peroxidation of cell membranes. By directly quenching these reactive molecules, Rutin provides a first line of defense against oxidative damage.

2.2.3.2 Upregulation of Endogenous Antioxidant Defenses

Beyond its direct scavenging activity, Rutin can also fortify the cell's intrinsic antioxidant capabilities. It has been shown to upregulate the expression and enhance the activity of key endogenous antioxidant enzymes, including Superoxide Dismutase (SOD), which converts superoxide to hydrogen peroxide; Catalase (CAT), which decomposes hydrogen peroxide into water and oxygen; and Glutathione Peroxidase (GSH-Px), which reduces hydrogen peroxide and lipid hydroperoxides using reduced glutathione (GSH) as a cofactor (Prasad and Prasad, 2019). This upregulation is often mediated through the activation of the Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway (Costa et al., 2016). Nrf2 is a transcription factor that controls the expression of a wide array of antioxidant and detoxification genes. By activating this pathway, Rutin effectively boosts the cell's overall resilience to oxidative stress.

2.2.3.3 Metal Chelation

Another important antioxidant mechanism of flavonoids, including Rutin, is their ability to chelate transition metal ions, such as iron (Fe^{2+}) and copper (Cu^{2+}) (Mira et al., 2002). These metals can participate in the Fenton reaction, a process that generates the highly destructive hydroxyl radical from hydrogen peroxide. By binding to these metal ions, Rutin sequesters them and prevents them from catalyzing the formation of free radicals, thereby

providing an additional layer of antioxidant protection. This chelating property is particularly relevant in the context of heavy metal toxicity, where it may contribute to the detoxification process.

2.2.4 Mechanisms of Anti-inflammatory Action

Inflammation is a critical component of many disease pathologies, including arsenic-induced tissue damage. Rutin exerts potent anti-inflammatory effects by intervening at several key points in the inflammatory cascade (Ganeshpurkar and Saluja, 2017).

2.2.4.1 Inhibition of Pro-inflammatory Mediators

Rutin has been demonstrated to effectively suppress the production and release of crucial molecules that drive the inflammatory response. It can inhibit the activity of pro-inflammatory enzymes such as Cyclooxygenase-2 (COX-2), which is responsible for producing inflammatory prostaglandins, and inducible Nitric Oxide Synthase (iNOS), which generates large, cytotoxic amounts of nitric oxide during inflammation (Choi et al., 2014; Kauss et al., 2024). Furthermore, Rutin can reduce the expression and secretion of potent pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α) and Interleukins (e.g., IL-1β, IL-6), which are central orchestrators of the inflammatory process (Muvhulawa et al., 2022).

2.2.4.2 Modulation of Inflammatory Signaling Pathways

The ability of Rutin to suppress these inflammatory mediators is largely due to its influence on the upstream signaling pathways that control their expression. The most significant of these is the Nuclear Factor-kappa B (NF-κB) pathway. In resting cells, NF-κB is held inactive in the cytoplasm. Inflammatory stimuli, such as those generated by arsenic-induced oxidative stress, lead to its activation and translocation into the nucleus, where it initiates the transcription of numerous pro-inflammatory genes (Lawrence, 2009). Rutin has been shown to inhibit the activation of NF-κB, effectively blocking this central switch for the inflammatory response (Sharma et al., 2021; Liu et al., 2022). By preventing the activation of NF-κB, Rutin can halt the inflammatory cascade at its source, leading to a broad-spectrum reduction in inflammation. This mechanism is fundamental to its protective effects in inflammatory conditions and is highly relevant to its potential role in mitigating arsenic-induced tissue damage.

2.3 Biomarkers of Inflammation and Oxidative Stress in Tissue Toxicity

To accurately assess the extent of tissue damage and to evaluate the efficacy of a therapeutic intervention, it is essential to use reliable and specific biochemical markers. This study employs three key biomarkers—RONS, MPO, and Nitrite—each of which provides a distinct window into the pathological processes of oxidative stress and inflammation induced by sodium arsenite.

2.3.1 Reactive Oxygen and Nitrogen Species (RONS)

Reactive Oxygen and Nitrogen Species (RONS) is a comprehensive term that encompasses both reactive oxygen species (ROS), such as the superoxide anion and hydroxyl radical, and reactive

nitrogen species (RNS), such as nitric oxide and peroxynitrite. These molecules play a dual role in biology. At low, physiological concentrations, they are essential for normal cellular processes, acting as signaling molecules in pathways that regulate cell growth, immunity, and homeostasis (Dröge, 2002). However, when their production overwhelms the cell's antioxidant capacity, a condition known as oxidative or nitrosative stress occurs.
In this state, high levels of RONS become highly destructive, indiscriminately attacking and damaging all major classes of biological macromolecules (Shi et al., 2004). They cause lipid peroxidation in membranes, leading to loss of integrity; they oxidize proteins, causing them to misfold and lose function; and they damage DNA, leading to mutations and cell death. This widespread damage is a fundamental mechanism in toxicology and the pathogenesis of numerous diseases, including those induced by toxicants like arsenic (Ren et al., 2020). Therefore, measuring the total level of RONS provides a direct and holistic assessment of the overall oxidative burden within a tissue. The 2′,7′-dichlorofluorescin diacetate (DCFH-DA) assay used in this study is a widely accepted method for quantifying total intracellular RONS levels, making it an excellent marker for the primary toxic insult caused by sodium arsenite (LeBel et al., 1992).

2.3.2 Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is a heme-containing peroxidase enzyme that is abundantly stored within the azurophilic (primary) granules of neutrophils, and to a lesser extent, monocytes. Its primary physiological function is as a critical component of the innate immune system's antimicrobial arsenal (Klebanoff, 2005). Upon activation during an inflammatory response, neutrophils release MPO into phagosomes and the extracellular space. There, MPO utilizes hydrogen peroxide (H_2O_2) to catalyze the formation of highly potent reactive oxidants, most notably hypochlorous acid (HOCl)—the active ingredient in bleach—which is extremely effective at killing invading pathogens (Davies and Kettle, 2003).
While essential for host defense, the extracellular release of MPO and its powerful products can also cause significant collateral damage to host tissues. MPO-derived oxidants contribute directly to tissue injury during inflammatory conditions (Khan et al., 2018). For this reason, the activity of MPO in a tissue is considered a highly specific and quantitative biomarker of neutrophil infiltration and acute inflammation (Loria et al., 2008). An elevation in tissue MPO activity provides direct evidence that a robust inflammatory response has been mounted, involving the recruitment and activation of neutrophils at that site. In the context of arsenic toxicity, measuring MPO activity serves as a direct indicator of the inflammatory damage occurring in the liver and kidneys.

2.3.3 Nitrite and Nitric Oxide (NO)

Nitric Oxide (NO) is a gaseous free radical that functions as a critical signaling molecule involved in a vast array of physiological processes, including vasodilation, neurotransmission, and immune regulation (Moncada et al., 1991). NO is synthesized by a family of enzymes called nitric oxide synthases (NOS). While the constitutive forms of NOS (eNOS and nNOS) produce small, controlled amounts of NO for signaling, the inducible form (iNOS) is expressed in response to inflammatory stimuli and produces large, sustained amounts of NO (Wink and Mitchell, 1998).
During inflammation, such as that induced by arsenic, the high levels of NO produced by iNOS have cytotoxic and pro-inflammatory effects (Tripathi et al., 2007). Furthermore, this excess NO

can react with superoxide radicals to form the highly damaging RNS, peroxynitrite (Beckman and Koppenol, 1996). However, NO itself is extremely unstable, with a biological half-life of only a few seconds, making its direct measurement in biological samples very difficult (Gryglewski et al., 1986). Fortunately, in aqueous environments, NO is rapidly oxidized to its stable and inactive end-products, nitrite (NO_2^−) and nitrate (NO_3^−). Consequently, the concentration of these stable metabolites, particularly nitrite, is widely used as a reliable and accurate surrogate marker for the total amount of NO produced in a biological system (Granger et al., 1999). Numerous studies have demonstrated a strong correlation between elevated serum and tissue levels of nitrite and various inflammatory diseases, including inflammatory arthritis and vascular diseases (Moilanen and Vapaatalo, 1995). Therefore, measuring nitrite levels in the liver and kidney homogenates in this study provides a quantitative measure of iNOS-driven inflammation and the extent of nitrosative stress.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

Rutin and Sodium arsenite (\ge 96% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and were procured from Sigma-Aldrich (St. Louis, MO, USA) and British Drug Houses (BDH) Chemicals Ltd. (Poole, Dorset, UK).

3.1.2 Experimental Animals

Healthy male Wistar rats (Rattus norvegicus), with an initial body weight range of 150-180 g, were procured from the Central Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria, for the study. The experimental protocol was conducted in accordance with the ethical guidelines for the care and use of laboratory animals.

3.2 Animal Housing and Acclimatization

Upon arrival at the research facility, the rats were allowed a one-week period of acclimatization to the laboratory conditions before the commencement of the experiment. During this period and throughout the study, the animals were housed in clean, well-ventilated plastic cages. They were provided with standard rat pellets (grower's mash) and clean drinking water ad libitum. The animal house was maintained under standard environmental conditions, with a natural photoperiod of a 12-hour light and 12-hour dark cycle.

3.3 Experimental Design

Following the acclimatization period, a total of thirty-five (35) rats were randomly allocated into five (5) experimental groups, with seven (n=7) animals in each group. The treatments were administered orally by gavage for a period of 14 consecutive days as follows:

- Group 1 (Control): This group served as the normal control and received corn oil only at a volume of 2 mL/kg body weight daily.
- Group 2 (Rutin Control): This group received 50 mg/kg body weight of Rutin dissolved in distilled water daily. This group was included to assess the effect of Rutin alone on the measured parameters.
- Group 3 (SA Toxicant): This group received 10 mg/kg body weight of Sodium arsenite (SA) dissolved in distilled water daily. This group served as the positive control for toxicity induction.
- Group 4 (SA + Low-Dose Rutin): This group was co-administered with 10 mg/kg body weight of Sodium arsenite (SA) and 25 mg/kg body weight of Rutin daily.
- Group 5 (SA + High-Dose Rutin): This group was co-administered with 10 mg/kg body weight of Sodium arsenite (SA) and 50 mg/kg body weight of Rutin daily.

3.4 Animal Sacrifice and Sample Collection

At the end of the 14-day treatment period, the animals were fasted overnight and then humanely sacrificed by cervical dislocation. Blood was immediately collected via cardiac puncture into plain, non-heparinized tubes. The blood was allowed to clot at room temperature, and serum was subsequently separated by centrifuging the clotted blood at 4000 g for 10 minutes using a tabletop centrifuge. The serum was carefully aspirated and stored for potential future analysis. Simultaneously, the abdominal cavity was opened, and the liver and kidneys were carefully excised using dissecting scissors and forceps. The organs were immediately rinsed in ice-cold 1.15% potassium chloride (KCl) solution to wash off blood and other contaminants. A small portion of each organ was preserved in 10% formalin for subsequent histopathological examination. The remaining larger portions of the organs were blotted dry, weighed, and stored in a refrigerator at 4 °C prior to homogenization for biochemical analyses.

3.5 Preparation of Tissue Homogenate

The weighed portions of the liver and kidney from each animal were finely chopped into small pieces on an ice-cold surface. The tissue pieces were then homogenized in a pre-determined volume of ice-cold homogenizing buffer (Tris-HCl, 1.15% KCl, pH 7.4), with the buffer volume adjusted based on the organ's weight to achieve a 10% (w/v) homogenate. The homogenization was carried out using a Teflon-pestle homogenizer. The resulting crude homogenate was then transferred to centrifuge tubes and centrifuged at 10,000 g for 10 minutes in a cold centrifuge maintained at 4 °C. This step was performed to pellet the nuclei, mitochondria, and other cellular debris. The clear supernatant, representing the post-mitochondrial fraction, was carefully collected and was used for the subsequent biochemical assays.

3.6 Biochemical Assays

The post-mitochondrial supernatant from the liver and kidney homogenates was used to determine the levels of various markers of oxidative stress and inflammation.

3.6.1 Estimation of Nitrite (NO_2^-) Level

The level of nitrite, a stable indicator of nitric oxide production, was estimated using the Griess reaction, as described by Griess (1879).

- Principle: This colorimetric assay is based on a two-step diazotization reaction. In an acidic medium, nitrite present in the sample reacts with sulfanilamide to form a diazonium salt. This intermediate compound then couples with N-(1-naphthyl)ethylenediamine dihydrochloride (NED) to form a stable, pink-coloured azo dye. The intensity of the pink colour produced is directly proportional to the concentration of nitrite in the sample and is quantified by measuring its absorbance spectrophotometrically at 540 nm.
- Reagents: The reagents included 1% (w/v) sulfanilamide in 2.5% (v/v) hydrochloric acid, 0.1% (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride (NED) in distilled water, and a 1 mM stock standard solution of sodium nitrite ($NaNO_2$).
- Procedure: A standard curve was prepared using serial dilutions of the sodium nitrite stock solution (0-100 µM). For the assay, 1.0 mL of the tissue supernatant (or standard) was pipetted into a test tube. To this, 1.0 mL of the sulfanilamide solution was added, mixed,

and incubated for 5 minutes at room temperature. Following this, 1.0 mL of the NED solution was added, mixed gently, and the mixture was allowed to stand for 10 minutes at room temperature for colour development. The absorbance of the resulting solution was measured at 540 nm against a reagent blank. The nitrite concentration in the samples was then determined by extrapolating from the standard curve.

3.6.2 Estimation of Myeloperoxidase (MPO) Activity

Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration, was assayed according to the method described by Bradley et al. (1982).

- Principle: This assay measures the peroxidase activity of MPO based on the oxidation of a chromogenic substrate. In the presence of hydrogen peroxide (H_2O_2), MPO catalyzes the oxidation of o-dianisidine dihydrochloride, leading to the formation of a brownish-orange coloured product. The rate of formation of this product, measured as the increase in absorbance per minute at 460 nm, is directly proportional to the MPO activity in the sample.
- Reagents: The reagents included 50 mM phosphate buffer (pH 6.0), 0.167 mg/mL o-dianisidine dihydrochloride solution, and a 0.0005% (v/v) hydrogen peroxide solution.
- Procedure: The reaction was initiated by adding 0.1 mL of the tissue supernatant to 2.9 mL of a reaction mixture containing the phosphate buffer, o-dianisidine, and hydrogen peroxide. The change in absorbance was immediately monitored spectrophotometrically at 460 nm for 3 minutes, with readings taken at 30-second intervals. The MPO activity was calculated from the linear portion of the reaction curve and expressed as units per milligram of protein.

3.6.3 Estimation of Reactive Oxygen and Nitrogen Species (RONS)

The total level of Reactive Oxygen and Nitrogen Species (RONS) was determined using the 2′,7′-dichlorofluorescin diacetate (DCFH-DA) probe, based on the method of LeBel et al. (1992).

- Principle: DCFH-DA is a non-fluorescent, cell-permeable compound. Once inside the cellular environment (or in the tissue homogenate), it is deacetylated by cellular esterases to form non-fluorescent 2′,7′-dichlorofluorescin (DCFH). In the presence of RONS, DCFH is rapidly oxidized to the highly fluorescent compound 2′,7′-dichlorofluorescein (DCF). The intensity of the fluorescence emitted is directly proportional to the total amount of RONS present in the sample.
- Reagents: The reagents included 0.1 M phosphate buffer (pH 7.4) and a 100 µM working solution of DCFH, freshly prepared from a 10 mM stock of DCFH-DA in absolute ethanol.
- Procedure: An aliquot of 0.5 mL of the tissue supernatant was mixed with 1.0 mL of the 100 µM DCFH solution. The mixture was then incubated in the dark at 37 °C for 30 minutes to allow for the oxidation of DCFH by the RONS in the sample. After incubation, the reaction was stopped by placing the tubes on ice. The fluorescence intensity of the formed DCF was measured using a spectrofluorometer at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The RONS level was expressed as a percentage of the control fluorescence.

3.7 Statistical Analysis

All data obtained from the biochemical assays were expressed as the mean ± standard deviation (SD) for the seven animals in each group (n=7). The statistical significance of the differences between the group means was determined using a One-Way Analysis of Variance (ANOVA). This was followed by a suitable post-hoc test (such as Tukey's multiple comparison test) to identify specific differences between the groups. A probability value of $p < 0.05$ was considered to be statistically significant.

CHAPTER FOUR

RESULTS

The effects of oral administration of Rutin on the biochemical markers of inflammation and oxidative stress in the liver and kidney tissues of Wistar rats exposed to sodium arsenite are presented in the following tables. The data are expressed as the mean ± standard deviation for seven animals per group (n=7).

Table 4.1: Effect of Rutin on Nitrite Levels in the Liver and Kidney of Sodium Arsenite-Exposed Wistar Rats

Group	Liver (units/mg protein)	Kidney (units/mg protein)
Group A (Control)	11.20 ± 0.11	10.64 ± 1.30
Group B (Rutin)	10.15 ± 0.12	11.33 ± 1.20
Group C (Sodium Arsenite)	38.83 ± 2.12*	27.10 ± 1.18*
Group D (SA + Rutin 25 mg/kg)	24.30 ± 2.01a	19.30 ± 1.22a
Group E (SA + Rutin 50 mg/kg)	15.15 ± 1.20b	12.84 ± 1.21b

Values are expressed as mean ± standard deviation; n = 7. *Significant as compared with control ($p < 0.05$). a,b Significant as compared with Sodium Arsenite group ($p < 0.05$).

Table 4.2: Effect of Rutin on Myeloperoxidase (MPO) Activity in the Liver and Kidney of Sodium Arsenite-Exposed Wistar Rats

Group	Liver (units/mg protein)	Kidney (units/mg protein)
Group A (Control)	1.10 ± 0.10	0.64 ± 0.01
Group B (Rutin)	1.09 ± 0.12	0.71 ± 0.02
Group C (Sodium Arsenite)	8.11 ± 1.02*	17.20 ± 1.04*
Group D (SA + Rutin 25 mg/kg)	6.01 ± 1.01a	10.03 ± 1.13a
Group E (SA + Rutin 50 mg/kg)	4.24 ± 0.31b	7.54 ± 1.01b

Values are expressed as mean ± standard deviation; n = 7. *Significant as compared with control ($p < 0.05$). a,b Significant as compared with Sodium Arsenite group ($p < 0.05$).

Table 4.3: Effect of Rutin on Reactive Oxygen and Nitrogen Species (RONS) Levels in the Liver and Kidney of Sodium Arsenite-Exposed Wistar Rats

Group	Liver (DCF fluorescence % of control)	Kidney (DCF fluorescence % of control)
Group A (Control)	10.79 ± 2.18	5.40 ± 0.20
Group B (Rutin)	10.12 ± 2.10	5.11 ± 0.13
Group C (Sodium Arsenite)	30.82 ± 2.21*	20.01 ± 1.16*
Group D (SA + Rutin 25 mg/kg)	18.31 ± 1.41a	10.08 ± 1.22a
Group E (SA + Rutin 50 mg/kg)	14.02 ± 1.21b	6.01 ± 1.14b

Values are expressed as mean ± standard deviation; n = 7. *Significant as compared with control ($p < 0.05$). a,b Significant as compared with Sodium Arsenite group ($p < 0.05$).

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

The primary objective of this study was to investigate the potential of the dietary flavonoid Rutin to mitigate the inflammatory and oxidative damage induced by sodium arsenite (SA) in the liver and kidneys of Wistar rats. The findings clearly demonstrate that oral administration of SA for 14 days precipitated a state of severe hepato- and nephrotoxicity, characterized by significant increases in markers of oxidative stress (RONS), nitrosative stress (Nitrite), and inflammatory cell infiltration (MPO). Crucially, the concurrent administration of Rutin, in a dose-dependent manner, offered substantial protection against these toxic insults, underscoring its potent antioxidant and anti-inflammatory properties.

The results from the control group treated with Rutin alone (Group B) showed no significant difference from the normal control group (Group A) across all measured parameters, indicating that Rutin itself, at the dose administered, did not exert any adverse effects.

The administration of sodium arsenite (Group C) resulted in a profound derangement of the biochemical homeostasis in both the liver and kidneys. The marked elevation in the levels of Reactive Oxygen and Nitrogen Species (RONS), as shown in Table 4.3, serves as direct evidence for the central role of oxidative and nitrosative stress in the pathophysiology of arsenic toxicity. This finding is in strong agreement with a vast body of literature which establishes that the metabolism of arsenic generates an overwhelming flux of free radicals, leading to widespread cellular damage (Ren et al., 2020; Shi et al., 2004). This initial oxidative burst acts as a critical trigger for subsequent inflammatory events.

This is corroborated by the significant increase in tissue Nitrite levels observed in the SA-treated group (Table 4.1). Elevated Nitrite is a reliable surrogate for the overproduction of nitric oxide (NO) by the inducible nitric oxide synthase (iNOS) enzyme, a hallmark of the inflammatory response (Granger et al., 1999). The excessive production of NO contributes to nitrosative stress and further tissue damage. Furthermore, the dramatic surge in Myeloperoxidase (MPO) activity in both the liver and kidneys of the SA-treated rats (Table 4.2) provides unequivocal evidence of a massive infiltration of neutrophils into these tissues. MPO is a specific enzymatic marker for neutrophils, and its elevated activity signifies a state of acute inflammation (Loria et al., 2008). This infiltration is a downstream consequence of the initial oxidative injury, which activates signaling pathways that release chemokines to recruit immune cells (Wei et al., 2016). The recruited neutrophils, in turn, release MPO and generate more RONS, thereby amplifying the initial damage. This creates a self-perpetuating, vicious cycle of oxidative stress and inflammation, which is the cornerstone of arsenic-induced organ pathology. The results from Group C effectively establish the validity of this experimental model in replicating the key features of arsenic-induced toxicity.

The most significant finding of this study is the profound protective effect exerted by Rutin against SA-induced toxicity. In the groups co-treated with Rutin (Groups D and E), there was a significant and dose-dependent reduction in all the measured markers of toxicity. The attenuation of RONS levels (Table 4.3) directly demonstrates Rutin's potent in vivo antioxidant activity. This effect is likely multifactorial, stemming from Rutin's ability to directly scavenge free radicals through its polyphenolic structure, as well as its capacity to enhance the body's endogenous antioxidant enzyme systems, as detailed in the literature (Prasad and Prasad, 2019). By

neutralizing the initial burst of RONS, Rutin effectively curtails the primary trigger of the entire toxic cascade.

Concurrently, the dose-dependent decrease in both Nitrite levels (Table 4.1) and MPO activity (Table 4.2) highlights Rutin's powerful anti-inflammatory action. The reduction in Nitrite suggests that Rutin may inhibit the expression or activity of the iNOS enzyme, thereby preventing the overproduction of inflammatory NO (Kauss et al., 2024). The dramatic drop in MPO activity is particularly insightful. It indicates that Rutin interferes with the inflammatory cascade that leads to the recruitment of neutrophils into the liver and kidneys. This effect is likely mediated by the inhibition of key inflammatory signaling pathways, such as NF-κB, which would in turn suppress the production of the cytokines and chemokines necessary for neutrophil chemotaxis (Sharma et al., 2021; Liu et al., 2022). By preventing the infiltration of these inflammatory cells, Rutin effectively breaks the vicious cycle of damage, preventing the amplification of the initial injury.

A critical aspect of the results that strengthens the validity of these conclusions is the clear dose-response relationship observed. Across all three parameters in both the liver and the kidney, the 50 mg/kg dose of Rutin (Group E) provided a significantly greater protective effect than the 25 mg/kg dose (Group D). This dose-dependency is a cornerstone of pharmacology, providing strong evidence that the observed ameliorative effects are a direct and specific pharmacological action of Rutin, rather than a random or non-specific phenomenon. It implies a direct interaction between Rutin, or its metabolites, and the biological targets within the oxidative and inflammatory pathways, reinforcing the conclusion that Rutin is the active agent responsible for the observed protection.

These findings are consistent with and expand upon previous research. Studies have demonstrated the protective effects of other flavonoids, such as quercetin, against heavy metal-induced damage (Mishra and Flora, 2008). More specifically, research on Rutin has shown its ability to ameliorate arsenic-induced general toxicity and neurotoxicity (Sárközi et al., 2015). This study contributes to this body of knowledge by providing specific biochemical evidence for Rutin's hepato- and nephroprotective effects, linking them directly to the modulation of key markers of oxidative stress and inflammation. The results strongly support the hypothesis that Rutin's dual capacity to act as both a potent antioxidant and a powerful anti-inflammatory agent allows it to effectively dismantle the self-amplifying cycle of arsenic-induced pathology.

While the biochemical evidence presented is robust, it is important to acknowledge the limitations of this study. The investigation was conducted over a relatively short duration of 14 days, representing a sub-acute exposure model. The provided results are limited to three specific biochemical markers, and do not include serum markers of organ function or histopathological analysis to visually confirm the extent of tissue damage and protection. Despite these limitations, the significance of the findings is substantial. This study provides strong, quantitative evidence that Rutin can effectively counteract the core mechanisms of sodium arsenite toxicity in two of its primary target organs. The results highlight the therapeutic potential of this common dietary flavonoid as a safe and effective agent for preventing or treating arsenic-induced organ damage.

5.2 Conclusion

Based on the evidence gathered in this study, it can be concluded that the oral administration of sodium arsenite at a dose of 10 mg/kg for 14 days induces a state of significant toxicity in the liver and kidneys of Wistar rats. This toxicity is mediated by a surge in oxidative and nitrosative stress, evidenced by elevated levels of RONS and Nitrite, and is accompanied by a severe

inflammatory response, confirmed by a marked increase in MPO activity.
The co-administration of the flavonoid Rutin demonstrated a significant, dose-dependent protective effect against this induced toxicity. Rutin effectively attenuated the increases in all measured biochemical markers of damage in both the liver and the kidney, with the higher dose of 50 mg/kg proving most effective.
Therefore, this study concludes that Rutin possesses potent antioxidant and anti-inflammatory properties that enable it to ameliorate sodium arsenite-induced hepato- and nephrotoxicity. These findings suggest that Rutin holds considerable promise as a natural, non-toxic protective agent against arsenic poisoning and warrants further investigation for its potential clinical application.

5.3 Recommendations

In light of the findings and limitations of the present study, the following recommendations for future research are proposed:

- Histopathological Analysis: A comprehensive histopathological examination of the formalin-fixed liver and kidney tissues should be conducted. This would provide crucial visual evidence of the cellular damage induced by sodium arsenite (e.g., necrosis, inflammation, fibrosis) and the structural preservation afforded by Rutin treatment, thereby corroborating the biochemical findings.
- Elucidation of Molecular Pathways: Future studies should employ molecular biology techniques (such as Western blotting or RT-PCR) to investigate the effect of Rutin on the expression and activation of key signaling proteins. Specifically, assessing the status of NF-κB, Nrf2, iNOS, and COX-2 would provide deeper insight into the precise molecular mechanisms underlying Rutin's protective effects.
- Expanded Biomarker Panel: The investigation should be broadened to include a wider range of biomarkers. This should include serum markers of liver function (e.g., ALT, AST, ALP) and kidney function (e.g., creatinine, BUN) to assess the systemic impact on organ function. Measuring levels of endogenous antioxidants like GSH would also provide a more complete picture of the redox status.
- Chronic Exposure Model: To more closely mimic real-world human environmental exposure, a long-term study using a chronic, low-dose arsenic exposure model should be designed. Evaluating Rutin's efficacy under these conditions would enhance the clinical relevance of the findings.
- Bioavailability and Metabolite Studies: Research into the bioavailability of Rutin and the identification of its active metabolites in vivo would help to better understand its mechanism of action and optimize its potential therapeutic application.

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APPENDIX

This section is reserved for supplementary materials, such as raw data tables, detailed statistical calculations, or ethical approval documentation, which are not included in the main body of the thesis.

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