# ANTIOXIDANT EFFECTS OF RUTIN ON SODIUM ARSENITE INDUCED OXIDATIVE STRESS IN THE LIVER OF WISTAR RATS

**BY**

**FAROJOYE OLADIMEJI BANKOLE**

**(BMS2101407)**

**DEPARTMENT OF MEDICAL BIOCHEMISTRY**

**SCHOOL OF BASIC MEDICAL SCIENCE**

**COLLEGE OF MEDICAL SCIENCE**

**UNIVERSITY OF BENIN**

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## CERTIFICATION

We the undersigned hereby certify that FAROJOYE OLADIMEJI BANKOLE (BMS2101407) 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 (B.Sc) Degree in Medical Biochemistry.

..... .....

**Dr. (Mrs).O.Ikponmwosa-Eweka** **(Date)**

**(Project Supervisor)**

..... .....

**Dr. N. B. Aguebor-Ogie** **(Date)**

**(Ag. Head of Department)**

..... .....

**External Examiner** **(Date)**

## DEDICATION

This report is dedicated to my beloved parents, Mr. and Mrs. FAROJOYE, whose love, guidance, and sacrifices have been my greatest motivation. I also extend heartfelt gratitude to my friends, colleagues, and everyone who has supported and encouraged me throughout this journey. Your belief in me made this achievement possible.

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## ABSTRACT

Arsenic exposure remains a major environmental health concern due to its ability to generate oxidative stress and induce tissue injury, particularly in the liver. This study investigated the protective potential of rutin against sodium arsenite-induced hepatic oxidative damage in Wistar rats. The experiment involved the administration of sodium arsenite to induce oxidative stress, while rutin was concurrently given at different doses to evaluate its antioxidant and hepatoprotective effects.

Following treatment, liver antioxidant status was assessed through the measurement of key biochemical parameters including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Histopathological examination of liver tissues was also carried out to determine structural alterations associated with arsenite toxicity and the possible protective influence of rutin.

The results indicated that exposure to sodium arsenite caused a pronounced decline in endogenous antioxidant defenses, reflected by reduced levels of GSH and decreased activities of SOD, CAT, and GPx. These biochemical disruptions were accompanied by noticeable histological abnormalities in hepatic tissue, suggesting oxidative damage and cellular degeneration. However, rats that received rutin alongside sodium arsenite demonstrated marked improvement in antioxidant enzyme activities and glutathione levels compared with animals treated with arsenite alone. The degree of improvement was more pronounced at higher rutin doses, indicating a dose-dependent protective effect.

The findings suggest that rutin exerts significant antioxidant activity capable of counteracting arsenite-induced oxidative stress in the liver. This protective action may be attributed to its ability to scavenge reactive oxygen species and enhance endogenous antioxidant defense mechanisms. Consequently, rutin may serve as a promising natural compound for reducing oxidative damage associated with heavy metal toxicity.

In conclusion, the study demonstrates that rutin effectively mitigates sodium arsenite-induced hepatic oxidative injury in Wistar rats by restoring antioxidant balance and improving liver tissue integrity.

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# CHAPTER ONE

# INTRODUCTION

## 1.0 Background of Study

Arsenic is a naturally occurring metalloid found throughout the earth's crust, and its persistence in the environment has made it a major global toxicological concern. Humans are mainly exposed to it through polluted groundwater, industrial discharges, certain foods, and occupations involving arsenic compounds (Chen & Costa, 2021; IARC, 2012). Among the different inorganic species, sodium arsenite is considered especially dangerous because it is easily absorbed and can readily stimulate the formation of reactive oxygen species (ROS). These reactive molecules disrupt normal cellular processes and contribute to oxidative damage in organs such as the liver and kidneys (Gong *et al.,* 2015; Gora *et al.,* 2014).

The liver, being the primary organ responsible for metabolizing and detoxifying xenobiotics, is particularly vulnerable to arsenic toxicity. Continuous exposure has been shown to upset hepatic redox homeostasis, resulting in mitochondrial dysfunction, lipid peroxidation, leakage of intracellular enzymes, and ultimately hepatocellular injury and necrosis (Ijaz *et al.,* 2022). The shift in the balance between ROS production and the body's antioxidant defenses—enzymatic systems like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), as well as non-enzymatic antioxidants such as reduced glutathione (GSH)—is central to the development of oxidative liver damage (Sohrab *et al.,* 2023; Goya *et al.,* 2025).

Growing interest has been directed toward naturally derived bioactive compounds with strong antioxidant potential. Rutin, a polyphenolic flavonoid abundant in many fruits and

medicinal plants, has been widely documented for its free-radical-neutralizing, metal-chelating, anti-inflammatory, and hepatoprotective activities (Ahmed *et al.,* 2022; Gur *et al.,* 2023; Prasad *et al.,* 2021). Evidence suggests that rutin supports endogenous antioxidant systems and regulates oxidative-stress-related pathways—particularly those involving Nrf2/HO-1 and NF-κB—making it a promising agent for counteracting heavy-metal-induced toxicity (Khan *et al.,* 2022).

In view of these findings, this present study evaluates whether rutin can mitigate sodium arsenite–induced oxidative stress in the liver of Wistar rats. The research aims to clarify the biochemical mechanisms involved and to determine the potential of rutin as a protective intervention against arsenic-associated hepatic injury.

### 1.1 Justification of the Study

The liver serves as the body's primary detoxification organ and is particularly vulnerable to damage from toxic substances such as arsenic. Chronic exposure to sodium arsenite leads to excessive production of reactive oxygen species (ROS), resulting in oxidative stress, lipid peroxidation, and impaired liver function. Conventional therapeutic interventions for heavy metal toxicity are often limited by side effects and poor efficacy. Hence, there is an increasing need to explore safer, natural alternatives with potent antioxidant properties. Rutin, a flavonoid abundantly found in medicinal plants, has been recognized for its strong radical-scavenging, anti-inflammatory, and metal-chelating activities (Ahmed *et al.,* 2022; Gur *et al.,* 2023). However, studies focusing specifically on the protective effects of rutin against sodium arsenite–induced hepatic oxidative damage remain limited. This research, therefore, seeks to bridge this gap by evaluating rutin's ability to restore antioxidant balance and protect liver tissues in arsenic-exposed Wistar rats.

## 1.2 Aim of the Study

To evaluate the antioxidant and hepatoprotective effects of rutin on sodium arsenite–induced oxidative stress in the liver of Wistar rats.

## 1.3 Objectives of the Study

- To assess the impact of sodium arsenite on liver antioxidant enzyme activities (SOD, CAT, GPx, GST, and GSH) in Wistar rats.
- To determine the protective effects of rutin supplementation on biochemical and oxidative stress parameters in the liver.
- To compare the dose-dependent efficacy of rutin in ameliorating hepatic oxidative damage.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Arsenic and Its Toxicological Significance

Arsenic is a naturally occurring metalloid widely distributed in the environment and recognized for its pronounced toxicity in both humans and animals. Its presence in ecosystems arises from natural geological activities as well as anthropogenic sources, making contamination a persistent global environmental concern. Human exposure commonly occurs through contaminated drinking water, food chains, industrial emissions, and occupational contact, and has been associated with oxidative stress, hepatic injury, and various malignancies (Ganie *et al.,* 2024).

Arsenic exists in organic and inorganic forms, with the inorganic species—particularly arsenite and arsenate—being considerably more toxic. Major contributors to environmental arsenic contamination include mineral deposits, agricultural chemicals, industrial effluents, and long-term accumulation in groundwater systems (Yang *et al.,* 2024). In many developing regions, especially across parts of Asia and Africa, prolonged consumption of arsenic-contaminated water has resulted in dermatological disorders, neurological deficits, cardiovascular complications, and increased cancer risk.

Sodium arsenite ($NaAsO_2$), a trivalent inorganic form, is especially hazardous due to its high reactivity and capacity to disrupt cellular redox balance. It interacts with sulfhydryl groups of proteins, interferes with mitochondrial function, and compromises DNA repair mechanisms, thereby promoting oxidative injury and organ dysfunction (Khan *et al.,* 2022). Chronic exposure

has been strongly linked to carcinogenesis and systemic toxicity (Ganie *et al.*, 2024; Yang *et al.*, 2024).

.


**Figure 1**: Chemical Structure of sodium Arsenite

**2.2 Biochemical Mechanisms of Arsenic-Induced Toxicity**

The pathological effects of arsenic are primarily mediated through oxidative stress and disturbance of intracellular signaling pathways. Following absorption, arsenic undergoes metabolic transformation and accumulates in tissues such as the liver, where it disrupts cellular homeostasis.

A central mechanism of toxicity involves excessive generation of reactive oxygen species (ROS), which overwhelms endogenous antioxidant defenses. This imbalance results in lipid peroxidation, protein oxidation, mitochondrial dysfunction, and activation of apoptotic pathways (Thakur *et al.*, 2021; Fatemi *et al.*, 2021). Experimental studies consistently demonstrate that arsenic exposure impairs antioxidant systems, leading to structural and biochemical evidence of liver injury (Ding *et al.*, 2022; Mirzaei *et al.*, 2023).


Figure 2: Sodium Arsenite Bottle

Arsenic-induced hepatotoxicity is characterized by depletion of reduced glutathione (GSH) and suppression of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The disruption of these protective systems amplifies oxidative damage and contributes to inflammation and tissue degeneration (Ganie *et al.*, 2024; Yang *et al.*, 2024; Khan *et al.*, 2022).

### 2.3 Effects of Sodium Arsenite on the Liver

The liver plays a central role in detoxification and is therefore highly susceptible to arsenic toxicity. Continuous exposure to sodium arsenite alters hepatic redox equilibrium and

compromises cellular integrity.

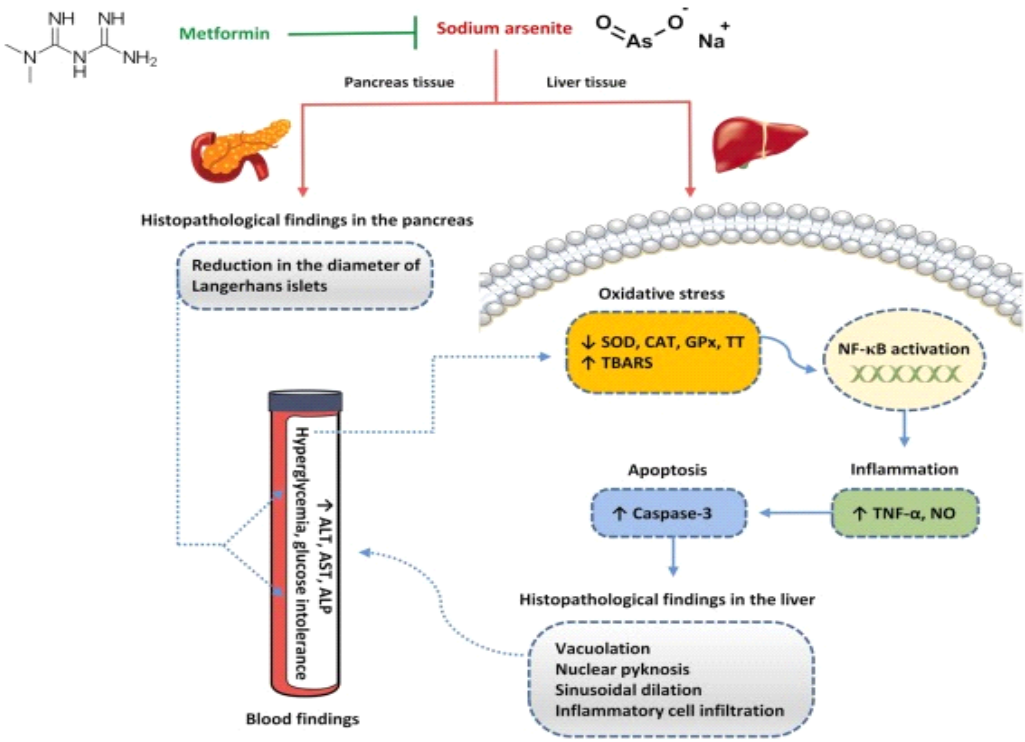


**Figure 3**: Effects of Sodium Arsenite on Liver

Mechanistically, arsenic promotes ROS overproduction while simultaneously weakening both enzymatic and non-enzymatic antioxidant defenses (Thakur *et al.*, 2021; Fatemi *et al.*, 2021). This results in membrane lipid peroxidation, leakage of intracellular enzymes, and progressive hepatocellular damage (Ding *et al.*, 2022; Mirzaei *et al.*, 2023).

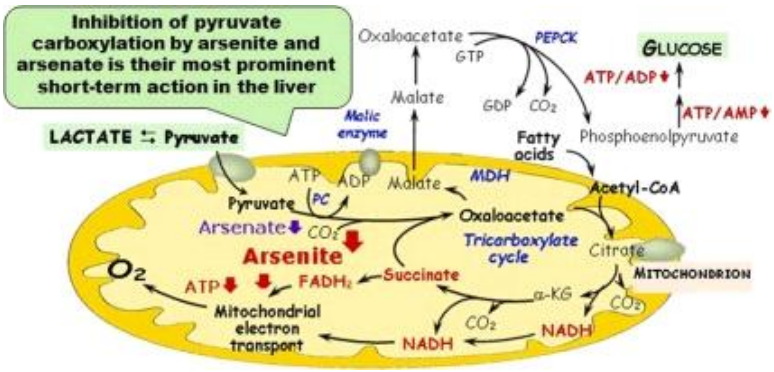

**Figure 4**: Molecular mechanism of Arsenite on The Liver

Histopathological findings from experimental models reveal hepatocyte degeneration, inflammatory infiltration, sinusoidal dilation, and necrotic changes following arsenic administration. These pathological alterations are closely associated with suppressed antioxidant enzyme activity and reduced glutathione levels (Ganie *et al.,* 2024; Yang *et al.,* 2024; Khan *et al.,* 2022).

### 2.4 Role of Antioxidants in Combating Oxidative Stress

Antioxidant systems are essential in preserving cellular redox balance and preventing oxidative injury. They function by neutralizing reactive oxygen species and limiting molecular damage.

The enzymatic antioxidant network includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST). These enzymes cooperate to detoxify superoxide radicals and hydrogen peroxide while facilitating the conjugation and elimination of harmful metabolites (Kiran *et al.,* 2023; Goyal *et al.,* 2025; Akbari *et al.,* 2022).

Non-enzymatic antioxidants, particularly reduced glutathione (GSH), serve as critical intracellular scavengers. GSH directly neutralizes ROS and acts as a substrate for GPx-mediated detoxification reactions. Disruption of these protective mechanisms enhances susceptibility to toxin-induced cellular injury.

## 2.5 Therapeutic Importance of Natural Antioxidants Against Heavy Metal Toxicity

Limitations associated with conventional therapies for heavy metal toxicity—including adverse effects and incomplete efficacy—have encouraged exploration of plant-derived antioxidants as safer alternatives.

Natural polyphenolic compounds exhibit strong radical-scavenging and metal-chelating capabilities. Among these, flavonoids have attracted considerable attention for their cytoprotective effects across various experimental models. Their mechanisms of action include direct neutralization of free radicals, modulation of antioxidant enzyme expression, and suppression of inflammatory mediators (Goyal *et al.*, 2023; Calabrese *et al.*, 2024; Henrotin *et al.*, 2022).

Evidence from heavy metal exposure studies indicates that phytochemicals can attenuate oxidative stress markers and restore impaired antioxidant defenses (Khan *et al.*, 2022).

## 2.6 Overview of Rutin

Rutin is a naturally occurring flavonoid glycoside widely distributed in fruits, vegetables, and medicinal plants. It has been extensively studied for its antioxidant, anti-inflammatory, and organ-protective properties.


**Figure 5**: Rutin

Pharmacologically, rutin demonstrates the ability to scavenge reactive oxygen species, chelate transition metals, and modulate endogenous antioxidant enzyme systems (Goyal *et al.*, 2023; Calabrese *et al.*, 2024; Henrotin *et al.*, 2022). It also regulates oxidative stress-related signaling pathways, particularly through activation of Nrf2/HO-1 and inhibition of NF-κB-mediated inflammatory cascades (Tian *et al.*, 2022; Rahmani *et al.*, 2023; Rakshit *et al.*, 2021).

These mechanisms contribute to its protective effects in models of metabolic, inflammatory, and toxin-induced organ damage.

## 2.7 Rutin and Organ Protection in Experimental Models

Preclinical investigations have consistently demonstrated the multi-organ protective effects of rutin. In hepatic models, rutin supplementation has been shown to normalize lipid peroxidation indices and restore the activity of antioxidant enzymes such as SOD, CAT, and GPx following toxic insult (Khan *et al.*, 2022).

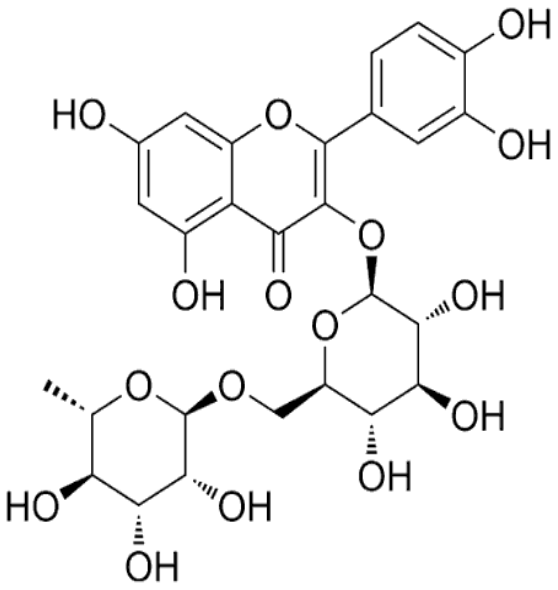


**Figure 6**: Chemical Structure of Rutin

Histologically, rutin administration reduces hepatocyte degeneration and inflammatory cell infiltration while preserving structural architecture. These findings highlight its ability to mitigate both biochemical and morphological damage induced by oxidative stress.

At the molecular level, activation of the Nrf2/HO-1 pathway enhances antioxidant gene expression, while concurrent suppression of NF-κB signaling reduces pro-inflammatory cytokine production (Tian *et al.*, 2022; Rahmani *et al.*, 2023; Rakshit *et al.*, 2021).

## 2.8 Rutin in the Management of Arsenic-Induced Hepatotoxicity

Although numerous studies support the antioxidant capacity of rutin, research specifically evaluating its protective effects against sodium arsenite-induced hepatotoxicity remains comparatively limited.


**Figure 7**: Sources of Rutin

Available evidence suggests that rutin can counteract arsenic-induced oxidative stress by restoring GSH levels and enhancing antioxidant enzyme activities (Khan *et al.,* 2022). It also reduces inflammatory responses and improves histopathological outcomes in arsenic-exposed tissues (Ganie *et al.*, 2024; Yang *et al.*, 2024; Khan *et al.*, 2022).

However, there remains insufficient data on dose-dependent responses and comprehensive biochemical assessments in Wistar rat models. These gaps justify further investigation into the hepatoprotective potential of rutin under controlled experimental conditions.

The present study was therefore designed to systematically evaluate the antioxidant and hepatoprotective effects of rutin against sodium arsenite-induced oxidative liver damage in Wistar rats, with emphasis on elucidating underlying biochemical mechanisms.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.0 Materials

1. Methanol

2. Chloroform

3. Formalin (10%) for tissue fixation

4. Normal saline

5. Distilled water

## 3.1 Apparatus

1. Oral gavage (for drug administration)

2.Refrigerator

3. Cages

4. Feeding bowls

5. Plain bottles

6. Dissecting set (scissors, forceps, scapel)

7. Electronic weighing balance

8. EDTA containers

9. Disposable gloves and nose masks

10. Syringes and needles

11. Glass slides and cover slips

12. Sample bottles and vials for blood and tissue storage

13. Wistar rat feeds

14. Cotton wool and methylated spirit

15. Spectrophotometer

16. Labels and markers pens

17. Iceblock

**Chemicals and Reagents**

All reagents utilized in this investigation were of analytical grade and prepared freshly prior to use. Rutin (≥96% purity) and sodium arsenite were procured from Sigma-Aldrich (St. Louis, MO, USA). Additional chemicals, including potassium chloride, Tris base, copper sulfate, potassium iodide, bovine serum albumin (BSA), and other assay reagents, were obtained from certified laboratory suppliers. Distilled water was used for all solution preparations.

**3.2 ANIMAL PROTOCOL**

Thirty-five (35) male Wistar rats weighing between 150 g and 180 g were employed for this study. The animals were sourced from the Animal House Facility, Department of Anatomy, University of Benin, Nigeria.

Upon procurement, the rats were acclimatized for seven days under standard laboratory conditions. They were housed in clean, well-ventilated cages and maintained on a 12-hour light/12-hour dark cycle. Commercially prepared rat feed and potable water were provided ad libitum throughout the experimental period.

### 3.3 EXPERIMENTAL DESIGN

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

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

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

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

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

Group 5: Orally co-administered 50 mg/kg of Rutin and 10 mg/kg of Sodium arsenite (SA) for 14 days. Following acclimatization, animals were randomly allocated into five groups (n = 7 per group). Treatments were administered orally once daily for a duration of fourteen (14) consecutive days.

- Group I (Control): Received corn oil at 2 mL/kg body weight.
- Group II (Rutin-treated): Administered rutin at 50 mg/kg body weight.
- Group III (Sodium arsenite-treated): Received sodium arsenite at 10 mg/kg body weight.
- Group IV (Low-dose co-treatment): Administered rutin (25 mg/kg) in combination with sodium arsenite (10 mg/kg).
- Group V (High-dose co-treatment): Administered rutin (50 mg/kg) alongside sodium arsenite (10 mg/kg).

All treatments were delivered via oral gavage using appropriately calibrated dosing equipment.

## 3.4 SACRIFICE OF EXPERIMENTAL ANIMALS

At the end of the treatment period, the animals were sacrificed following standard ethical procedures. Blood samples were collected into plain tubes and allowed to clot at room temperature. Serum was separated by centrifugation at 4000 rpm for 10 minutes and stored for subsequent biochemical evaluation.

The liver, kidneys, and heart were carefully excised, rinsed in ice-cold 1.15% potassium chloride solution to eliminate residual blood, and gently blotted dry. Portions designated for histological analysis were fixed in formalin, while remaining tissues were processed for biochemical assays.

### 3. 5 Homogenizations

Liver samples were homogenized in cold Tris-HCl buffer containing 1.15% potassium chloride (pH 7.4) using a Teflon homogenizer to ensure uniform disruption of tissue architecture. The homogenate was subjected to centrifugation at 10,000 × g for 10 minutes at 4°C to obtain the post-mitochondrial fraction. The resulting supernatant was carefully collected and preserved for antioxidant enzyme assays.

### 3.61 Protein Determination

Total protein content in liver homogenates was quantified using a modified Biuret reaction. This assay is based on the formation of a violet-colored complex between copper ions and peptide bonds under alkaline conditions. Absorbance readings were obtained at 540 nm using a spectrophotometer, and protein concentrations were extrapolated from a standard calibration curve prepared with bovine serum albumin.

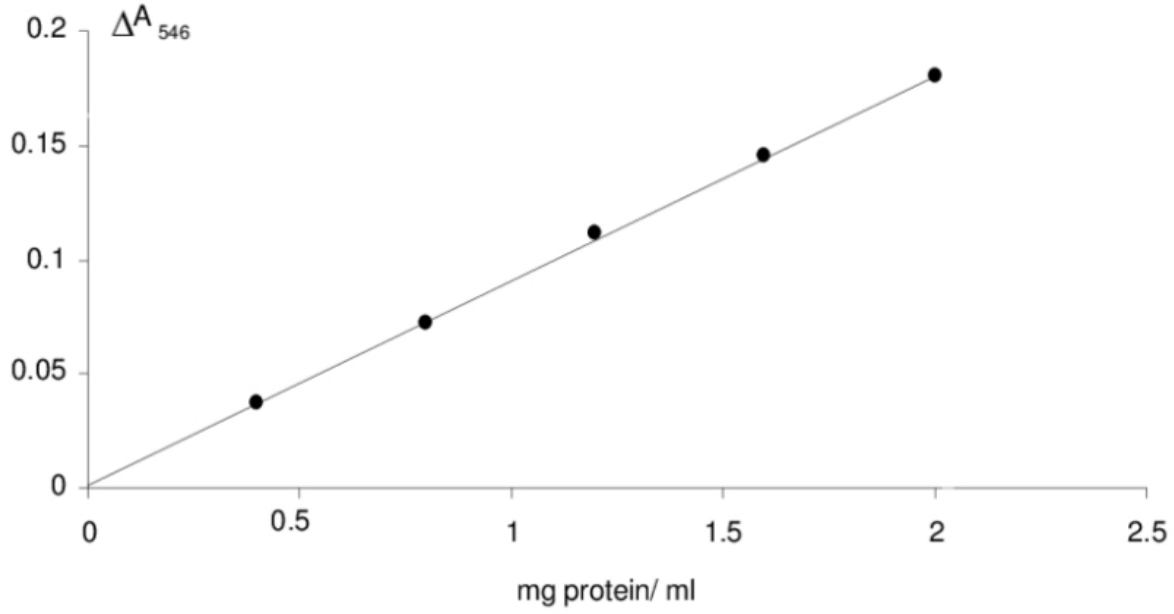


**Figure 8: Standard curve for protein determination by Biuret`s method**

### 3.62 ESTIMATION OF REDUCED GLUTATHIONE (GSH) LEVEL

Reduced glutathione levels were assessed using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid). In this reaction, sulfhydryl groups present in glutathione interact with the reagent to produce a yellow chromophore measurable at 412 nm. Concentrations were calculated using a standard curve derived from known GSH standards.

### 3.63 ESTIMATION OF GLUTHATHIONE PEROXIDASE (GPx)

Glutathione peroxidase activity was evaluated by monitoring its catalytic role in reducing hydrogen peroxide and organic peroxides. Enzyme activity was determined spectrophotometrically and normalized relative to protein concentration in the sample.

### 3.64 Estimation of Catalase (CAT) Activity

Catalase activity was measured based on its ability to decompose hydrogen peroxide into water and oxygen. The rate of hydrogen peroxide breakdown was monitored spectrophotometrically over a fixed time interval. Enzyme activity was expressed per milligram of protein.

### 3.65 Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined using the epinephrine auto-oxidation method. Under alkaline conditions, epinephrine undergoes oxidation to form adrenochrome; SOD inhibits this process. The degree of inhibition was measured at 480 nm, and enzyme activity was expressed as the amount required to produce 50% inhibition of epinephrine oxidation.

### 3.7 Statistical Analysis

Experimental data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc testing where necessary. Statistical significance was accepted at $p < 0.05$. Analyses were conducted using SPSS software (version 16.0).

# CHAPTER FOUR

## RESULTS

This chapter presents the findings of the experimental study investigating the protective effects of rutin against sodium arsenite–induced oxidative stress in the liver of Wistar rats. The results are organized to show variations in key biochemical parameters, including reduced glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), across different experimental groups. Data are displayed in tables and figures, and statistical analyses were performed to assess the significance of the observed changes, highlighting the hepatoprotective potential of rutin.

### 4.1 Glutathione

The table below shows that sodium arsenite causes a severe reduction in liver reduced glutathione (GSH), dropping from normal control level (~64.19) to 21.26 units/mg protein, confirming strong oxidative stress. Rutin alone does not actually affect GSH, as seen in the similarities between the control and rutin group. However, when rutin is co-administrated with sodium arsenite, GSH levels significantly improve in a dose-dependent manner. the 25mg/kg dose partially restores GSH, while the 50mg/kg dose produce a stronger recovery, though still below control values. Overall, the results indicate that rutin effectively protects the liver by enhancing antioxidant defense against arsenite-induced oxidative stress

| **GSH (units/mg protein)** | **Grp A** | **Grp B** | **Grp C** | **Grp D** | **Grp E** |
|---|---|---|---|---|---|
| **Liver** | 64.19± 1.60 | 65.37± 1.28 | 21.26± 0.33 | 31.53± 0.62 | 40.4± 1.53 |

Grp A = Control, Grp B = Rutin, Grp C = Sodium Arsenite, Grp D = Sodium Arsenite + Rutin (25 mg/kg), Grp E = Sodium Arsenite + Rutin (50 mg/kg). Values are expressed as mean ± standard deviation; n = 7. *Significant compared with control ($p < 0.05$). a,b Significant compared with Sodium Arsenite ($p < 0.05$).

### 4.2 Glutathione S-Transferase

The GST results show that sodium arsenite markedly reduce glutathione S-transferase activity in the liver, dropping from about 59-60 nmole/min/mg protein in control and rutin only groups to 24.7 nmole/min/mg protein. This significant decrease indicates oxidative stress and detoxification capacity caused by arsenite. Co-treatment with rutin leads to a dose-dependent improvement: the 25mg/kg dole elevates GST to 35.2, while the 50 mg/kg dose further increases activity to 45.6. Although these values remain below control levels, they represent significant recovery compared to the arsenite group. Overall' findings shows that rutin helps restore GST activity, supportiung its protective antioxidant and detoxification-enhancing effects against sodium arsenite toxicity.

| **GST(nmoley/min/mg protein**) | **Grp A** | **Grp B** | **Grp C** | **Grp D** | **Grp E** |
|---|---|---|---|---|---|
| **Liver** | 59.30± 2.0 | 60.05± 1.85 | 24.7± 2.0 | 35.2± 3.2 | 45.6± 2.8 |

Grp A = Control, Grp B = Rutin, Grp C = Sodium Arsenite, Grp D = Sodium Arsenite + Rutin (25 mg/kg), Grp E = Sodium Arsenite + Rutin (50 mg/kg). Values are expressed as mean ± standard deviation; n = 7. *Significant compared with control ($p < 0.05$). a,b Significant compared with Sodium Arsenite ($p < 0.05$).

## 4.3 GLUTATHIONE PEROXIDASE ENZYME (GPX)

The GPx results show a sharp contrast to the other antioxidant markers. While the control and rutin-only group have low, normal GPx activity (~2.2-3.0 µg/mol/mg protein), sodium arsenite exposure causes a dramatic increase to 12.43. This spike reflects a compensatory response, where liver upregulates GPx in reaction to severe oxidative stress. Rutin co-treatment reduces this elevated GPx activity in a dose-dependent manner. the 25 mg/kg dose lowers GPx to 10.6, while the 50 mg/kg dose brings it further down to 7.08. Although still higher than control values, these reductions indicate that rutin helps normalize oxidative stress levels, reducing the need for extreme GPx upregulation.

| **GPX (µg/mol/mg protein)** | **Grp A** | **Grp B** | **Grp C** | **Grp D** | **Grp E** |
|---|---|---|---|---|---|
| **Liver** | 2.21± 0.22 | 2.96± 0.48 | 12.43± 1.13 | 10.61±1 .15 | 7.08± 0.12 |

Grp A = Control, Grp B = Rutin, Grp C = Sodium Arsenite, Grp D = Sodium Arsenite + Rutin (25 mg/kg), Grp E = Sodium Arsenite + Rutin (50 mg/kg). Values are expressed as mean ± standard deviation; n = 7. *Significant compared with control ($p < 0.05$). a,b Significant compared with Sodium Arsenite ($p < 0.05$).

## 4.4 CATALASE

The catalase activity result show that sodium arsenite markedly impairs the liver's antioxidant defense. Catalase levels fall sharply from over 126-130 µmol $H_2O_2$ consumed/min/mg protein in the control and rutin-only groups to just 51.35 in the sodium arsenite group, indicating significant oxidative stress and reduced enzymatic detoxification of hydrogen peroxide. Co-administration of rutin leads to a dose-dependent restoration of catalase activity. the 25 mg/kg dose increases activity to 62.57, while the 50mg/kg dose raises it further to 102.0. Although still below control values, these improvements demonstrate that rutin partially reverses arsenite-induced oxidative damage and strengthens the catalase-mediated antioxidant system in the liver.

| **CAT (µmol $H_2O_2$ consumed/min/mg protein)** | **Grp A** | **Grp B** | **Grp C** | **Grp D** | **Grp E** |
|---|---|---|---|---|---|
| **Liver** | 126.04± 2.34 | 130.43± 2.73 | 51.35± 2.18 | 62.57± 1.02 | 102.0±12.78 |

Grp A = Control, Grp B = Rutin, Grp C = Sodium Arsenite, Grp D = Sodium Arsenite + Rutin (25 mg/kg), Grp E = Sodium Arsenite + Rutin (50 mg/kg). Values are expressed as mean ± standard deviation; n = 7. *Significant compared with control ($p < 0.05$). a,b Significant compared with Sodium Arsenite ($p < 0.05$).

### 4.5 Superoxide Dismutase

The SOD results show that sodium arsenite significantly suppresses superoxide dismutase activity in the liver, reducing it from about 0.375-0.399 units/mg protein in the control and rutin-only groups to 0.102 units/mg protein. This sharp decline indicates impaired enzymatic defense against superoxide radicals and confirms strong oxidative stress. Co-treatment with rutin improves SOD activity in a dose-dependent manner: the 25 mg/kg dose increases SOD to 0.147, while the 50 mg/kg dose further elevates it to 0.198. Although these values do not fully reach control levels, the significant recovery demonstrates that rutin helps restore antioxidant capacity and mitigates arsenic-induced oxidative damage in hapatic tissues.

| **SOD (units/mg protein)** | **Grp A** | **Grp B** | **Grp C** | **Grp D** | **Grp E** |
|---|---|---|---|---|---|
| **Liver** | 0.375± 0.02 | 0.399± 0.05 | 0.102± 0.02 | 0.147± 0.03 | 0.198±0.05 |

Grp A = Control, Grp B = Rutin, Grp C = Sodium Arsenite, Grp D = Sodium Arsenite + Rutin (25 mg/kg), Grp E = Sodium Arsenite + Rutin (50 mg/kg). Values are expressed as mean ± standard deviation; n = 7. *Significant compared with control ($p < 0.05$). a,b Significant compared with Sodium Arsenite ($p < 0.05$).

# CHAPTER FIVE

## 5.1 Discussion

The present investigation evaluated the protective potential of rutin against sodium arsenite-induced hepatic oxidative damage in Wistar rats. The findings demonstrate that arsenite exposure significantly disrupted antioxidant homeostasis, while co-administration of rutin mitigated these alterations in a dose-dependent manner.

Administration of sodium arsenite alone resulted in marked suppression of endogenous antioxidant defense mechanisms. The observed decline in reduced glutathione (GSH) concentration, alongside decreased activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), indicates severe oxidative imbalance. These changes reflect an overproduction of reactive oxygen species (ROS) that overwhelms cellular detoxification systems. Similar patterns of antioxidant depletion following arsenic exposure have been documented in earlier studies (Ganie *et al.,* 2024; Yang *et al.,* 2024; Khan *et al.,* 2022).

The reduction in GSH levels is particularly significant because glutathione plays a central role in maintaining intracellular redox equilibrium. Its depletion suggests excessive utilization in neutralizing free radicals generated by arsenite metabolism. Furthermore, impairment of enzymatic antioxidants such as SOD and CAT compromises the conversion of superoxide radicals and hydrogen peroxide into less harmful intermediates, thereby promoting lipid peroxidation and cellular injury (Thakur *et al.,* 2021; Fatemi *et al.,* 2021).

Histopathological observations further corroborated the biochemical findings. Sodium arsenite-treated rats exhibited structural alterations consistent with hepatocellular damage, including

inflammatory infiltration and degenerative changes. These morphological disruptions are consistent with reports describing arsenic-induced hepatic degeneration mediated through oxidative stress mechanisms (Ding *et al.,* 2022; Mirzaei *et al.,* 2023).

In contrast, rutin administration demonstrated significant protective effects. Rats co-treated with rutin showed restoration of antioxidant enzyme activities and improved glutathione levels compared to arsenite-only treated animals. This improvement suggests that rutin enhances cellular antioxidant capacity, thereby limiting oxidative injury. The hepatoprotective effect observed in this study aligns with previous findings highlighting the antioxidant and cytoprotective properties of rutin in toxin-induced organ damage models (Khan *et al.,* 2022).

The protective mechanism of rutin may be attributed to multiple biochemical pathways. As a polyphenolic flavonoid, rutin possesses strong free radical scavenging activity, enabling direct neutralization of reactive oxygen species. Additionally, rutin has been shown to modulate redox-sensitive transcription factors, including activation of the Nrf2/HO-1 signaling pathway and suppression of NF-κB-mediated inflammatory responses (Tian *et al.,* 2022; Rahmani *et al.,* 2023; Rakshit *et al.,* 2021). Through these mechanisms, rutin enhances antioxidant gene expression while simultaneously reducing inflammatory cascades that exacerbate tissue injury.

Dose-dependent responses observed in the co-treatment groups further support the biological relevance of rutin's protective action. The higher dose demonstrated greater normalization of antioxidant parameters compared to the lower dose, suggesting that the magnitude of hepatoprotection may be directly related to rutin concentration. Such dose-responsive effects have also been reported in related experimental models evaluating flavonoid-mediated protection against oxidative toxins (Goyal *et al.,* 2023; Calabrese *et al.,* 2024; Henrotin *et al.,* 2022).

Collectively, the results of this study reinforce the central role of oxidative stress in arsenite-induced hepatotoxicity and demonstrate that rutin effectively attenuates this damage by restoring antioxidant balance. While the current findings provide strong biochemical evidence of protection, further investigations involving molecular assays and long-term exposure models may provide deeper insights into the mechanistic pathways involved.

Overall, the study supports the therapeutic potential of rutin as a natural antioxidant agent capable of mitigating arsenic-induced hepatic injury. Its ability to modulate redox signaling and preserve structural integrity highlights its promise as a complementary strategy in managing heavy metal toxicity.

### 5.2 Conclusion

Sodium arsenite exposure causes significant oxidative stress in the liver of Wistar rats, marked by the depletion of GSH and the impairment of antioxidant enzymes (GST, GPx, CAT, SOD). Rutin supplementation successfully reduces these harmful effects, restoring antioxidant capacity in a dose-dependent way and indicating a protective mechanism that involves ROS scavenging and boosting the body's natural antioxidant defenses. These findings demonstrate rutin's potential as a natural therapeutic agent for treating arsenic-induced hepatotoxicity and support further research into its clinical and pharmacological uses.

## REFERENCES


Ahmed, O. M., Elkomy, M. H., Fahim, H. I., Ashour, M. B., Naguib, I. A., Alghamdi, B. S., Mahmoud, H. U. R., and Ahmed, N. A. (2022). Rutin and quercetin counter doxorubicin-induced liver toxicity in Wistar rats via their modulatory effects on inflammation, oxidative stress, apoptosis, and Nrf2. *Oxidative Medicine and Cellular Longevity*, 2022, 2710607.

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., and Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, 48(3), 611–633.

Calabrese, E. J., Pressman, P., Hayes, A. W., Dhawan, G., Kapoor, R., Agathokleous, E., and Calabrese, V. (2024). RUTIN, a widely consumed flavonoid, that commonly induces hormetic effects. *Food and Chemical Toxicology*, 187, 114626.

Chen, Q. Y., and Costa, M. (2021). Arsenic: A global environmental challenge. *Annual Review of Pharmacology and Toxicology*, 61(1), 47–63. https://doi.org/10.1146/annurev-pharmtox-031920-111805

Ding, G., Huang, J., Lin, Q., Xia, R., Wu, S., and Zhang, L. (2022). Effects of chronic exposure to sodium arsenite on liver damage in rats. *Journal of Preventive Medicine*, 887–892.

Fatemi, I., Khalili, H., Mehrzadi, S., Basir, Z., Malayeri, A., and Goudarzi, M. (2021). Mechanisms involved in the possible protective effect of chrysin against sodium arsenite-induced liver toxicity in rats. *Life Sciences*, 267, 118965.

Ganie, S. Y., Javaid, D., Hajam, Y. A., and Reshi, M. S. (2024). Arsenic toxicity: sources, pathophysiology and mechanism. *Toxicology Research*, 13(1), tfad111.

Gaur, Y. K., Kumar, S., Gupta, J. K., Singh, K., Saha, S., and Mukherjee, S. (2024). Impact of the Rutin on Diabetic Complications. *Current Functional Foods*, 2(1), e030823219378.

Gora, R. H., Kerketta, P., Baxla, S. L., Toppo, R., Prasad, R., Patra, P. H., and Roy, B. K. (2014). Ameliorative effect of Tephrosia purpurea in arsenic-induced nephrotoxicity in rats. *Toxicology International*, 21(1), 78–83. https://doi.org/10.4103/0971-6580.128792

Goyal, J., and Verma, P. K. (2023). An overview of biosynthetic pathway and therapeutic potential of rutin. *Mini Reviews in Medicinal Chemistry*, 23(14), 1451–1460.

Goyal, S., Thirumal, D., Singh, S., Kumar, D., Singh, I., Kumar, G., and Sindhu, R. K. (2025). Basics of antioxidants and their importance. Antioxidants: *Nature's Defense Against Disease*, 1–20.

Henrotin, Y. E., Michlmayr, C., Rau, S. M., Quirke, A.-M., Bigoni, M., and Ueberall, M. A. (2022). Combination of enzymes and rutin to manage osteoarthritis symptoms: Lessons from a narrative review of the literature. *Rheumatology and Therapy*, 9(5), 1305–1327.

Ijaz, M. U., Jabeen, F., Ashraf, A., Imran, M., Ehsan, N., Samad, A., Saleemi, M. K., and Iqbal, J. (2022). Evaluation of possible protective role of chrysin against arsenic-induced nephrotoxicity in rats. *Toxin Reviews*, 41(4), 1237–1245. https://doi.org/10.1080/15569543.2020.1836920

Khan, S. S., Sharma, A., and Flora, S. J. S. (2022). Phytochemicals in the management of arsenic toxicity. *Chemical Research in Toxicology*, 35(6), 916–934.

Kıran, T. R., Otlu, O., and Karabulut, A. B. (2023). Oxidative stress and antioxidants in health and disease. *Journal of Laboratory Medicine,* 47(1), 1–11.

Mirzaei, M., Moosavi, M., Mansouri, E., Mohtadi, S., and Khodayar, M. J. (2023). Diosmin exerts hepatoprotective and antihyperglycemic effects against sodium arsenite-induced toxicity through the modulation of oxidative stress and inflammation in mice. *Journal of Trace Elements in Medicine and Biology*, 78, 127154.

Rahmani, S., Naraki, K., Roohbakhsh, A., Hayes, A. W., and Karimi, G. (2023). The protective effects of rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. *Food Science & Nutrition*, 11(1), 39–56.

Rakshit, S., Shukla, P., Verma, A., Nirala, S. K., and Bhadauria, M. (2021). Protective role of rutin against combined exposure to lipopolysaccharide and D-galactosamine-induced dysfunctions in liver, kidney, and brain: *Hematological, biochemical, and ... Journal of Food Biochemistry*, 45(2), e13605.

Thakur, M., Rachamalla, M., Niyogi, S., Datusalia, A. K., and Flora, S. J. S. (2021). Molecular mechanism of arsenic-induced neurotoxicity including neuronal dysfunctions. International Journal of Molecular Sciences, 22(18), 1007Tian, C., Shao, Y., Jin, Z., Liang, Y., Li, C., Qu, C., Sun, S., Cui, C., & Liu, M. (2022). The protective effect of rutin against lipopolysaccharide induced acute lung injury in mice based on the pharmacokinetic and pharmacodynamic combination model. *Journal of Pharmaceutical and Biomedical Analysis*, 209, 114480.

Tian, C., Shao, Y., Jin, Z., Liang, Y., Li, C., Qu, C., Sun, S., Cui, C., and Liu, M. (2022). The protective effect of rutin against lipopolysaccharide induced acute lung injury in mice

based on the pharmacokinetic and pharmacodynamic combination model. *Journal of Pharmaceutical and Biomedical Analysis*, 209, 114480.