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Hepatorenal protective effect of rutin against sodium arsenite-induced oxido-inflammatory stress and Nrf-2/HO-1 pathway

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ABSTRACT

Background: Arsenic is a persistent environmental contaminant that poses a serious public health risk due to its well-documented hepatotoxic and nephrotoxic properties. Exposure to sodium arsenite ($NaAsO_2$), a soluble inorganic arsenical compound, induces multi-organ injury through mechanisms involving oxidative stress, NF-κB-driven inflammation, and dysregulation of the Nrf2/HO-1 antioxidant pathway. **Objective:** This study investigated the hepatorenal protective effects of rutin (RUT) against $NaAsO_2$-induced hepatorenal toxicity in male rats, and sought to elucidate the underlying mechanisms with specific reference to oxidative stress, inflammatory signalling, and the Nrf2/HO-1 and NF-κB pathways. **Materials and Methods:** Forty male Wistar rats were randomly allocated into five groups (n = 8 per group) and treated orally for 14 days as follows: Group I, vehicle control; Group II, RUT alone (50 mg/kg); Group III, $NaAsO_2$ alone (10 mg/kg); Group IV, $NaAsO_2$ (10 mg/kg) + RUT (25 mg/kg); and Group V, $NaAsO_2$ (10 mg/kg) + RUT (50 mg/kg). Hepatotoxicity and nephrotoxicity were evaluated using serum biochemical markers (ALP, ALT, urea, and creatinine), histopathological examination of liver and kidney tissues, and biochemical assessment of oxidative stress indices (MDA, RONS, NO, and GSH) and inflammatory signalling proteins (NF-κB, RANTES, and Nrf2/HO-1). **Results:** $NaAsO_2$ exposure significantly impaired hepatic and renal redox homeostasis, markedly elevating lipid peroxidation (MDA), reactive oxygen and nitrogen species (RONS), nitric oxide (NO), NF-κB, and RANTES, while depleting GSH content and suppressing the Nrf2/HO-1 pathway. These biochemical perturbations were accompanied by significant histopathological alterations in both liver and kidney tissues. Co-administration of RUT dose-dependently and significantly ameliorated all these changes, suppressing NF-κB activation and its downstream pro-inflammatory targets, restoring GSH levels, and upregulating Nrf2/HO-1 expression. Histological examination confirmed that RUT markedly attenuated $NaAsO_2$-induced structural disruption in hepatic and renal tissues. **Conclusion:** Rutin confers potent hepatorenal protection against $NaAsO_2$-induced toxicity through coordinated free-radical scavenging, NF-κB-mediated suppression of inflammation, and activation of the Nrf2/HO-1 antioxidant pathway. These findings suggest that RUT represents a promising therapeutic candidate for mitigating arsenic-induced hepatorenal organ damage.

Keywords: Rutin, Sodium Arsenite, NF-κB, Nrf2/HO-1 Pathway, Oxidative Stress, Hepatorenal Injury.

INTRODUCTION

Arsenic compounds are well-known toxins that are found freely in the environment, particularly in groundwater. Studies have shown that exposure to arsenic occurs through multiple routes, including oral and occupational exposure [1,2]. Arsenic compounds are one of the predominant substances used in the production of herbicides, insecticides, and food preservatives, and are often found as by-products in fossil fuels [3]. Both clinical trials and laboratory experiments show that arsenic is toxic to the skin, kidney, and liver [4]. Also, sodium arsenite has been found to damage the uterus and prostate in humans [4,5]. Sodium arsenite ($NaAsO_2$) metabolism promotes oxido-inflammatory stress through the generation of reactive oxygen species (ROS) and free radicals. These agents can initiate cellular damage via lipid peroxidation and DNA adduct formation, leading to multi-organ failure affecting the hepatic, renal, cardiac, and central nervous systems [6,7]. Oxidative stress associated with $NaAsO_2$ metabolism can inhibit antioxidant enzyme activities and diminish reduced glutathione concentration in the cell. Arsenic compounds can increase ROS production, creating an environment in which oxidative stress exacerbates

inflammation and induces organ dysfunction [8,9]. Owing to their role in metabolic activities and waste removal, the hepatorenal system is the ideal system to study $NaAsO_2$ toxicity in animal model [10].

Growing evidence suggests that bioactive plant molecules may be effective cytoprotective agents against oxidative and systemic damage caused by arsenic. Furthermore, given that many modern pharmaceuticals are derived from natural sources, the use of phytochemicals to mitigate xenobiotic-induced tissue damage is an ideal therapeutic strategy [11]. Rutin (rutoside) is a natural flavonoid characterized by its distinct powdered yellow appearance.

Evidence has shown that its pharmacological profile is mediated by the four hydroxyl groups and the rutinose moiety at the C-3 position, which allows it to effectively scavenge free radicals [11]. Rutin possesses a wide array of medicinal properties, specifically acting as an antioxidant, anti-inflammatory agent, lowering blood pressure, and conferring cardio protection. Additionally, rutin multi-targeted therapeutic properties include neuroprotective, antidiabetic, and can inhibit ROS and trigger apoptosis in pancreatic cells [12 – 17]. While the therapeutic properties of rutin are well documented, its specific effect at low doses on acute arsenic-mediated hepatorenal damage has not been established. The present investigation, therefore, aims to elucidate the mitigatory role of rutin against liver and kidney injury induced by sodium arsenite in male rats.

MATERIAL AND METHODS

Reagents and kits

Rutin from AmBeed Scientific (ArlingtonHts, IL60004, USA) CAS (153-18-4), Sodium arsenite from Molychem (Mumbai, India) CAS (7784-46-5). Hepatorenal function markers were determined by measuring the release of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, and creatinine using a commercially available Randox kit (Ardmore, UK) according to the manufacturer's instructions. RANTES (CSB-E07398), NF-kB (CSB-E13539), iNOS (CSB-E08325), COX-2 (CSB-E13399), NRF-2 (CSB-EL015752) AND HO-1 (CSB-E08267) from Cusabio technology (Wuhan, China). Trichloroacetic acid (TCA), thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), and dipotassium phosphate (K_2HPO_4) were bought from BDH (Poole, United Kingdom).

Experimental animals

Forty male albino rats with a weight of 185±5 g were bought from the animal colony of the Anatomy Department, University of Benin, Nigeria. The rats were allowed a 7-day acclimation under standard photoperiod conditions. Fresh drinking water and rat chow were adequately provided for their nourishment. Following approval from the University of Benin Ethics Committee (APP No. LS25046), guidance was provided in accordance with the NIH Procedural Manual for animal handling and care.

Treatment and design

The animals were subjected to the following treatment regimen for 14 days:
Control group: Received distilled water (2 mL/kg) via oral gavage daily.
Rutin-only group: Received rutin at a dose of 50 mg/kg orally.
$NaAsO_2$-only group: Received sodium arsenite at a dose of 10 mg/kg via oral gavage.
$NaAsO_2$ + RUT 1 group: Received a combination of rutin (25 mg/kg) and sodium arsenite (10 mg/kg) via oral gavage.
$NaAsO_2$ + RUT 2 group: Received a combination of rutin (50 mg/kg) and sodium arsenite (10 mg/kg) via oral gavage.

Sodium arsenite was dissolved in distilled water, while rutin was prepared in warm distilled water with a few drops of dimethyl sulfoxide (DMSO) to aid dissolution.

Animal sacrifice

Following a 24 h post treatment period, animals were weighed and euthanized via cervical dislocation under the influence of ketamine/xylazine (100/10 mg/kg). Cardiac blood was collected, and the liver and kidneys were harvested. These tissues were further homogenized in ice-cold buffer and centrifuged at 12,000 g for 15 min at 4 °C. The resultant supernatants were stored at -20 °C for subsequent biochemical assays.

Liver and kidney function examination

Liver enzymes (AST, ALT, and ALP) and renal markers (urea and creatinine) were determined using a commercial kit from Randox Technology (Ardmore, UK).

Determination of oxidative stress biomarkers

Oxidative stress biomarkers in the hepatorenal supernatant, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) activities, reduced glutathione (GSH), and lipid peroxidation (LPO) levels, were assayed using established spectrophotometric methods. Briefly, SOD activity [18] was measured at 480 nm via epinephrine oxidation in carbonate buffer (pH 10.2). CAT activity [19] was determined by monitoring H_2O_2 breakdown at 240 nm. The reaction mixture consists of 1.8 mL of phosphate buffer (50 mM, pH 7.0), 180 µL of H_2O_2 (300 mM), and 20 µL of supernatant. The rate of H_2O_2 decomposition was measured at 240 nm over a 3 min period. Following the protocol of Habig *et al* [20], GST activity was determined by adding 20 µL of supernatant to a reaction mix containing GSH and CDNB in phosphate buffer (pH 7.0). The change in absorbance was recorded at 340 nm over 5 min. GPX activity was assessed according to Rotruck *et al* [21], by incubating 20 µL of supernatant with a reaction mixture containing phosphate buffer, GSH, sodium azide, and H_2O_2 at 37 °C. After 3 min, the reaction was stopped with TCA and spun at 3000 rpm for 5 min. The residual GSH was then determined by adding K_2HPO_4 and DTNB to the resulting supernatant, and the absorbance was measured spectrophotometrically at 412 nm. Reduced glutathione (GSH) was determined following Jollow *et al* [22]. Hepatorenal samples were precipitated with TCA and centrifuged (3000 rpm, 5 min). The resultant supernatant was incubated with Ellman's reagent in phosphate buffer, and absorbance was measured at 412 nm after 15 min. Lipid peroxidation (LPO) was quantified as malondialdehyde (MDA) levels, as described by Adedara *et al* [23]. Briefly, 100 µL of hepatorenal supernatant was heated at 85 °C for 1 h with a reaction cocktail (10% TCA, 0.75% TBA, and 0.1 M HCl). After cooling and centrifugation, the absorbance of the supernatant was measured at 532 nm.

Determination of reactive oxygen and nitrogen species levels

Reactive oxygen and nitrogen species (RONS) levels in the hepatorenal system were quantified by measuring the oxidation of 2'7'-dichlorohydrofluorescin diacetate (DCFH-DA) to the fluorescent compound DCF [24,25]. Succinctly, 10 µL of hepatorenal homogenate was incubated with 5 µL of DCFH-DA (5 µM as the final concentration) in 0.1 M potassium phosphate buffer (pH 7.4). DCF fluorescence was monitored (excitation wavelength 488 nm; emission wavelength 525 nm) for 10 min at 30-second intervals using a plate reader. The results were expressed as a percentage of the control group.

Assessment of pro-inflammatory indices

Myeloperoxidase (MPO) activity and nitric oxide (NO) levels were determined using established protocols. MPO activity was assessed

according to Bradly *et al* [26], while NO concentration was quantified as nitrite using Griess reagent [27]. Furthermore, regulated on activation, normal T-cell expressed and secreted (RANTES), inducible nitric oxide synthase (iNOS), cyclooxygenase -2 (COX-2), and nuclear factor kappa-light chain enhancer of activated B cells (NF-kB) were evaluated in the hepatorenal samples with ELISA kits (Cusabio, China) according to the manufacturer's protocol.

Determination of molecular antioxidant defense system

According to the manufacturer's instructions, the levels of the antioxidant genes nuclear factor erythroid 2-related factor 2 (NRF-2) and heme oxygenase-1 (HO-1) were estimated in liver and kidney tissue using ELISA kits (Cusabio, China).

Assessment of histopathology of the hepatorenal samples

Following excision of the organs, representative liver and kidney samples were fixed in 10% phosphate buffered formalin. After 48 h, the tissues were subjected to the standard laboratory tissue processing sequence: dehydration, xylene clearing, and paraffin embedding. Sections were cut at 5 µm using a rotary microtome and stained with hematoxylin and eosin. Histopathological evaluation was subsequently performed under a light microscope by a pathologist unaware of the treatment groups.

Data analysis

The experimental groups were compared using GraphPad PRISM 8 with one-way analysis of variance and Bonferroni's post hoc test. $P < 0.05$ was used to determine statistical significance.

RESULTS

Dietary rutin (RUT) abates $NaAsO_2$ –induced dose dependent spike in hepatorenal function biomarkers

The effect of RUT on the serum ALT, ALP, AST, urea, and creatinine levels. Figure 1 data showed that $NaAsO_2$ administration significantly increased hepatorenal toxicity biomarkers in rats relative to controls ($p < 0.05$). However, administration of dietary RUT 25 and 50 mg/kg to $NaAsO_2$-exposed rats markedly decreased the $NaAsO_2$ –associated spike in serum enzymes in a graded dose pattern.

RUT ameliorated $NaAsO_2$ –induced oxidative stress and RONs generation

The protective effects of RUT on oxidative stress indices and RONS were observed. Figure 2A and 2B demonstrated the influence of RUT on oxidative stress parameters, level of RONS, and antioxidant enzymes in the hepatorenal system of $NaAsO_2$ –challenged rats. $NaAsO_2$ exposure caused a significant ($p < 0.05$) decrease in the activities of CAT, SOD, GST, and GPX in the liver and kidney of rats. Furthermore, GSH levels were markedly diminished, with a concomitant significant increase in LPO and RONS concentrations. However, RUT administration significantly reversed the observed hepatorenal oxidative damage, as evidenced by a diminution in LPO and RONS concentrations relative to the $NaAsO_2$-only cohort.

RUT abrogated the $NaAsO_2$–induced increase in the level of inflammatory and pro-inflammatory cytokines

The chemopreventive effect of RUT on $NaAsO_2$-induced inflammation on the hepatorenal system. Figures 3 and 4 indicate the preventive role of RUT in reducing inflammation in $NaAsO_2$–treated animals. In the $NaAsO_2$-only group, the hepatorenal tissues showed a marked increase in inflammation, as evidenced by elevated MPO activity and by concentrations of nitrite, RANTES, NF-κB, iNOS, and COX-2 compared with controls. However, the administration of RUT at 25 and 50 mg/kg significantly suppressed the $NaAsO_2$ –induced increase in MPO activity, nitrite, RANTES, NF-kB, iNOS, and COX-2 concentration when compared with the RUT-only group.

RUT improves the levels of antioxidant enzyme regulator in $NaAsO_2$ – induced hepatorenal dysfunction

The level of NRF-2 and HO-1 in the liver and kidney of rats treated with $NaAsO_2$ and RUT is depicted in Figure 5. In $NaAsO_2$-treated rats only, the hepatorenal concentrations of NRF-2 and HO-1 were significantly lower than in the control cohort ($p < 0.05$). However, the administration of dietary RUT at 25 and 50 mg/kg caused a marked increase in the hepatorenal concentration of NRF-2 and HO-1 signaling when compared to the $NaAsO_2$-only treated group.

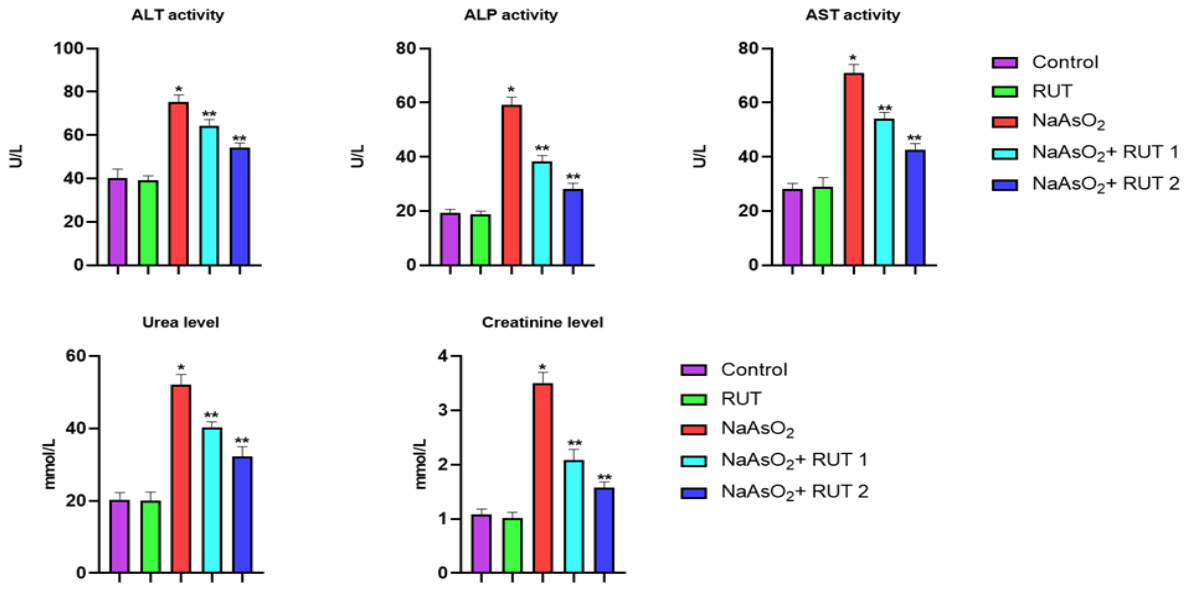

Figure 1: Effect of RUT on hepatorenal serum enzyme activities in the liver and kidney of $NaAsO_2$-exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent reversal of serum enzyme markers

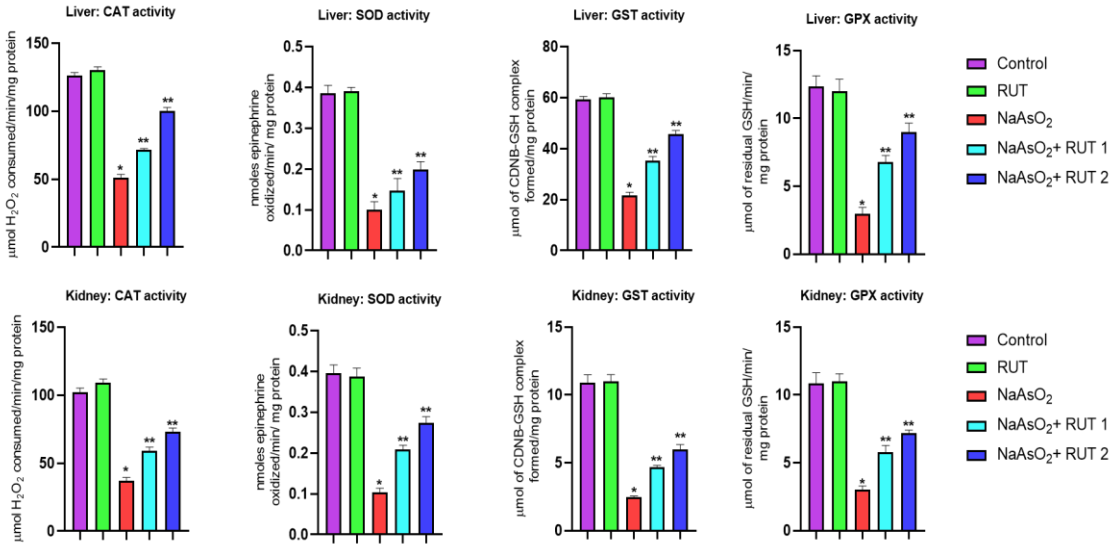

Figure 2A: Effect of RUT on CAT, SOD, GST and GPX activities in the liver and kidney of $NaAsO_2$-exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent recovery in CAT, SOD, GST and GPX activities

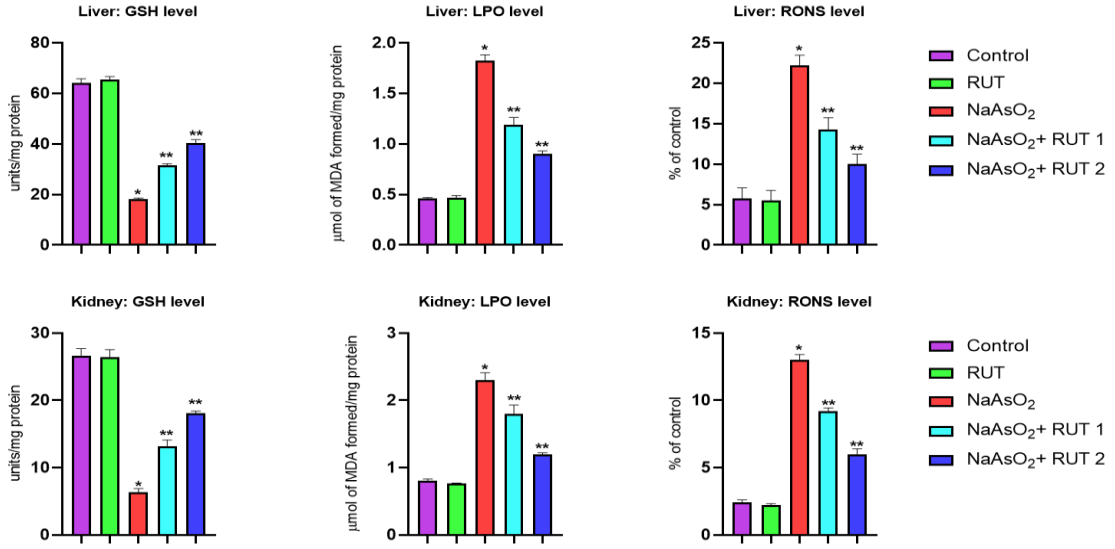

Figure 2B: Effect of RUT on GSH, LPO, and RONS levels in the liver and kidney of $NaAsO_2$-exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent increase in GSH, and aborted LPO, and RONS levels

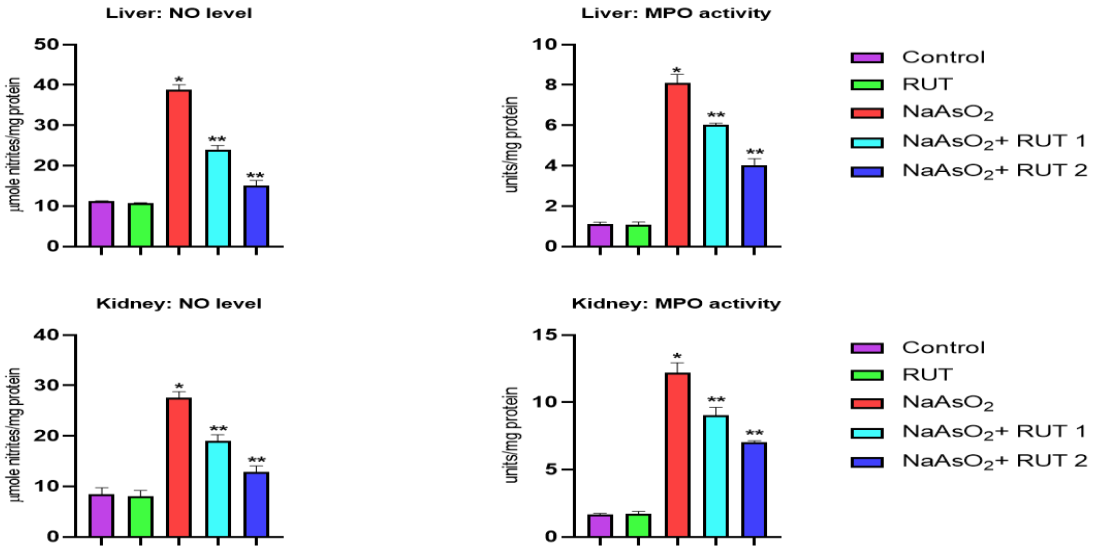

Figure 3: Effect of RUT on nitrite level concentration and MPO activities in the liver and kidney of $NaAsO_2$-exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent decrease in nitrite level concentration and MPO activities

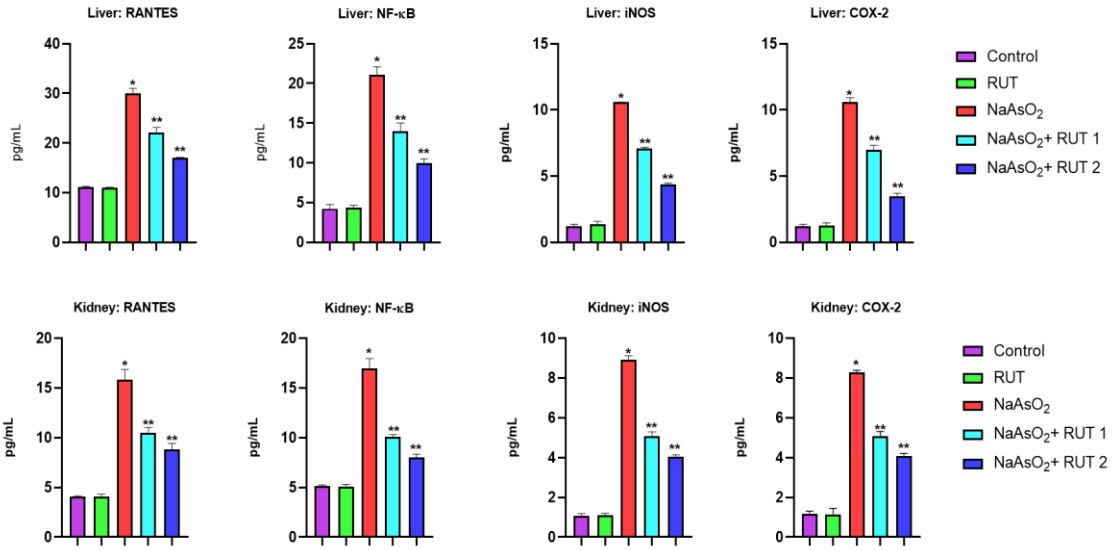

Figure 4: Effect of RUT on the concentration of RANTES, NF-κB, iNOS and COX-2 in the liver and kidney of $NaAsO_2$ -exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent decrease in RANTES, NF-κB, iNOS and COX-2

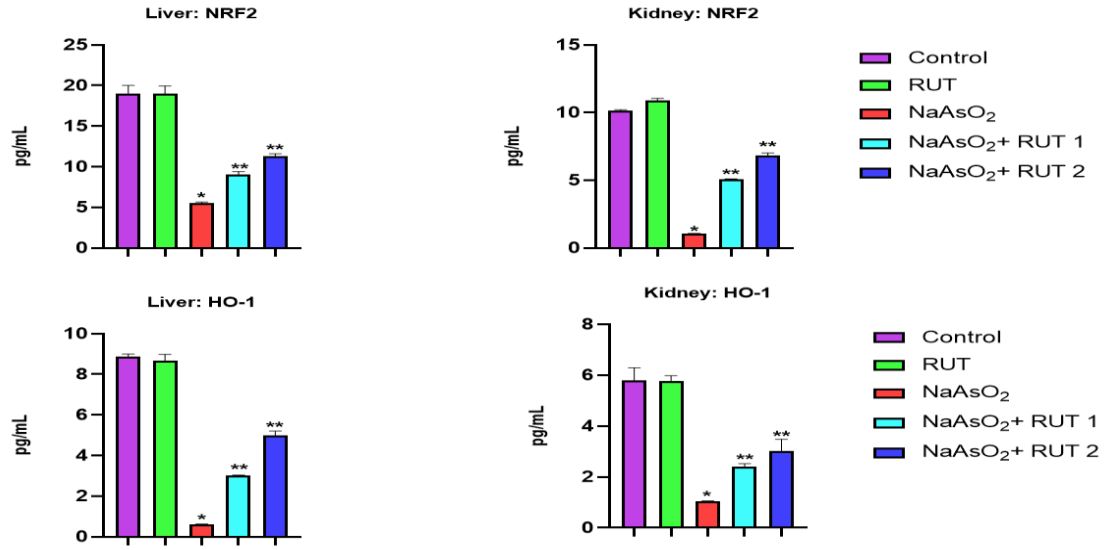

Figure 5: Effect of RUT on NRF2 and HO-1 concentration in the liver and kidney of $NaAsO_2$ -exposed rats. Data shows mean ± SD of 8 rats. *Significantly differs from control ($p < 0.05$); **significantly differs from $NaAsO_2$ alone ($p < 0.05$). Administration of RUT (50 mg/kg) elicited a dose-dependent recovery in NRF2 and HO-1 concentration

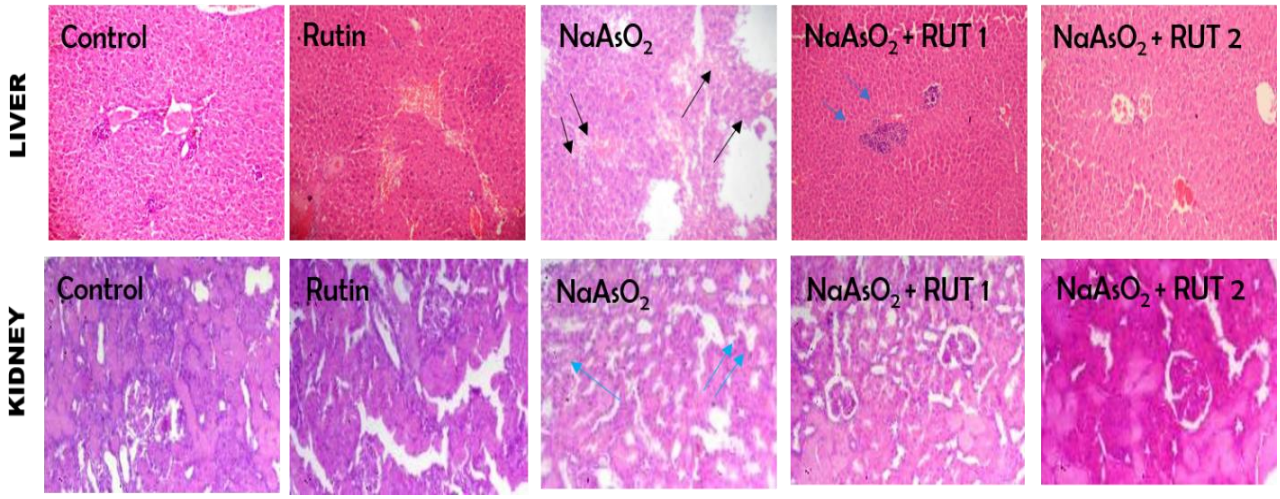

Figure 6: Representative photomicrographs of the liver and kidneys show that control rats and rats treated with RUT alone exhibit normal histological architecture in both organs. In contrast, rats treated with $NaAsO_2$ alone displayed severe disseminated congestion and marked infiltration of inflammatory cells in the liver and kidney. However, cotreatment with RUT 1 or RUT 2 markedly ameliorated these pathological alterations, with both organs showing near-normal morphology

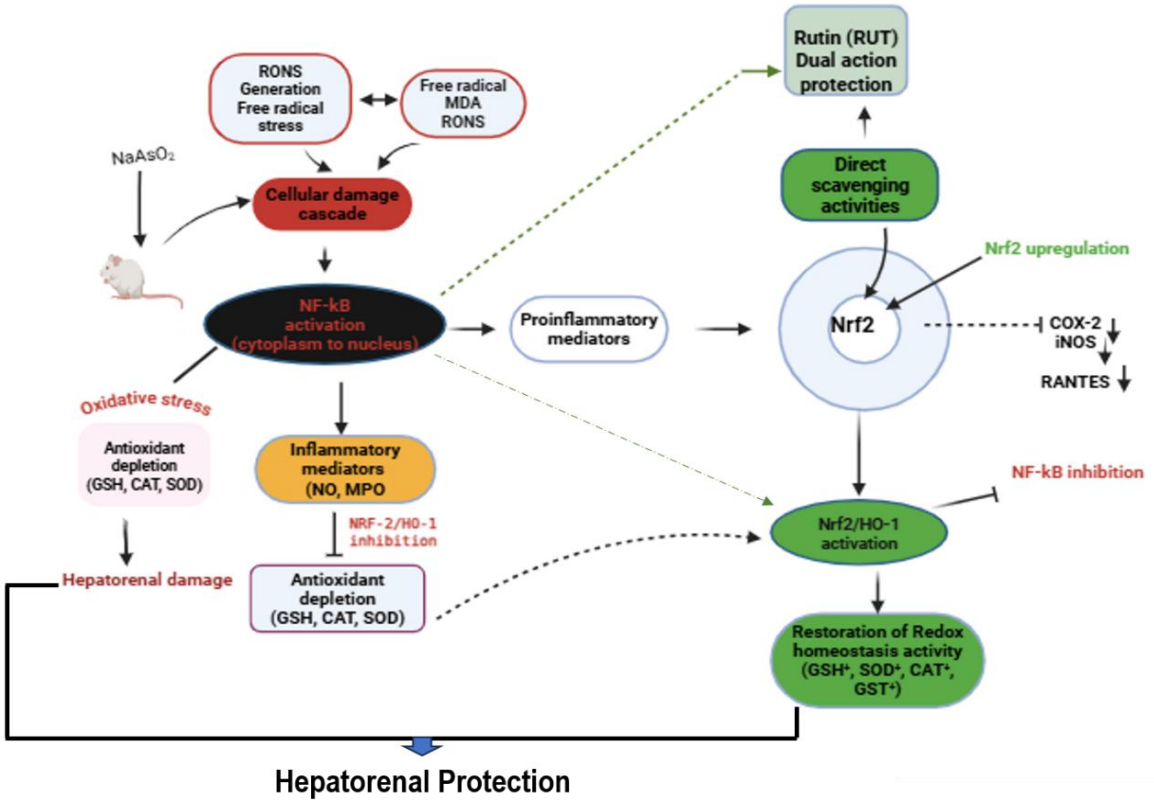

Figure 7: The proposed mechanistic pathway of $NaAsO_2$-induced hepatorenal toxicity and chemoprotective role of Rutin

Histopathological assessment of the hepatorenal architecture following $NaAsO_2$ and RUT treatment

Figure 6 shows liver and kidney histology for control group and other groups that received RUT and/or $NaAsO_2$. Histological examination of rats treated with RUT revealed preserved hepatic architecture characterized by intact central veins, tightly packed hepatic cords and clear sinusoidal spaces. Similarly, RUT administration maintained renal integrity, with a near-normal Bowman's space and healthy proximal and distal tubules. In contrast to the control group, $NaAsO_2$ exposure resulted in central vein disruption and dilated sinusoidal spaces in the liver. In a similar pattern, renal sections revealed distorted brush borders and degenerated macula densa.

DISCUSSION

Pesticides and metals represent common environmental contaminants that can alter human health [4,10]. The bioaccumulation of these pollutants in the cell can disrupt essential metabolic pathways at the cellular level [4]. The clinical and experimental acute arsenic poisoning targets multiple organ systems, resulting in hepatic and renal impairments, gastric distress, cardiovascular abnormalities and nervous system breakdown [8,9]. Despite the known systemic toxicity associated with arsenic, the exact pathological mechanism affecting the hepatorenal axis remains poorly understood. Our study therefore evaluated the low-dose-dependent protective effect of dietary RUT against $NaAsO_2$-induced oxidative injury in hepatic and renal tissues, specifically those involved in inflammation (RANTES, NF-kB, iNOS, COX-2) and antioxidant defense (NRF-2, HO-1), and to determine RUT's therapeutic efficacy. In this present study, we investigated the natural pharmacological activities of RUT against $NaAsO_2$-associated hepatorenal oxido-inflammatory damage and antioxidant enzyme regulator dysfunction in rats. Early indicators of hepatorenal health and function are measured in serum activities of ALP, AST, ALT, urea, and creatinine. Though urea and creatinine can be found in many cells within the body, their increased levels might point to impaired renal function [28]. The results from our study demonstrated that $NaAsO_2$ intoxication caused significant damage in the liver and kidney of rats, as observed in the unabated ALP, AST, and ALT functions, which corroborated the sustained increases seen in the urea and creatinine levels. However, RUT administration to rats restored hepatorenal health as evidenced by decreased activities of ALP, AST, ALT in the liver, and urea and creatinine in the kidney of the dose-dependent cotreated rats. Previously published reports have shown that polyphenolic compounds can perturb xenobiotic-induced increases in serum liver and kidney functions [29].

Enzymic antioxidants SOD and CAT are involved in the catalytic degradation of superoxide radicals into hydrogen peroxide and further neutralized to regular oxygen and water, thereby protecting cells from harmful toxic effects [30,31]. In the present study, we observed a marked decrease in SOD and CAT activities in the $NaAsO_2$-only-treated cohort, suggesting inhibited enzyme activity. Furthermore, we opined that harmful toxins, such as superoxide radicals and hydrogen peroxide, might have accumulated in the hepatorenal axis of $NaAsO_2$-only-treated rats. However, there was a significant dose-dependent augmentation to near normal in SOD and CAT activities in the liver and kidneys of rats co-exposed to $NaAsO_2$ and RUT supplementation, suggestive of RUT antioxidant ability. A similar trend was obtained by Akanda *et al* [32].

GSH, a known low-molecular-weight antioxidant, efficiently preserves the intracellular redox environment. GPX, which is a selenium-rich enzyme, can conjugate hydrogen peroxide and lipid hydroxides by reacting with GSH. Furthermore, GST facilitates cellular detoxification by catalyzing the conjugation of GSH to electrophilic xenobiotics [33-35]. As seen in the study, the significant diminution in the GSH concentration, as well as GST and GPX activities, could only suggest that these cellular antioxidant defense molecules were consumed while preserving the hepatorenal integrity of rats challenged with $NaAsO_2$ alone. Administration of RUT, however, in a dose-dependent manner significantly upregulated to near normal the status of these GST, GPX, and GSH concentrations.

$NaAsO_2$ alone treatment in our study increased oxidative stress in the hepatorenal tissues of challenged rats, as evidenced by a marked increase in MDA, a product of lipid peroxidation, and RONS levels compared to the control. Furthermore, arsenic compound intoxication

has been reported to generate RONS and lipid peroxides when in contact with the membrane lipids [35]. Also, previous studies have shown that the oxidation of arsine intermediates, a byproduct of arsenite metabolism, initiates a cascade of free radical chain reaction leading to lipid peroxidation [36,37]. However, RUT administration in a dose-dependent manner significantly attenuated the elevation of MDA and RONS in the hepatorenal system of $NaAsO_2$-exposed rats. This suggests RUT's potent antioxidant and radical-scavenging activity.

The depletion of the antioxidant defense system renders hepatorenal tissue highly susceptible to NO-mediated nitrosative stress and compromises cellular integrity, while neutrophil-derived myeloperoxidase (MPO) can promote oxidative stress and inflammatory milieu via its enzymatic activity [38,39]. Also, free radicals are important mediators in the pathogenesis of inflammation-driven tissue impairment underlying a cascade of oxidative damage that alters cellular homeostasis [34,38,40]. NF-κB is a redox-sensitive regulator of immune responses, inflammation, cell proliferation, and survival that is activated by oxidative stress, leading to the expression of genes that promote these processes [40,41]. Phosphorylation of the RelA/p65 is required for NF-κB to activate expression of downstream genes involved in inflammatory and other responses [42]. The NF-κB/COX-2 axis is a fundamental pathway in chronic inflammation, and evidence indicates that NF-κB-induced COX-2 expression is a critical event in inflammation-mediated tissue damage. It is often driven by the transcriptional activation of COX-2 gene in response to oxidative stress and environmental toxins [43]. iNOS level was measured to verify that nitrosative stress contributes to arsenite-induced hepatorenal injury. Arsenic acts as a significant iNOS stimulant, leading to RONS damage as seen in toxicity studies in renal and hepatic systems [44,45]. Furthermore, RANTES, a low-molecular-weight protein also known as CCL5, is directly involved in attracting immune cells, including T lymphocytes, monocytes, and eosinophils, to sites of inflammation or tissue injury [46,47]. Multiple lines of evidence have shown that RANTES plays a key role in both human liver disease and experimental animal models [48]. RANTES expression is significantly elevated during ischemia-reperfusion injury in various tissues, including the kidneys, lungs, heart, intestine, brain, and skeletal muscles [49]. In this study, $NaAsO_2$-exposure alone elicited significant inflammatory response in the hepatorenal tissues, evidenced by aberrant levels of MPO, NO, iNOS, NF-κB, COX-2, and RANTES. These chains of events, we opined, contributed to the aggravated inflammatory damage. However, our data showed suppressed MPO activity, NO generation, iNOS, COX-2, NF-κB, and RANTES production in the hepatorenal organ of $NaAsO_2$ rats administered graded doses of RUT, underscoring RUT's potential as a strong anti-inflammatory agent against $NaAsO_2$-induced organ damage.

NRF-2 serves as a master regulator of redox homeostasis by directly controlling the transcription of various cytoprotective genes, most notably HO-1. This NRF-2/HO-1 signaling axis exerts potent antioxidant and anti-inflammatory effects by neutralizing free radical-induced damage and actively suppressing the NF-κB pathway [50,51]. Previous studies have demonstrated that the NRF-2/HO-1 pathway inhibits NF-κB activation, with HO-1 playing a central role in NRF-2-mediated NF-κB suppression [52]. The physiological importance of this mechanism is further evidenced by NRF-2 deficient murine models, which exhibit a marked increase in susceptibility to cytokine induced inflammatory stress [53]. In the study, we observed downregulation of NRF-2/HO-1 levels in the liver and kidneys of the $NaAsO_2$-alone-treated group compared to the control. This could be attributed to the significant induction of oxido-inflammatory stress responses in the exposed cohort. Conversely, dietary RUT, in a dose-dependent treatment, elicited a marked increase in NRF-2 and HO-1 protein levels in the co-administered groups. This suggests RUT's free radical scavenging and chemopreventive capabilities against toxic xenobiotic exposures. Other reports have shown that low-dose quercetin and other antioxidants exert beneficial effects via NRF-2/ARE activation, induction of antioxidant enzymes, and inhibition of NF-κB-induced proinflammatory genes [54]. In our study, this was observed as a significant decrease in NF-κB activity and associated proteins, driven by NRF-2 and HO-1 induction.

Histological analysis of liver and kidney tissues from $NaAsO_2$-exposed rats revealed severe congestion and inflammatory cell infiltration in the liver, along with glomerular congestion and infiltrates of congested cells in the kidney. Dietary RUT treatment substantially mitigated these hepatorenal histopathological changes, restoring tissue architecture to levels comparable to sham controls. These findings corroborate the earlier biochemical evidence of RUT anti-oxidant-inflammatory protective effects against $NaAsO_2$-induced hepatorenal injury.

CONCLUSION

Overall, our study demonstrated that $NaAsO_2$ treatment elicited hepatorenal oxidative damage in rats by boosting inflammatory mediators and disrupting antioxidant defenses. Rutin supplementation markedly protected against this injury by antagonizing inflammatory overactivation and strengthening redox master regulators. Accordingly, RUT presents a significant dietary intervention against arsenic-related toxicities.

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Author contribution

Omorede Ikponmwosa-Eweka: work supervision, conducted the experiment and analyzed the data, and revised the final version of the manuscript. Ikenna Chukwuemeka Maduako: conceptualized the research idea, experimental design, and drafted the original manuscript. All authors read and approved the manuscript.

Ethical approval: Following approval from the University of Benin Ethics Committee with number (APP No. LS25046), guidance was provided in accordance with the NIH (1985) Procedural Manual for animal handling and care.

Conflict of interest

The authors declared no conflict of interest.

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