



Research Article

Protective Effects of Rutin and Vitamin C against Sodium Arsenite-Induced Splenic Oxidative and Hematological Toxicity in Wistar Rats

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ABSTRACT

Sodium arsenite, a trivalent inorganic arsenic compound, induces oxidative and inflammatory injury in lymphoid organs such as the spleen. This study evaluated the protective effects of rutin and vitamin C, administered individually and in combination at low and high doses, against sodium arsenite-induced splenic toxicity in male Albino Wistar rats. Adult rats were assigned to six groups (n = 7) and orally treated for 14 days, comprising a 7-day antioxidant pre-treatment followed by 7 days of co-administration with sodium arsenite. Splenic nitrite, myeloperoxidase, reactive oxygen and nitrogen species, lipid peroxidation, glutathione, glutathione-S-transferase, glutathione peroxidase, catalase, and superoxide dismutase were assessed alongside haematological indices and histological changes. Sodium arsenite significantly increased inflammatory and oxidative markers, depleted glutathione, glutathione-S-transferase, catalase, and superoxide dismutase, while increasing glutathione peroxidase, and produced anaemia and a neutrophilic, lymphopenic stress leukogram (p < 0.05). Vitamin C and rutin provided partial protection, whereas their high-dose combination produced the greatest improvement, restoring most biochemical and haematological indices toward control values, although glutathione remained reduced. Histologically, the high-dose combination preserved splenic architecture, while sodium arsenite caused marked disruption of white and red pulp. Overall, rutin and vitamin C provided dose-dependent protective effects, with the high-dose combination offering the most effective protection against sodium arsenite-induced splenic oxidative, inflammatory, haematological, and structural injury.

Keywords: Haematology; Oxidative stress; Rutin; Sodium arsenite; Spleen toxicity; Vitamin C

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INTRODUCTION

Arsenic is one of the most extensively studied environmental toxicants, and inorganic arsenic compounds remain a persistent public health hazard across large parts of the world. Groundwater arsenic contamination affects an estimated 106 countries, with roughly 230 million people chronically exposed through drinking water, food, and industrial routes (Bhat *et al.*, 2024). Of the two principal inorganic species, trivalent arsenite is considerably more

reactive and toxicologically potent than pentavalent arsenate, owing to its strong affinity for sulfhydryl groups on proteins and enzymes. Sodium arsenite is therefore widely used to model arsenic-induced organ toxicity in experimental animals (Oyagbemi *et al.*, 2017).

At the cellular level, arsenite disrupts mitochondrial respiration and generates excess reactive oxygen and nitrogen species, overwhelming endogenous antioxidant defences and triggering lipid

peroxidation, protein oxidation, and DNA damage (Redza-Dutordoir & Averill-Bates, 2016). This oxidative burden activates intrinsic apoptotic pathways and pro-inflammatory signalling cascades, and it is now recognised as a principal mechanism underlying arsenic-induced injury in multiple organ systems, including lymphoid tissue (Jamal *et al.*, 2020).

The spleen is the largest secondary lymphoid organ in the body and performs two closely linked functions: the red pulp filters senescent erythrocytes and blood-borne debris, while the white pulp initiates adaptive immune responses to circulating antigens (Mebius & Kraal, 2005). Because the spleen receives a substantial fraction of cardiac output and is richly populated with phagocytic and lymphoid cells, it is particularly vulnerable to blood-borne toxicants such as arsenic, and injury to splenic tissue has direct implications for systemic immune competence.

A growing body of evidence confirms that arsenic is immunotoxic. Dangleben *et al.* (2013) reviewed multiple lines of evidence showing that arsenic exposure impairs both innate and adaptive immunity through apoptosis induction, oxidative stress, and disrupted lymphocyte activation. In rodent models, chronic sodium arsenite exposure produces time-dependent activation of NF- κ B and MAPK signalling in the spleen, accompanied by dysregulated cytokine expression and immunosuppression (Yan *et al.*, 2020). More recently, Maity *et al.* (2024) demonstrated that thirty days of sodium arsenite exposure in Wistar rats caused structural alterations, reduced splenocyte counts, depleted SOD and catalase activity, elevated malondialdehyde, and increased pro-apoptotic and pro-inflammatory markers in the spleen. Comparable oxidative and apoptotic splenic injury, mediated through disrupted Nrf2/HO-1 signalling, has also been reported in non-mammalian models of arsenic exposure (Zheng *et al.*, 2025), underlining the conserved nature of this toxicity across species.

Because conventional chelation therapy carries its own toxicity and does not fully reverse arsenic-induced oxidative damage, attention has turned to dietary antioxidants capable of counteracting the oxidative and inflammatory cascade triggered by arsenic. Rutin and vitamin C are two such agents with well-characterised, complementary mechanisms of action.

Rutin, a quercetin glycoside abundant in citrus fruits, buckwheat, and many leafy vegetables, exerts antioxidant, anti-inflammatory, and antiapoptotic effects through activation of the Nrf2/antioxidant

response element pathway, suppression of NF- κ B-driven cytokine release, and inhibition of caspase-dependent apoptosis; it additionally chelates transition metal ions, limiting metal-catalysed lipid peroxidation (Rahmani *et al.*, 2023). In a closely related study, Ikponmwosa-Eweka *et al.* (2026) reported that rutin (25 and 50 mg/kg) significantly and dose-dependently reversed sodium arsenite-induced haematological derangement and hepatic oxidative stress in Wistar rats, restoring glutathione, glutathione-S-transferase, catalase, and superoxide dismutase activities while moderating an arsenite-induced surge in glutathione peroxidase activity evidence directly supporting the plausibility of rutin as a protective agent against sodium arsenite toxicity in the present splenic model.

Vitamin C (L-ascorbic acid) is a water-soluble antioxidant that scavenges reactive oxygen and nitrogen species directly, serves as an enzymatic cofactor, and regenerates other antioxidants from their oxidised forms. Co-administration of ascorbic acid (vitamin C) and α -tocopherol (vitamin E) has previously been shown to prevent arsenic-induced protein oxidation and DNA damage in rats (Kadirvel *et al.*, 2007), and combined ascorbic acid and α -tocopherol supplementation markedly reduced oxidative stress-induced inflammation in the arsenic-exposed thymus and spleen (Maity *et al.*, 2024).

Rutin acts mainly in lipid-associated cellular compartments, while vitamin C acts predominantly in the aqueous cytosolic and extracellular compartments, so their combined administration may offer broader antioxidant coverage than either agent alone; combinations of water-soluble and flavonoid antioxidants have produced synergistic protection against oxidative injury in other metal-toxicity models (Zamani *et al.*, 2020). However, no study to date has examined whether rutin and vitamin C, given individually and in combination, can protect splenic tissue against sodium arsenite-induced toxicity. This study investigated the effects of rutin and vitamin C, alone and combined at two dose levels, on splenic inflammatory, oxidative stress, antioxidant, and haematological parameters in sodium arsenite-exposed Wistar rats, hypothesising that combined administration would confer superior protection relative to either agent given alone.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium arsenite ($\geq 96\%$ purity), L-ascorbic acid (vitamin C), and rutin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reduced glutathione

(GSH), hydrogen peroxide (H₂O₂), sodium azide (NaN₃), trichloroacetic acid (TCA), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent) were also obtained from Sigma-Aldrich. Bovine serum albumin (BSA) and Folin-Ciocalteu reagent for protein determination were sourced from British Drug Houses (Dorset, Poole, UK). Reagents for automated haematological analysis (isotonic diluent, lysing agent, cleaner, and rinse solutions) were obtained from Mindray Medical International Ltd. (Shenzhen, China). All other chemicals and reagents used were of analytical grade.

Experimental Animals and Ethical Considerations

Healthy adult male Wistar rats (150–180 g) were obtained from the Central Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria. Animals were acclimatized for two weeks prior to the commencement of the study, during which they were housed in clean, well-ventilated polypropylene cages (seven rats per cage) under a natural 12-hour light/12-hour dark photoperiod, with free access to standard commercial rat pellets and water *ad libitum*. All procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies (Tveden-Nyborg *et al.*, 2025) and was approved by the Institutional Animal Ethics Committee (CMS/REC/2026/1003).

Induction of Splenic Toxicity

Sodium arsenite was dissolved in distilled water and administered orally by gastric gavage at a dose of 10 mg/kg body weight during the co-administration phase of the treatment protocol (see Experimental Design).

Experimental Design

A total of forty-two male Wistar rats were randomly allocated into six groups of seven animals each: Group I, normal control; Group II, sodium arsenite (SA) only; Group III, SA + vitamin C (100 mg/kg); Group IV, SA + rutin (100 mg/kg); Group V, SA + vitamin C (50 mg/kg) and rutin (50 mg/kg) in combination (low-dose combination); Group VI, SA + vitamin C (100 mg/kg) and rutin (100 mg/kg) in combination (high-dose combination). Treatment with Vitamin C and rutin was administered orally by gastric gavage over 14 consecutive days, comprising a 7-day pre-treatment phase in which animals received the antioxidant supplements (vitamin C and/or rutin) alone, followed by a 7-day co-administration phase in

which sodium arsenite was given alongside the antioxidant treatments; the normal control and SA-only groups received an equivalent volume of distilled water during the pre-treatment phase. Vitamin C and rutin were freshly prepared as aqueous suspensions immediately before each administration, and doses were adjusted weekly according to individual body weight.

Sample Collection and Tissue Preparation

At the end of the 14-day treatment period, animals were fasted overnight (approximately 12 hours) with free access to water. Rats were anaesthetized with chloroform vapour, and blood was collected by cardiac puncture. Approximately 3 mL of blood was transferred into dipotassium ethylenediaminetetraacetic acid (K₂EDTA) tubes, gently inverted 8–10 times, and maintained at room temperature for haematological analysis within 4 h of collection. The spleen was then carefully excised, rinsed in ice-cold 1.15% potassium chloride (KCl) solution to remove residual blood, blotted dry, and weighed. Splenic tissue was homogenized (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1.15% KCl using a Teflon-glass homogenizer maintained at 4 °C. Homogenates were centrifuged at 10,000 × g for 15 min at 4 °C to obtain the post-mitochondrial supernatant, which was collected and stored at –20 °C until biochemical analysis.

Biochemical Assays

Determination of Nitrite level

Splenic nitrite, the stable end-product of nitric oxide, was quantified by the Griess reaction (Green *et al.*, 1982). Tissue supernatant (0.5 mL) was incubated with 0.5 mL of Griess reagent for 30 minutes at room temperature, and absorbance was measured at 542 nm against a sodium nitrite standard curve.

Determination of Myeloperoxidase (MPO) Activity

MPO activity, an index of neutrophil infiltration, was assayed spectrophotometrically using 3,3',5,5'-tetramethylbenzidine as the substrate, following the method of Granell (2003) in the supplied protocol. Tissue was homogenized in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide, then centrifuged at 13,000 rpm for 15 minutes at 4 °C. The reaction mixture (supernatant, tetramethylbenzidine, and H₂O₂ in phosphate buffer, pH 5.4) was read at 630 nm, and activity was expressed as μmol H₂O₂ consumed/min/mg protein.

Determination of Reactive Oxygen and Nitrogen Species (RONS)

RONS were quantified fluorometrically using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA)

assay (LeBel et al., 1992; Wang & Joseph, 1999). Tissue supernatant was incubated with 10 μ M DCFH-DA for 30 minutes at 37 °C in the dark, and fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm; RONS levels were expressed as DCF fluorescence (% of control).

Assessment of Lipid Peroxidation (LPO)

Lipid peroxidation was quantified as thiobarbituric acid reactive substances (TBARS) following Varshney and Kale (1990). Samples were reacted with trichloroacetic acid and thiobarbituric acid, heated at 80 °C for 45 minutes, and the resulting pink chromogen was measured at 532 nm. Results were expressed as malondialdehyde equivalents (μ mol MDA/mg protein) using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Estimation of Reduced Glutathione (GSH)

GSH content was determined using the method of Jollow et al. (1974). The homogenate was deproteinized with 4% Sulphosalicylic acid, and the resulting supernatant was reacted with Ellman's reagent (DTNB). Absorbance was measured at 412 nm, which was proportional to GSH content.

Determination of Glutathione Peroxidase (GPx) Activity

GPx activity was determined according to Rotruck et al. (1973), based on the enzyme-catalyzed reduction of H_2O_2 with GSH as the hydrogen donor. Residual GSH was quantified using Ellman's reagent at 412 nm, and activity was expressed as μ mol GSH oxidized/min/mg protein.

Estimation of Catalase (CAT) Activity

CAT activity was determined by the method of Cohen et al. (1970), based on the rate of hydrogen peroxide decomposition. Residual H_2O_2 was titrated with potassium permanganate, and absorbance was measured at 480 nm; activity was expressed as U/mg protein.

Determination of Glutathione-S-Transferase (GST)

Splenic GST activity was determined according to Habig et al. (1974), based on the enzyme-catalyzed conjugation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB). The reaction mixture contained tissue supernatant, phosphate buffer (pH 6.5), GSH, and CDNB. The rate of formation of S-2,4-dinitrophenylglutathione was monitored spectrophotometrically at 340 nm, and GST activity was expressed as nmol of CDNB-GSH conjugate formed per min per mg protein.

Determination of Superoxide Dismutase (SOD) Activity

Splenic SOD activity was assayed using the epinephrine auto-oxidation inhibition method described by Misra and Fridovich (1972). The assay relies on SOD's ability to inhibit the auto-oxidation of adrenaline to adrenochrome at pH 10.2. Absorbance was monitored at 480 nm, and one unit of SOD activity was defined as the amount of enzyme required to achieve 50% inhibition of epinephrine auto-oxidation per milligram of protein.

Protein Determination

Protein concentration in tissue homogenates was determined by the modified Lowry method described by Bradford et al (1976), using bovine serum albumin as the standard, with absorbance measured at 750 nm.

Hematological Analysis

Whole blood collected in K_2EDTA tubes was analyzed on an automated hematology analyzer (Mindray BC-2800, Shenzhen, China), calibrated for rodent blood, to determine red blood cell (RBC) count, hemoglobin (Hb) concentration, total white blood cell (WBC) count, and differential leukocyte counts (neutrophils, lymphocytes, and monocytes, expressed as percentages). The neutrophil-to-lymphocyte ratio was calculated from the differential counts.

Histopathological Examination

Spleen tissue samples for histological examination were fixed in 10% neutral buffered formalin and processed by standard paraffin-embedding techniques. Tissue sections were cut with a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E) for general morphological assessment. Stained sections were examined under a light microscope by a pathologist blinded to the groupings, and representative photomicrographs were captured for each experimental group to assess splenic histoarchitecture, including the white pulp, red pulp, and central artery.

Data Analysis

All data were expressed as mean $\pm$ standard deviation (SD) for each group ($n = 7$). Differences among treatment groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. Analyses were performed using GraphPad Prism software (version 8.0; GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Effect of Vitamin C and Rutin, Administered Individually and in Combination, on Splenic Inflammatory and Oxidative Stress Markers of Sodium Arsenite-Exposed Rats

Table 1 shows the effect of sodium arsenite administration, alone and combined with vitamin C and rutin, on splenic nitrite, myeloperoxidase (MPO), reactive oxygen and nitrogen species (RONS), and lipid peroxidation (LPO) content. Relative to the normal control group, rats given sodium arsenite alone showed a significant ($p < 0.05$) rise in splenic nitrite (19.67 ± 1.24 vs. 8.07 ± 0.61 units/mg protein), MPO (4.05 ± 0.51 vs. 0.55 ± 0.05 units/mg protein), RONS (15.41 ± 1.10 vs. $5.06 \pm 1.05\%$ of control), and LPO (32.26 ± 0.18 vs. 2.00 ± 0.04 μmol MDA/mg protein), confirming the induction of splenic oxidative and inflammatory injury. Individual treatment with vitamin C (100 mg/kg) or rutin (100 mg/kg) produced only a modest lowering of these markers relative to the sodium arsenite-only group. Combined treatment, by contrast, produced a clear dose-dependent improvement: the low-dose combination (vitamin C 50 mg/kg + rutin 50 mg/kg) significantly reduced nitrite, MPO, RONS, and LPO relative to sodium arsenite alone, and the high-dose combination (vitamin C 100 mg/kg + rutin 100 mg/kg) reduced all four markers to values statistically indistinguishable from the normal control group, with LPO in particular falling to 2.04 ± 0.40 μmol MDA/mg protein, essentially matching the control value of 2.00 ± 0.04 .

Effect of Vitamin C and Rutin, Administered Individually and in Combination, on Splenic Antioxidant Status of Sodium Arsenite-Exposed Rats

Table 2 presents the effect of the treatments on splenic antioxidant enzyme activities and glutathione content. Sodium arsenite exposure alone caused a significant ($p < 0.05$) depletion of GSH (4.04 ± 0.43 vs. 23.68 ± 0.77 units/mg protein), GST (7.60 ± 1.50 vs. 24.60 ± 0.95 nmol/min/mg protein), CAT (6.76 ± 0.40 vs. 34.08 ± 0.63 μmol H_2O_2 consumed/min/mg protein), and SOD (0.267 ± 0.05 vs. 0.570 ± 0.05 units/mg protein), reflecting exhaustion of the splenic antioxidant defence system. GPx activity moved in the opposite direction, rising from 1.55 ± 0.06 to 8.06 ± 0.04 $\mu\text{g}/\text{mol}/\text{mg}$ protein following sodium arsenite exposure, a pattern that may reflect compensatory upregulation of this peroxide-detoxifying enzyme under acute peroxide overload. Vitamin C and rutin, administered individually, partially restored GSH, GST, CAT, and SOD activities and partially lowered the elevated GPx activity, but values in these groups remained significantly different from control. The combination treatments produced a clearer restoration of antioxidant status: the low-dose combination significantly improved all five parameters relative to the sodium arsenite group, and the high-dose combination restored GST (24.00 ± 1.00), CAT (33.37 ± 0.73), SOD (0.567 ± 0.03), and GPx (1.75 ± 0.07) to values statistically comparable with the normal control group. Splenic GSH content in the high-dose combination group (17.94 ± 0.97) improved substantially relative to the sodium arsenite group but remained significantly below the normal control value (23.68 ± 0.77), making glutathione repletion the slowest-recovering parameter of the antioxidant panel assessed.

Table 1: Effect of vitamin C and rutin, administered individually and in combination, on splenic inflammatory and oxidative stress markers following sodium arsenite exposure

Parameter	Normal	SA	Vit C (100) + SA	Rutin (100) + SA	Vit C (50) + Rut (50) + SA	Vit C (100) + Rut (100) + SA
Nitrite (units/mg protein)	8.07 ± 0.61	$19.67 \pm 1.24^*$	15.70 ± 0.23	15.00 ± 0.67	$11.12 \pm 1.01^{**}$	$7.62 \pm 0.52^{***}$
MPO (units/mg protein)	0.55 ± 0.05	$4.05 \pm 0.51^*$	3.00 ± 0.01	3.02 ± 0.15	$2.12 \pm 0.15^{**}$	$0.55 \pm 0.06^{***}$
RONS (% of control)	5.06 ± 1.05	$15.41 \pm 1.10^*$	9.15 ± 0.70	9.45 ± 0.18	$7.01 \pm 0.60^{**}$	$5.39 \pm 1.09^{***}$
LPO (μmol MDA formed/mg protein)	2.00 ± 0.04	$32.26 \pm 0.18^*$	24.02 ± 1.01	23.82 ± 1.20	$20.20 \pm 0.84^{**}$	$2.04 \pm 0.40^{***}$

(*) $p < 0.05$ vs Normal; (**) $p < 0.05$ vs SA (low-dose combination); (***) $p < 0.05$ vs SA (high-dose combination). MPO: myeloperoxidase; RONS: reactive oxygen and nitrogen species; LPO: lipid peroxidation; SA: sodium arsenite; Vit C: vitamin C; Rut: rutin. Data presented as mean $\pm$ SD; $n = 7$.

Table 2: Effect of vitamin C and rutin, administered individually and in combination, on splenic antioxidant status following sodium arsenite exposure

Parameter	Normal	SA	Vit C (100) + SA	Rutin (100) + SA	Vit C (50) + Rut (50) + SA	Vit C (100) + Rut (100) + SA
GSH (units/mg protein)	23.68±0.77	4.04±0.43*	9.93±0.21	10.11±0.15	15.70±0.23**	17.94±0.97***
GST (nmol/min/mg protein)	24.60±0.95	7.60±1.50*	12.00±1.21	12.85±1.50	17.35±2.00**	24.00±1.00***
GPx (µg/mol/mg protein)	1.55±0.06	8.06±0.04*	6.00±0.26	5.59±0.19	5.05±0.21**	1.75±0.07***
CAT (µmol H ₂ O ₂ consumed/min/mg protein)	34.08±0.63	6.76±0.40*	10.13±0.77	9.93±0.35	21.13±0.67**	33.37±0.73***
SOD (units/mg protein)	0.570±0.05	0.267±0.05*	0.333±0.04	0.303±0.06	0.354±0.12**	0.567±0.03***

(*) $p < 0.05$ vs Normal; (**) $p < 0.05$ vs SA (low-dose combination); (***) $p < 0.05$ vs SA (high-dose combination). GSH: reduced glutathione; GST: glutathione-S-transferase; GPx: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase; SA: sodium arsenite; Vit C: vitamin C; Rut: rutin. Data presented as mean $\pm$ SD; $n = 7$.

Effect of Vitamin C and Rutin, Administered Individually and in Combination, on Haematological Indices of Sodium Arsenite-Exposed Rats

Table 3 shows the effect of the treatments on peripheral haematological indices. Sodium arsenite exposure alone caused a significant ($p < 0.05$) reduction in red blood cell count (RBC: 2.02 ± 0.65 vs. $3.10 \pm 0.55 \times 10^6/\mu\text{L}$) and haemoglobin concentration (Hb: 3.45 ± 0.55 vs. 7.42 ± 1.15 g/dL), indicative of anaemia, alongside a significant elevation in total white blood cell count (WBC: 10.95 ± 0.65 vs. $5.02 \pm 1.00 \times 10^3/\mu\text{L}$). This leukocytosis was accompanied by a marked neutrophilia (42.05 ± 1.55 vs. $20.05 \pm 1.60\%$) and reciprocal lymphopenia (6.09 ± 0.65 vs. $30.11 \pm 1.65\%$), producing a substantial rise in the neutrophil-to-lymphocyte ratio (4.45 ± 0.30 vs. 0.45 ± 0.10), together with a significant reduction in monocyte percentage (0.75 ± 0.10 vs. $1.85 \pm 0.10\%$). Individual treatment with vitamin C or rutin produced partial improvement in these indices relative to the sodium arsenite-only group. Combined treatment again produced a clearer restoration: the low-dose combination significantly improved all indices relative to sodium arsenite alone, and the high-dose combination normalised RBC (3.08 ± 0.60), Hb (7.45 ± 1.00), WBC (5.25 ± 1.10), neutrophil percentage (19.12 ± 1.55), lymphocyte percentage (26.02 ± 1.19), neutrophil-to-lymphocyte ratio (0.42 ± 0.01), and monocyte percentage (1.65 ± 0.05) to values

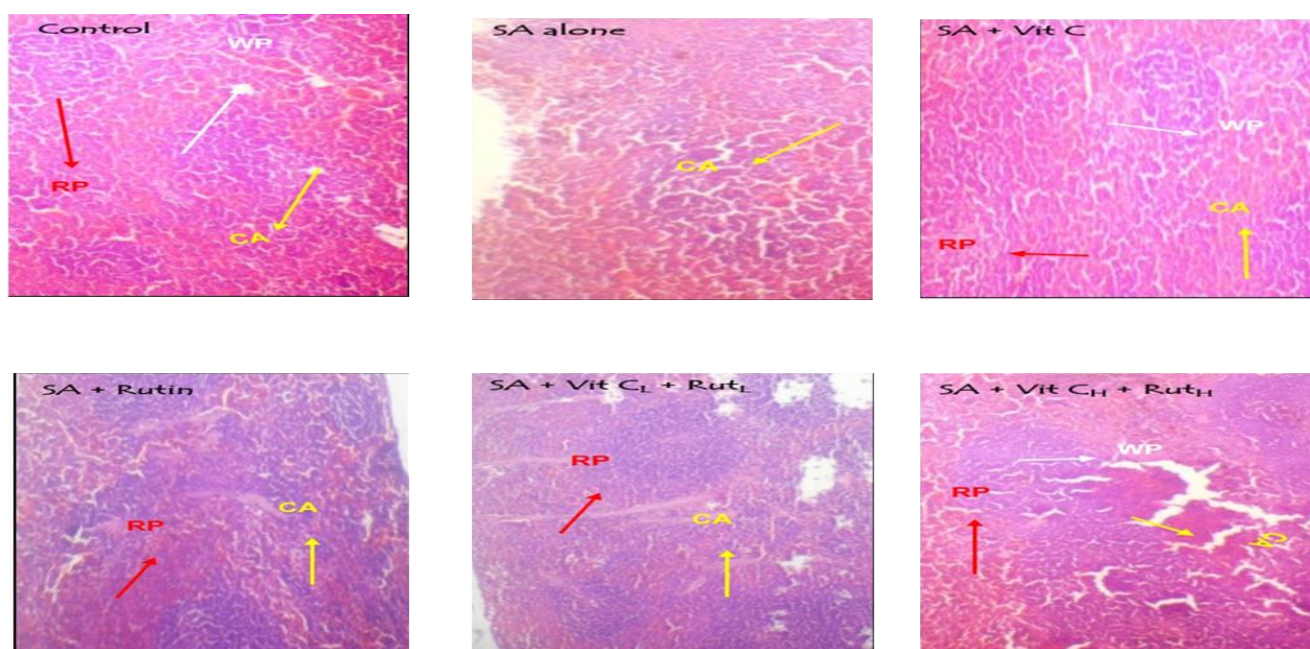
statistically comparable with the normal control group.

Qualitative histological assessment showed that the Control section displayed clean, well-organised splenic architecture, with a distinct white pulp (WP) nodule, a clearly visible central artery (CA), and evenly distributed red pulp (RP) with no evidence of congestion. The SA-alone section showed no clearly demarcated white pulp nodule within the field examined, with dense, closely packed red pulp and a central artery traversing the section. The SA + Vitamin C section showed a discernible white pulp nodule with reasonably organised surrounding red pulp. The SA + Rutin section showed darker, denser, and more congested-appearing red pulp, with no white pulp nodule captured in the field examined. The SA + Vitamin CL + RutinL section similarly showed dense, dark red pulp without a distinct white pulp nodule, alongside streaky pale bands consistent with trabecular or vascular structures. The SA + Vitamin CH + RutinH section showed a discernible white pulp nodule, a visible central artery, and organised red pulp. Ranked from most to least normal-appearing on visible architecture, the overall pattern was Control = SA + Vitamin CH + RutinH > SA + Vitamin C > SA + Rutin > SA + Vitamin CL + RutinL > SA alone, reflecting a graded, dose-related pattern of protection against sodium arsenite-induced splenic injury.

Table 3: Effect of vitamin C and rutin, administered individually and in combination, on haematological indices following sodium arsenite exposure

Parameter	Normal	SA	Vit C (100) + SA	Rutin (100) + SA	Vit C (50) + Rut (50) + SA	Vit C (100) + Rut (100) + SA
RBC ($10^6/\mu\text{L}$)	3.10 $\pm$ 0.55	2.02 $\pm$ 0.65*	2.34 $\pm$ 0.55	2.33 $\pm$ 0.60	2.73 $\pm$ 0.71**	3.08 $\pm$ 0.60***
Hb (g/dL)	7.42 $\pm$ 1.15	3.45 $\pm$ 0.55*	5.05 $\pm$ 0.60	5.00 $\pm$ 0.55	6.00 $\pm$ 0.05**	7.45 $\pm$ 1.00***
WBC ($10^3/\mu\text{L}$)	5.02 $\pm$ 1.00	10.95 $\pm$ 0.65*	8.00 $\pm$ 1.10	6.99 $\pm$ 0.47	6.36 $\pm$ 0.70**	5.25 $\pm$ 1.10***
Neutrophils (%)	20.05 $\pm$ 1.60	42.05 $\pm$ 1.55*	37.44 $\pm$ 1.60	35.59 $\pm$ 1.85	25.51 $\pm$ 1.05**	19.12 $\pm$ 1.55***
Lymphocytes (%)	30.11 $\pm$ 1.65	6.09 $\pm$ 0.65*	20.14 $\pm$ 0.80	20.10 $\pm$ 1.00	26.10 $\pm$ 1.35**	26.02 $\pm$ 1.19***
Neutrophil/Lymphocyte Ratio	0.45 $\pm$ 0.10	4.45 $\pm$ 0.30*	2.50 $\pm$ 0.16	2.53 $\pm$ 0.29	1.53 $\pm$ 0.02**	0.42 $\pm$ 0.01***
Monocytes (%)	1.85 $\pm$ 0.10	0.75 $\pm$ 0.10*	1.00 $\pm$ 0.10	1.03 $\pm$ 0.15	1.19 $\pm$ 0.11**	1.65 $\pm$ 0.05***

(*) $p < 0.05$ vs Normal; (**) $p < 0.05$ vs SA (low-dose combination); (***) $p < 0.05$ vs SA (high-dose combination). RBC: red blood cell; Hb: haemoglobin; WBC: white blood cell; SA: sodium arsenite; Vit C: vitamin C; Rut: rutin. Data presented as mean $\pm$ SD; $n = 7$.

**Figure 1: Effect of vitamin C and rutin, administered individually and in combination, on splenic histoarchitecture of sodium arsenite-exposed rats. Photomicrographs, H&E stain, light microscopy**

CA: central artery; WP: white pulp; RP: red pulp; SA: sodium arsenite; Vit C: vitamin C; Rut: rutin; L: low dose; H: high dose

DISCUSSION

Sodium arsenite exposure produced marked splenic oxidative and inflammatory injury in the Wistar rats used in this study, reflected in elevated nitrite, MPO, RONS, and lipid peroxidation alongside depletion of the main enzymatic and non-enzymatic antioxidant defences. Vitamin C and rutin, given together, reduced this injury in a dose-related manner, and the high-dose combination brought most parameters back to values statistically comparable with the normal control group. These findings agree with earlier reports describing sodium arsenite as a potent

inducer of oxidative-inflammatory damage in the spleen and thymus (Maity *et al* 2024), and they support combined antioxidant supplementation as a reasonable strategy against arsenic toxicity.

The rise in splenic nitrite and MPO in the arsenite-exposed rats points to increased nitric oxide production and neutrophil infiltration. MPO is stored almost exclusively in neutrophil azurophilic granules, so its tissue activity is commonly used as a marker of neutrophil recruitment during inflammation. The parallel rise in RONS and LPO suggests that arsenite generated enough reactive oxygen and nitrogen

species to overwhelm the spleen's antioxidant capacity and trigger lipid peroxidation, a process that damages membrane integrity and can itself propagate further radical generation. A similar pattern has been reported in the arsenic-exposed murine spleen, where chronic exposure triggered sustained NF- κ B and MAPK activation together with a pro-inflammatory cytokine profile (Yan *et al.*, 2020), and in the arsenic-exposed rat thymus and spleen, where oxidative stress and NF- κ B-mediated inflammation preceded structural tissue damage (Maity *et al.*, 2024).

Beyond its direct oxidative effects, arsenic is a recognized immunotoxicant that can impair both innate and adaptive immune function through apoptosis induction and disrupted lymphocyte signalling (Dangleben *et al.*, 2013). Reactive oxygen species generated during arsenite metabolism are known to activate the intrinsic mitochondrial apoptotic pathway (Redza-Dutordoir & Averill-Bates, 2016), and comparable apoptotic and endoplasmic-reticulum stress responses have been documented in the arsenic-exposed spleen of other vertebrate models, alongside disrupted Nrf2/HO-1 antioxidant signalling and histological injury (Zheng *et al.*, 2025). Apoptotic and Nrf2/NF- κ B pathway markers were not measured directly in the present study, so this cannot be confirmed here; however, the splenic oxidative injury and antioxidant depletion seen in the sodium arsenite-only group would be compatible with an underlying apoptotic and pro-inflammatory process, and the disorganized histoarchitecture noted in the SA-alone section of Figure 1, relative to Control, is broadly in line with this picture.

The haematological changes seen in sodium arsenite-exposed rats extend the localised splenic oxidative and inflammatory injury described above to the systemic level. The marked reduction in red blood cell count and haemoglobin concentration matches previous reports of sodium arsenite-induced anaemia in Wistar rats (Ola-Davies & Akinrinde, 2016). A plausible explanation is the spleen's role in filtering, and when damaged prematurely destroying, oxidatively compromised erythrocytes (Mebius & Kraal, 2005): the elevated splenic lipid peroxidation and depleted antioxidant capacity documented above would be expected to increase erythrocyte membrane fragility and accelerate splenic clearance of red cells. The accompanying leukocytosis, driven by a marked neutrophilia with reciprocal lymphopenia, raised the neutrophil-to-lymphocyte ratio substantially; this ratio is a recognized, clinically validated index of systemic inflammation and

physiological stress (Zahorec, 2021) and likely reflects, at the systemic level, the elevated splenic MPO activity reported above, both pointing to heightened neutrophil mobilization in response to arsenite-induced tissue injury. The combination treatments restored these haematological indices in a pattern that closely mirrored the biochemical findings, with the high-dose vitamin C-rutin combination normalising red cell, white cell, and differential counts to values statistically comparable with the normal control group. This offers whole-organism support for the protective effect of the combined regimen, in line with the tissue-level biochemical protection detailed above.

The depletion of GSH, GST, CAT, and SOD activities in the sodium arsenite group reflects the classic exhaustion of enzymatic and non-enzymatic antioxidant reserves that accompanies sustained oxidative challenge. Glutathione in particular is consumed both directly, in neutralizing reactive electrophiles, and indirectly, as a substrate for glutathione-dependent enzymes such as GST and GPx. GPx activity moved in the opposite direction to the other antioxidant enzymes, rising in the arsenite-only group; this could represent a short-term compensatory upregulation of peroxide-detoxifying capacity in response to the hydrogen peroxide and lipid hydroperoxide burden generated by arsenite, a response that would be unsustainable without adequate glutathione and cofactor supply. Ikponmwosa-Eweka *et al.* (2026) reported a near-identical pattern in sodium arsenite-exposed Wistar rats, where hepatic GPx activity rose sharply after arsenite exposure and was then moderated toward control values by rutin co-treatment; they interpreted this as a compensatory response that resolved once antioxidant supplementation reduced the underlying oxidative burden. GPx activity in the present high-dose combination group also normalized, alongside restoration of GSH, GST, CAT, and SOD, which fits this interpretation: combined rutin and vitamin C treatment appears to have relieved the peroxide burden enough that compensatory GPx upregulation was no longer needed.

Rutin's contribution to protection fits its well-documented ability to activate the Nrf2/antioxidant response element pathway, upregulating endogenous antioxidant enzyme expression while suppressing NF- κ B-driven transcription of pro-inflammatory mediators and inhibiting caspase-3/-9-dependent apoptosis (Rahmani *et al.*, 2023). Its metal-chelating activity may also have limited

arsenite-catalyzed radical generation directly at the tissue level, complementing these enzymatic and transcriptional effects. Ikponmwosa-Eweka *et al.* (2026) reported similar dose-related restoration of GSH, GST, CAT, SOD, and GPx with rutin monotherapy against sodium arsenite in Wistar rats, which suggests that the extra benefit seen here with combination treatment reflects a genuine contribution from vitamin C rather than rutin acting alone.

Vitamin C alone produced comparatively modest protection. This fits its role as a direct but rapidly consumed radical scavenger: as a water-soluble antioxidant, ascorbate is preferentially depleted in the aqueous phase and offers limited protection against lipid-phase peroxidative damage without a complementary lipophilic or flavonoid antioxidant. Earlier work similarly found that ascorbic acid needed to be paired with a second antioxidant, rather than given alone, to substantially reduce arsenic-induced protein oxidation, DNA damage, and splenic inflammation in rats (Kadirvel *et al.*, 2007; Maity *et al.*, 2024).

The near-complete normalization of splenic biochemical and haematological markers achieved with the vitamin C-rutin combination, compared with either agent alone, points to a complementary or synergistic mode of action. Rutin acts mainly through transcriptional upregulation of antioxidant enzymes and radical/metal scavenging in lipid-associated compartments, while vitamin C works through rapid aqueous-phase radical scavenging; together, they would be expected to cover more of the cell than either agent alone. A similar pattern of synergistic antioxidant protection has been reported for other water-soluble/flavonoid antioxidant combinations against metal-induced organ toxicity (Zamani *et al.*, 2020), which supports the plausibility of an additive or synergistic interaction here, although the present data cannot establish its precise molecular basis.

The histopathological findings provide morphological context for the biochemical and haematological changes described above. The organized white pulp, red pulp, and central artery architecture seen in the Control section matches the low nitrite, MPO, RONS, and LPO values and the preserved antioxidant enzyme activities measured in this group. The absence of a discernible white pulp nodule and the dense, closely packed red pulp in the SA-alone section, similarly, line up with the marked oxidative, inflammatory, and haematological derangement documented in that group, echoing previous reports linking sodium arsenite-induced oxidative stress to disrupted splenic microarchitecture (Maity *et al.*,

2024; Zheng *et al.*, 2025). The treated groups showed a graded pattern of architectural preservation that closely paralleled their biochemical and haematological protection.

Ranked from most normal-looking to most affected-looking, based purely on visible architecture, the overall pattern was: G1 (control) = G6 (high-dose combination) > G3 (vitamin C alone) > G4 (rutin alone) > G5 (low-dose combination) > G2 (SA alone, worst-affected). This is a biologically coherent pattern: sodium arsenite alone caused the most apparent white pulp depletion and congestion, while the high-dose vitamin C + rutin combination showed the strongest protective and recovery effect, closely approaching control. It also fits a picture in which the antioxidant combination outperforms either agent given alone, which in turn outperforms the untreated toxin group.

A strength of the present study is the inclusion of both individual and combined, dose-ranging antioxidant treatment cohorts alongside a comprehensive biochemical index spanning inflammatory, oxidative, antioxidant, haematological, and histopathological parameters, which together allow the interaction between rutin and vitamin C to be characterized.

Limitations include the restriction of the study to male rats, the absence, at this stage, of molecular-level data (e.g., Nrf2, NF- κ B, or apoptotic protein expression) to confirm the proposed mechanisms, and the relatively short treatment duration typical of subacute toxicity models, which may not fully capture the consequences of chronic environmental arsenic exposure.

CONCLUSION

Sodium arsenite exposure produced measurable splenic toxicity in male Wistar rats, evidenced by significant increases in nitrite levels, myeloperoxidase activity, reactive oxygen and nitrogen species generation, and lipid peroxidation, alongside marked reductions in glutathione content and the activities of glutathione-S-transferase, catalase, and superoxide dismutase. Hematological assessment revealed anemia accompanied by a neutrophilia and lymphopenia consistent with an oxidative stress response. Histopathological examination of splenic tissue was concordant with these biochemical and hematological findings, demonstrating progressive disruption of splenic architecture in arsenic-exposed animals. Rutin and vitamin C, given individually, offered partial protection against these changes, while their combined administration produced a considerably greater, dose-dependent protective

effect, with the high-dose combination restoring most biochemical and haematological parameters to values statistically comparable with the normal control group. Taken together, these findings support rutin and vitamin C, particularly in combination, as promising adjunctive agents against arsenic-induced splenic toxicity. Further studies incorporating molecular pathway analysis, extended exposure duration, and dose-optimisation are needed to clarify the mechanistic basis of this protective interaction and its translational relevance.

REFERENCES

- Bhat, A., Ravi, K., Tian, F., & Singh, B. (2024). Arsenic contamination needs serious attention: An opinion and global scenario. *Pollutants*, 4(2), 196–211. <https://doi.org/10.3390/pollutants4020013>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cohen, G., Dembiec, D., & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34(1), 30–38. [https://doi.org/10.1016/0003-2697\(70\)90083-7](https://doi.org/10.1016/0003-2697(70)90083-7)
- Dangleben, N. L., Skibola, C. F., & Smith, M. T. (2013). Arsenic immunotoxicity: A review. *Environmental Health*, 12, 73. <https://doi.org/10.1186/1476-069X-12-73>
- Granell, S., Gironella, M., Bulbena, O., Panés, J., Mauri, M., Sabater, L., Aparisi, L., Gelpí, E., & Closa, D. (2003). Heparin mobilizes xanthine oxidase and induces lung inflammation in acute pancreatitis. *Critical Care Medicine*, 31(2), 525–530. <https://doi.org/10.1097/01.CCM.0000050444.15655.FB>
- Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry*, 126(1), 131–138. [https://doi.org/10.1016/0003-2697\(82\)90118-x](https://doi.org/10.1016/0003-2697(82)90118-x)
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249(22), 7130–7139. [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8)
- Ikponmwosa-Eweka, O., Maduako, I. C., Ohenhen, A. B., & Amiebenomo, R. A. (2026). Protective effects of rutin against sodium arsenite-induced hematological alterations and oxidative stress in Wistar rats. *FUDMA Journal of Sciences*, 10(2), 419–424. <https://doi.org/10.33003/fjs-2026-1002-4569>
- Jamal, Z., Das, J., Ghosh, S., Gupta, A., Chattopadhyay, S., & Chatterji, U. (2020). Arsenic-induced immunomodulatory effects disorient the survival-death interface by stabilizing the Hsp90/Beclin1 interaction. *Chemosphere*, 238, 124647. <https://doi.org/10.1016/j.chemosphere.2019.124647>
- Jollow, D. J., Mitchell, J. R., Zampaglione, N., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis: Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11(3), 151–169. <https://doi.org/10.1159/000136485>
- Kadirvel, R., Sundaram, K., Mani, S., Samuel, S., Elango, N., & Panneerselvam, C. (2007). Supplementation of ascorbic acid and α -tocopherol prevents arsenic-induced protein oxidation and DNA damage induced by arsenic in rats. *Human & Experimental Toxicology*, 26(12), 939–946. <https://doi.org/10.1177/0960327107087909>
- LeBel, C. P., Ischiropoulos, H., & Bondy, S. C. (1992). Evaluation of the probe 2',7'-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology*, 5(2), 227–231. <https://doi.org/10.1021/tx00026a012>
- Maity, J., Pal, P., Pal, R., & Mukhopadhyay, P. K. (2024). Co-administration of L-ascorbic acid and α -tocopherol alleviates arsenic-induced immunotoxicities in the thymus and spleen by dwindling oxidative stress-induced inflammation. *Biological Trace Element Research*, 202(5), 2199–2227. <https://doi.org/10.1007/s12011-023-03841-7>
- Mebius, R. E., & Kraal, G. (2005). Structure and function of the spleen. *Nature Reviews Immunology*, 5(8), 606–616. <https://doi.org/10.1038/nri1669>
- Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170–3175. [https://doi.org/10.1016/S0021-9258\(19\)45228-9](https://doi.org/10.1016/S0021-9258(19)45228-9)
- Ola-Davies, O. E., & Akinrinde, A. S. (2016). Acute sodium arsenite-induced hematological and biochemical changes in Wistar rats: Protective effects of ethanol extract of *Ageratum conyzoides*. *Pharmacognosy Research*, 8(Suppl 1), S26–S30. <https://doi.org/10.4103/0974-8490.178645>
- Oyagbemi, A. A., Omobowale, T. O., Akinrinde, A. S., Saba, A. B., Ogunpolu, B. S., & Daramola, O. (2017). Lack of reversal of sodium arsenite-induced hypertension and cardiovascular abnormalities by L-arginine and kolaviron in male Wistar rats. *Cardiovascular Toxicology*, 17(4), 438–448. <https://doi.org/10.1007/s12012-017-9402-y>

- Rahmani, S., Naraki, K., Roohbakhsh, A., Hayes, A. W., & 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. <https://doi.org/10.1002/fsn3.3041>
- Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1863(12), 2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>
- Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 179(4073), 588–590. <https://doi.org/10.1126/science.179.4073.588>
- Tveden-Nyborg, P., Bergmann, T. K., Jessen, N., Simonsen, U., & Lykkesfeldt, J. (2025). BCPT 2026 policy for experimental and clinical studies. *Basic & Clinical Pharmacology & Toxicology*, 137(6), e70159. <https://doi.org/10.1111/bcpt.70159>
- Varshney, R., & Kale, R. K. (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *International Journal of Radiation Biology*, 58(5), 733–743. <https://doi.org/10.1080/09553009014552121>
- Wang, H., & Joseph, J. A. (1999). Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine*, 27(5-6), 612–616. [https://doi.org/10.1016/s0891-5849\(99\)00107-0](https://doi.org/10.1016/s0891-5849(99)00107-0)
- Yan, N., Xu, G., Zhang, C., Liu, X., Li, X., Sun, L., Wang, D., Duan, X., & Li, B. (2020). Chronic arsenic exposure induces the time-dependent modulation of inflammation and immunosuppression in spleen. *Cell & Bioscience*, 10, 91. <https://doi.org/10.1186/s13578-020-00448-6>
- Zahorec, R. (2021). Neutrophil-to-lymphocyte ratio, past, present and future perspectives. *Bratislavské Lekárske Listy*, 122(7), 474–488. https://doi.org/10.4149/BLL_2021_078
- Zamani, M. M., Mortazavi, S. H., Monajjemzadeh, M., Piranfar, V., Aalidaeijavadi, Z., & Bakhtiarian, A. (2020). Protective effect of combined long time administration of selenium and vitamin C on liver and kidney toxicity of cadmium in rats. *Iranian Journal of Pathology*, 15(4), 271–280. <https://doi.org/10.30699/IJP.2020.135777.2489>
- Zheng, P., Zhou, B., Zheng, Y., Ke, Y., Zhang, W., Xing, M., & Zhao, H. (2025). Unveiling the toxic mechanism of arsenic on the spleen of *Cyprinus carpio* and the antagonistic role of zinc via the P38 MAPK/Nrf2/HO-1 pathway. *Biological Trace Element Research*, 203(11), 5788–5799. <https://doi.org/10.1007/s12011-025-04603-3>