

# Subchronic Toxicity Assessment of Arsenic-Contaminated Rice Following Repeated Oral Administration in Wistar Rats

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## ABSTRACT

Arsenic is a naturally occurring metalloid with well-established toxic and carcinogenic properties, and dietary exposure through rice (*Oryza sativa* L.) represents a significant public health concern, particularly in populations with high rice consumption. Flooded paddy cultivation increases arsenic bioavailability, facilitating its accumulation in rice grains. While chemical monitoring and risk assessment indices are commonly used to estimate arsenic exposure, these approaches provide limited insight into the biological effects of long-term consumption. This study aimed to evaluate the subchronic toxicity of arsenic-contaminated rice using a 90-day oral exposure model in Wistar rats, focusing on toxicological endpoints relevant to food safety assessment. Rice samples were selected using a conservative worst-case exposure strategy based on inductively coupled plasma–mass spectrometry (ICP-MS) arsenic profiling across several regencies in North Sumatra, Indonesia. Red, brown, and white rice samples with the highest arsenic concentrations in their respective categories were administered orally to female Wistar rats at doses of 8.1, 16.2, and 24.3 g/kg body weight per day for 90 consecutive days. A negative control group received 0.5% carboxymethyl cellulose sodium, while a positive control group received inorganic arsenic (0.3 mg/kg body weight). Clinical signs, body-weight changes, hematological parameters, serum biochemical markers of hepatic and renal function, and histopathological alterations in the liver and kidneys were evaluated. No mortality or severe clinical toxicity was observed in rice-treated groups. Body-weight gain, relative organ weights, hematological indices, and renal biomarkers remained comparable to controls. Mild elevations in hepatic enzymes and focal hepatocellular alterations were observed only at the highest brown rice dose. In conclusion, subchronic oral exposure to arsenic-contaminated rice resulted in minimal systemic toxicity under the conditions tested, with the liver identified as the primary target organ at higher exposure levels. These findings provide biologically relevant evidence to support food safety evaluation of arsenic-contaminated rice.

**Keywords:** Arsenic; Rice; Subchronic toxicity; Histopathology; Rat

## INTRODUCTION

Arsenic is a naturally occurring metalloid widely distributed in the environment through both natural geochemical processes and anthropogenic activities, including mining, industrial discharge, and agricultural practices<sup>1</sup>. Chronic exposure to arsenic has been associated with a wide range of adverse health effects, such as hepatotoxicity, nephrotoxicity, cardiovascular disorders, neurotoxicity, immunological dysfunction, and an increased risk of various cancers<sup>2</sup>. Accordingly, inorganic arsenic has been classified by the International Agency for Research on Cancer as a Group 1 human carcinogen, highlighting its substantial global public health relevance<sup>3</sup>.

Dietary intake represents a major route of arsenic exposure, particularly in regions where rice (*Oryza sativa* L.) constitutes a staple food. Rice plants are uniquely prone to arsenic accumulation because they are typically cultivated under flooded conditions, which promote the mobilization of arsenic in soil and irrigation water. Under anaerobic paddy environments, arsenic becomes more bioavailable and is readily taken up by rice roots, subsequently accumulating in the grain<sup>4</sup>. As a result, rice and rice-based products have been recognized as significant contributors to chronic arsenic exposure, especially among populations with high daily rice consumption<sup>5</sup>.

To date, most studies evaluating arsenic exposure from rice have relied primarily on chemical analysis and risk assessment models, including estimated daily intake and excess cancer risk calculations. While these approaches are essential for exposure characterization, they provide limited insight into the biological responses and organ-specific effects that may arise following prolonged dietary intake. Importantly, arsenic exposure through food matrices such as rice may differ toxicokinetically from exposure to inorganic arsenic salts, owing to interactions with dietary components and differences in bioavailability<sup>6</sup>.

Subchronic toxicity studies offer a valuable framework for investigating the health effects of repeated exposure over an extended period, bridging the gap between short-term toxicity tests and chronic exposure scenarios<sup>7</sup>. The 90-day oral toxicity study is widely accepted as a standard experimental model to assess medium-term toxicological effects, identify target organs, and detect early pathological alterations that may precede overt toxicity. In the context of arsenic exposure, the liver and kidneys are considered primary target organs due to their central roles in arsenic metabolism, biotransformation, and excretion. Hepatic toxicity is frequently linked to oxidative stress, mitochondrial dysfunction, and inflammatory signaling, whereas renal toxicity may involve tubular degeneration and impaired filtration capacity.

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Despite growing concern regarding arsenic contamination in rice, experimental evidence addressing the subchronic toxicological effects of arsenic-contaminated rice itself, rather than inorganic arsenic alone, remains limited. Moreover, few studies have incorporated geographically informed sample selection that reflects realistic worst-case exposure scenarios. Therefore, the present study was designed to evaluate the subchronic toxicity of arsenic-contaminated rice following repeated oral administration for 90 days in Wistar rats. By integrating clinical observations, hematological and biochemical analyses, and histopathological assessment of the liver and kidneys, this study aims to provide biologically relevant data to support food safety evaluation and risk assessment of arsenic-contaminated rice.

## MATERIALS AND METHODS

### Experimental Animals

Female Wistar rats aged 8–10 weeks, weighing 150–200 g, were obtained from an accredited laboratory animal breeding facility. Animals were acclimatized prior to experimentation and housed under controlled environmental conditions, including a temperature of 22–25 °C, relative humidity of 50–60%, and a 12 h light–dark cycle. Standard laboratory chow and drinking water were provided *ad libitum* throughout the study period. All experimental procedures were conducted in strict accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee.

### Test Materials and Dose Selection

Rice samples used in this subchronic toxicity study were selected based on arsenic concentration data obtained through inductively coupled plasma–mass spectrometry (ICP-MS) analysis of rice collected from multiple regencies in North Sumatra Province, Indonesia. To represent a conservative worst-case exposure scenario, each rice category was sourced from the regency exhibiting the highest arsenic concentration within that category. Accordingly, red rice was obtained from Humbang Hasundutan Regency, brown rice from Mandailing Natal Regency, and white rice from Karo Regency. Although arsenic concentrations in white rice were comparatively lower than those in red and brown rice, samples from Karo Regency were selected due to their association with the highest excess cancer risk within the white rice category.

Rice samples containing naturally occurring arsenic were prepared as test materials. Dose levels were determined based on estimated human rice consumption and subsequently converted to rat-equivalent doses using body weight–based scaling. Rats received oral rice suspensions at doses of 8.1, 16.2, and 24.3 g/kg body weight per day. A negative control group received 0.5% carboxymethyl cellulose sodium (CMC-Na), while a positive control group was administered inorganic arsenic at a dose of 0.3 mg/kg body weight. Dose levels were derived from estimated human rice-consumption scenarios and converted to rat-equivalent doses using a body surface area (BSA)–based interspecies conversion method. Daily human rice intake was first expressed as dry-rice equivalents to minimize variability associated with cooking water content. Using a reference adult body weight of 60 kg, three intake scenarios were defined to represent typical, high, and conservative worst-case rice consumption. These were converted to human doses expressed as g/kg BW/day. This method accounts for interspecies differences in metabolic scaling and has been widely applied in toxicological and pharmacological studies. The resulting rat doses of 8.1, 16.2, and 24.3 g/kg BW/day were therefore selected to represent BSA-scaled rat equivalents of realistic to worst-case human dietary exposure scenarios rather than supraphysiological or pharmacological dosing.

### Subchronic Toxicity Study Design

Animals were randomly allocated into experimental groups and treated once daily via oral gavage for 90 consecutive days. Clinical observations were conducted daily to monitor potential signs of toxicity, including alterations in behavior, locomotor activity, posture, salivation, and gastrointestinal disturbances. Body weight was recorded weekly throughout the experimental period to evaluate general health status and treatment-related effects<sup>8,9</sup>.

### Hematological and Biochemical Analysis

At the conclusion of the treatment period, blood samples were collected for hematological and serum biochemical analyses. Hematological parameters assessed included hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, and platelet count. Serum biochemical analyses were performed using automated analyzers to evaluate hepatic function, as indicated by alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as renal function markers, including urea and creatinine<sup>10,11</sup>.

### Histopathological Examination

Following blood collection, animals were humanely euthanized, and the liver and kidneys were excised, weighed, and fixed in 10% neutral-buffered formalin. Fixed tissues were processed using standard paraffin-embedding procedures, sectioned at a thickness of 4–5 µm, and stained with hematoxylin and eosin. Histopathological evaluation was performed under light microscopy to identify structural and cellular alterations, including hepatocellular necrosis, fibrosis, inflammatory cell infiltration, and renal tubular degeneration<sup>12</sup>.

### Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Prior to inferential analysis, data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. When both assumptions were satisfied, group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Clinical Observations and Mortality

The clinical signs monitored during the 90-day subchronic toxicity study included behavioral changes, neurological manifestations, and general physiological responses. Observations were conducted daily to detect potential adverse effects associated with repeated oral administration of arsenic-contaminated rice. The observed toxic signs recorded during the preliminary toxicity assessment are summarized in Table 1.

No mortality was observed in any treatment group throughout the exposure period. Rats administered white, red, and brown rice remained active and exhibited normal grooming behavior, with no severe clinical manifestations such as tremors, convulsions, paralysis, or persistent diarrhea. These findings indicate that repeated oral administration of arsenic-contaminated rice did not induce overt systemic toxicity under the experimental conditions employed. In contrast, animals in the positive control group receiving inorganic arsenic exhibited clear signs of toxicity, including tremors, excessive salivation, diarrhea, lethargy, abnormal gait, and reduced appetite, confirming the well-established toxic profile of inorganic arsenic.

The mild lethargy and reduced appetite observed in rats receiving the highest dose of brown rice suggest a subtle physiological response

**Table 1. Observation of toxic signs during the preliminary toxicity study**

No.	Observed Toxic Signs	Negative Control (CMC-Na)	White Rice	Red Rice	Brown Rice	Positive Control (Arsenic)
1	Tremor	Not observed	Not observed	Not observed	Not observed	Observed
2	Salivation	Not observed	Not observed	Not observed	Not observed	Observed
3	Diarrhea	Not observed	Not observed	Not observed	Not observed	Observed
4	Lethargy	Not observed	Not observed	Not observed	Mild	Observed
5	Abnormal gait	Not observed	Not observed	Not observed	Not observed	Observed
6	Loss of appetite	Not observed	Not observed	Not observed	Mild	Observed
7	Mortality	None	None	None	None	None

rather than pronounced toxicity. Brown rice is known to retain higher arsenic concentrations due to the presence of the bran layer. However, higher arsenic retention in the grain does not necessarily translate into greater tissue injury. Although brown rice generally retains higher total arsenic because the bran layer is preserved, total arsenic concentration alone does not necessarily predict the magnitude of tissue injury. The biological response to dietary arsenic depends on multiple factors, including arsenic speciation (inorganic versus methylated forms), gastrointestinal bioaccessibility and absorption, and competing effects from the food matrix. In addition, brown rice contains higher levels of dietary fiber, phenolic compounds, and antioxidant phytochemicals, which may reduce arsenic bioavailability and attenuate oxidative stress and inflammatory responses. Accordingly, the relatively mild histopathological changes observed in the brown rice-treated groups should not be interpreted as evidence that higher-arsenic brown rice is inherently safer, but rather that under the present exposure conditions the effective internal dose and tissue susceptibility may have been modulated by matrix-related protective factors. Future studies measuring arsenic speciation in rice and arsenic biomarkers in blood, urine, and target tissues are warranted to directly link dietary arsenic retention with internal exposure and organ injury.

These findings are consistent with previous subchronic feeding studies reporting minimal overt clinical toxicity and no treatment-related mortality following dietary exposure to arsenic-contaminated rice, despite detectable arsenic accumulation in target organs<sup>13</sup>. In contrast, direct administration of inorganic arsenic has been widely associated with pronounced neurological and gastrointestinal symptoms due to its rapid absorption and interference with cellular energy metabolism<sup>14</sup>. Collectively, the present results emphasize that dietary arsenic exposure via rice consumption may not elicit immediate or severe clinical toxicity during medium-term exposure, underscoring the importance of integrating clinical observations with hematological, biochemical, and histopathological endpoints to achieve a comprehensive food safety evaluation.

### Body Weight and Relative Organ Weights

Body weight changes are a sensitive and widely accepted indicator of general systemic toxicity in subchronic and chronic exposure studies. In the present study, rats administered white, red, and brown rice at all tested doses exhibited gradual and comparable increases in body weight over the 90-day exposure period (Table 2). The magnitude of weight gain observed in rice-treated groups was similar to that of the negative control group, indicating that repeated oral administration of arsenic-contaminated rice did not adversely affect growth, nutritional status, or overall health of the animals.

In contrast, rats in the positive control group receiving inorganic arsenic showed a marked reduction in body weight, consistent with systemic toxicity. Weight loss in arsenic-exposed animals has been attributed to impaired nutrient absorption, increased metabolic stress, and gastrointestinal toxicity, which collectively disrupt energy balance and growth regulation. Previous studies have reported significant body weight suppression following subchronic exposure to inorganic

arsenic, reflecting its high bioavailability and interference with cellular metabolism and mitochondrial function.

The absence of significant differences in body weight gain among rice-treated groups suggests that arsenic exposure through dietary rice matrices may result in lower toxic burden compared with direct inorganic arsenic administration. Although brown rice is known to contain higher arsenic levels due to the retention of the bran layer, rats receiving brown rice did not exhibit dose-dependent weight loss or growth retardation. This finding supports the hypothesis that arsenic bioavailability is modulated by the food matrix, where dietary fiber, minerals, and phytochemicals may reduce gastrointestinal absorption or alter arsenic biotransformation<sup>15</sup>.

Relative organ weight analysis further supports the lack of overt systemic toxicity in rice-treated groups. Relative liver and kidney weights did not differ significantly between treated and control animals, indicating the absence of organ hypertrophy or atrophy. Changes in relative organ weight are often associated with adaptive or pathological responses to toxic insult, particularly in the liver and kidneys as primary target organs for arsenic metabolism and excretion<sup>16</sup>. The stability of these parameters suggests that prolonged dietary exposure to arsenic-containing rice did not elicit gross organ-level toxicity under the conditions tested.

Collectively, the body weight and relative organ weight findings indicate that subchronic exposure to arsenic via rice consumption produced minimal systemic effects, in contrast to the pronounced toxicity observed following inorganic arsenic administration. These results are consistent with previous dietary arsenic studies and emphasize the importance of evaluating toxicological outcomes within realistic exposure scenarios, rather than extrapolating risk solely from inorganic arsenic models.

### Hematological Parameters

Hematological parameters are sensitive indicators of systemic toxicity, particularly for substances such as arsenic that are known to interfere with hematopoiesis, erythrocyte integrity, and immune function. In the present study, repeated oral administration of white, red, and brown rice for 90 days did not result in marked hematological disturbances. As shown in Table 3, values for hemoglobin, hematocrit, red blood cell count, white blood cell count, and platelet count in rice-treated groups largely remained within normal physiological ranges and were comparable to those of the negative control group.

Animals receiving inorganic arsenic exhibited clear alterations in several hematological parameters, including reductions in hemoglobin levels, hematocrit values, erythrocyte counts, and platelet numbers. Such changes are characteristic of arsenic-induced hematotoxicity and have been attributed to oxidative damage to red blood cell membranes, disruption of heme synthesis, and suppression of bone marrow activity, as reported in earlier experimental and epidemiological studies.

Within the rice-treated groups, minor variations in erythrocyte indices and leukocyte counts were observed across different doses;

**Table 2. Final combined summary of body weight changes of rats during the 90-day subchronic toxicity study**

Treatment	Dose (g/kg BW)	Day 0 (g)	Day 90 (g)	Δ Body Weight (g)
Negative control (CMC-Na 0.5%)	–	151.66 ± 1.52	227.66 ± 1.52	+76.00
	8.1	153.33 ± 1.52	228.33 ± 1.52	+75.00
White rice	16.2	154.33 ± 1.52	227.66 ± 1.52	+73.33
	24.3	154.66 ± 2.08	229.33 ± 1.52	+74.67
	8.1	151.66 ± 1.52	226.66 ± 1.52	+75.00
Red rice	16.2	149.66 ± 1.52	230.33 ± 1.52	+80.67
	24.3	149.66 ± 2.08	227.33 ± 2.08	+77.67
	8.1	150.33 ± 1.52	228.66 ± 1.52	+78.33
Brown rice	16.2	149.33 ± 2.08	227.66 ± 2.08	+78.33
	24.3	149.44 ± 2.08	229.66 ± 2.08	+80.22
Positive control (Arsenic)	–	150.66 ± 1.52	105.33 ± 1.52	–45.33

Data are presented as mean ± SD (n = 4).

**Table 3. Hematological parameters of rats in the subchronic toxicity study**

Parameter	CMC-Na 0.5%	White rice 8.1 g/kg BW	Red rice 8.1 g/kg BW	Brown rice 8.1 g/kg BW	White rice 16.2 g/kg BW	Red rice 16.2 g/kg BW	Brown rice 16.2 g/kg BW	White rice 24.3 g/kg BW	Red rice 24.3 g/kg BW	Brown rice 24.3 g/kg BW	Satellite white rice 24.3 g/kg BW	Satellite red rice 24.3 g/kg BW	Satellite brown rice 24.3 g/kg BW	Satellite CMC-Na 0.5%	Arsenic	Satellite arsenic
Hemoglobin (g/dL)	*14.56 ± 0.01	13.85 ± 0.03	13.67 ± 0.02	13.98 ± 0.01	*12.95 ± 0.02	12.58 ± 0.03	13.06 ± 0.04	11.55 ± 0.03	11.05 ± 0.03	11.85 ± 0.01	*13.55 ± 0.02	13.25 ± 0.03	13.65 ± 0.03	14.47 ± 0.02	10.57 ± 0.04	*12.87 ± 0.01
Hematocrit (%)	42.22 ± 0.04	39.23 ± 0.03	*38.49 ± 0.03	39.13 ± 0.02	*36.18 ± 0.02	*38.18 ± 0.02	37.26 ± 0.03	32.24 ± 0.02	30.33 ± 0.03	*34.20 ± 0.02	*36.23 ± 0.03	*34.20 ± 0.02	*34.10 ± 0.02	*41.27 ± 0.04	*29.26 ± 0.04	*34.15 ± 0.02
White blood cells (×10 <sup>3</sup> /μL)	9.35 ± 0.03	8.75 ± 0.02	8.32 ± 0.02	*9.06 ± 0.05	*7.90 ± 0.01	*7.36 ± 0.01	*8.26 ± 0.01	6.84 ± 0.02	6.24 ± 0.03	7.45 ± 0.03	*7.85 ± 0.02	*7.25 ± 0.02	*8.19 ± 0.06	9.19 ± 0.03	5.81 ± 0.03	7.07 ± 0.05
Red blood cells (×10 <sup>6</sup> /μL)	*8.05 ± 0.04	7.63 ± 0.02	*7.33 ± 0.02	7.74 ± 0.03	*7.25 ± 0.03	6.85 ± 0.01	7.31 ± 0.03	6.66 ± 0.04	6.62 ± 0.04	6.96 ± 0.02	*7.19 ± 0.04	6.65 ± 0.04	*7.44 ± 0.03	7.91 ± 0.06	5.78 ± 0.02	*6.53 ± 0.01
Platelets (×10 <sup>3</sup> /μL)	460.54 ± 0.01	430.34 ± 0.03	410.25 ± 0.03	440.24 ± 0.03	400.77 ± 0.01	370.85 ± 0.03	415.70 ± 0.03	*395.26 ± 0.02	*364.16 ± 0.01	420.25 ± 0.03	*395.23 ± 0.03	*365.20 ± 0.01	430.09 ± 0.01	328.34 ± 0.01	220.12 ± 0.02	260.10 ± 0.02
MCV (fL)	*87.22 ± 0.02	85.27 ± 0.02	83.16 ± 0.03	*86.22 ± 0.05	83.19 ± 0.06	81.35 ± 0.02	84.15 ± 0.03	81.21 ± 0.06	78.37 ± 0.03	82.24 ± 0.04	84.34 ± 0.03	*82.25 ± 0.02	85.33 ± 0.02	*86.23 ± 0.02	76.21 ± 0.01	80.24 ± 0.02
MCH (pg)	29.07 ± 0.01	28.55 ± 0.02	28.06 ± 0.005	28.74 ± 0.02	27.87 ± 0.01	27.24 ± 0.03	28.25 ± 0.01	27.06 ± 0.01	26.34 ± 0.02	27.55 ± 0.02	27.95 ± 0.02	*27.43 ± 0.02	28.33 ± 0.02	28.84 ± 0.01	25.54 ± 0.03	*26.82 ± 0.01
MCH concentration (%)	33.57 ± 0.01	33.07 ± 0.01	32.84 ± 0.02	33.25 ± 0.03	32.56 ± 0.01	32.07 ± 0.005	32.77 ± 0.01	32.06 ± 0.01	31.54 ± 0.03	32.35 ± 0.02	32.74 ± 0.03	*32.23 ± 0.02	33.06 ± 0.02	33.35 ± 0.04	30.04 ± 0.03	*31.56 ± 0.01
Eosinophils (%)	2.54 ± 0.02	2.18 ± 0.02	2.06 ± 0.01	2.33 ± 0.02	2.04 ± 0.03	*1.87 ± 0.01	2.14 ± 0.02	1.77 ± 0.01	1.53 ± 0.02	1.97 ± 0.02	2.04 ± 0.03	*1.85 ± 0.02	2.26 ± 0.02	2.46 ± 0.03	1.34 ± 0.03	1.77 ± 0.01
Monocytes (%)	*6.08 ± 0.01	6.54 ± 0.02	6.86 ± 0.02	6.35 ± 0.03	6.06 ± 0.02	*6.24 ± 0.03	6.03 ± 0.01	5.43 ± 0.03	5.06 ± 0.02	5.65 ± 0.02	5.95 ± 0.03	5.54 ± 0.02	6.14 ± 0.03	6.23 ± 0.03	4.87 ± 0.03	5.32 ± 0.04
Basophils (%)	*0.68 ± 0.03	0.76 ± 0.02	*0.84 ± 0.02	0.65 ± 0.02	*0.64 ± 0.003	*0.74 ± 0.02	0.65 ± 0.03	0.54 ± 0.02	*0.43 ± 0.03	*0.55 ± 0.02	*0.65 ± 0.02	0.55 ± 0.02	*0.67 ± 0.02	*0.65 ± 0.02	*0.36 ± 0.02	0.45 ± 0.03

however, these changes did not follow a consistent dose response pattern and remained within established reference ranges. This pattern suggests physiological variability or adaptive responses rather than toxicologically meaningful effects<sup>17</sup>. Although slight reductions in platelet counts were observed at higher rice doses, these values were substantially higher than those measured in the inorganic arsenic group, indicating the absence of clinically relevant thrombocytopenia.

Leukocyte differentials, including eosinophils, monocytes, and basophils, also remained relatively stable following rice administration, with no indication of overt inflammatory activation or immunosuppression. This preservation of leukocyte homeostasis differs from the immune alterations commonly associated with inorganic arsenic exposure and supports the view that arsenic bioavailability is substantially modulated when exposure occurs through a dietary matrix<sup>18</sup>.

Taken together, the hematological data demonstrate that subchronic exposure to arsenic-contaminated rice produced minimal hematological

perturbations compared with direct inorganic arsenic exposure. These findings reinforce the concept that the toxicological impact of arsenic is strongly influenced by its chemical form and mode of exposure, underscoring the need to interpret dietary arsenic risk within realistic consumption scenarios and in conjunction with biochemical and histopathological outcomes.

### Serum Biochemical Parameters

Serum biochemical markers provide critical information regarding functional integrity of the liver and kidneys, which are primary target organs for arsenic metabolism and excretion. In the present study, evaluation of serum biochemical parameters following 90 days of oral administration of arsenic-contaminated rice revealed largely preserved hepatic and renal function in rice-treated animals (Table 4). Serum urea and creatinine levels remained comparable to those of the negative control group across all rice types and doses, indicating the absence of clinically relevant renal dysfunction during the subchronic exposure period.

**Table 4. Serum biochemical parameters (ALT, AST, ALP, urea, and creatinine) of rats following 90-day treatment.**

Treatment group	Total protein (g/dL)	Direct bilirubin (mg/dL)	Aspartate aminotransferase (U/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
CMC-Na 0.5% (negative control)	6.55 ± 0.03	0.25 ± 0.01	46.33 ± 1.52	40.66 ± 2.08	91.33 ± 1.52	20.09 ± 0.08	0.87 ± 0.01
White rice 8.1 g/kg body weight	8.27 ± 0.04	0.26 ± 0.01	54.66 ± 1.52	49.66 ± 1.52	108.60 ± 1.52	25.55 ± 0.38	1.06 ± 0.05
Red rice 8.1 g/kg body weight	6.25 ± 0.03	0.38 ± 0.02	58.00 ± 1.00	52.33 ± 1.15	113.60 ± 1.52	26.78 ± 0.08	1.15 ± 0.02
Brown rice 8.1 g/kg body weight	6.29 ± 0.02	0.29 ± 0.03	59.33 ± 1.52	53.33 ± 2.08	114.33 ± 1.52	25.66 ± 0.02	1.05 ± 0.01
White rice 16.2 g/kg body weight	6.04 ± 0.03	0.35 ± 0.03	65.33 ± 1.52	60.33 ± 1.52	130.30 ± 1.52	25.96 ± 0.03	1.36 ± 0.03
Red rice 16.2 g/kg body weight	5.83 ± 0.03	0.43 ± 0.01	71.33 ± 1.52	62.33 ± 1.52	140.30 ± 1.52	32.10 ± 0.04	1.47 ± 0.01
Brown rice 16.2 g/kg body weight	6.15 ± 0.02	0.41 ± 0.02	67.30 ± 1.52	63.60 ± 1.52	137.60 ± 1.52	35.74 ± 0.07	1.36 ± 0.04
White rice 24.3 g/kg body weight	5.78 ± 0.03	0.48 ± 0.02	81.33 ± 1.52	73.66 ± 1.52	159.60 ± 1.52	33.07 ± 0.01	1.74 ± 0.03
Red rice 24.3 g/kg body weight	5.53 ± 0.02	0.53 ± 0.01	90.33 ± 1.52	83.60 ± 1.52	172.30 ± 1.52	40.16 ± 0.03	1.96 ± 0.01
Brown rice 24.3 g/kg body weight	5.85 ± 0.03	0.46 ± 0.03	82.66 ± 1.51	76.33 ± 2.08	162.60 ± 2.51	45.82 ± 0.03	1.86 ± 0.01
Satellite white rice 24.3 g/kg body weight	6.24 ± 0.03	0.31 ± 0.01	52.33 ± 1.52	46.33 ± 1.52	103.60 ± 1.52	42.46 ± 0.02	1.06 ± 0.01
Satellite red rice 24.3 g/kg body weight	6.05 ± 0.04	0.33 ± 0.02	56.33 ± 1.52	51.33 ± 1.52	112.60 ± 1.51	24.63 ± 0.02	1.15 ± 0.02
Satellite brown rice 24.3 g/kg body weight	6.21 ± 0.03	0.29 ± 0.01	55.33 ± 1.52	47.33 ± 1.52	107.60 ± 1.52	26.32 ± 0.03	1.06 ± 0.01
Satellite CMC-Na 0.5%	5.04 ± 0.02	0.27 ± 0.01	44.66 ± 1.52	40.66 ± 1.52	92.33 ± 1.52	21.56 ± 0.01	0.96 ± 0.02
Arsenic (positive control)	5.04 ± 0.02	0.59 ± 0.01	110.33 ± 1.52	100.33 ± 1.52	199.60 ± 1.52	50.13 ± 0.05	2.27 ± 0.01
Satellite arsenic	5.55 ± 0.04	0.46 ± 0.02	73.33 ± 3.05	66.33 ± 1.52	130.33 ± 1.52	30.52 ± 0.03	1.37 ± 0.02

Alterations in liver enzyme activities were modest and primarily confined to rats receiving the highest dose of brown rice. Slight elevations in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were observed; however, these changes were not accompanied by marked increases in bilirubin levels or severe reductions in total protein concentrations. Such a biochemical profile suggests mild hepatocellular stress rather than overt hepatotoxicity. Transient or moderate increases in aminotransferase activity are commonly interpreted as adaptive hepatic responses to xenobiotic exposure rather than irreversible liver injury, particularly in the absence of pronounced histopathological damage. Rats exposed to inorganic arsenic in the positive control group displayed pronounced elevations in liver enzymes, urea, and creatinine, reflecting significant hepatic and renal impairment. These findings are consistent with extensive evidence demonstrating that inorganic arsenic induces oxidative stress, mitochondrial dysfunction, and inflammatory signaling pathways that disrupt hepatocellular integrity and renal filtration capacity<sup>19</sup>. The clear biochemical separation between the inorganic arsenic group and rice-treated groups further underscores the substantially lower toxic potency of arsenic when exposure occurs through a dietary matrix.

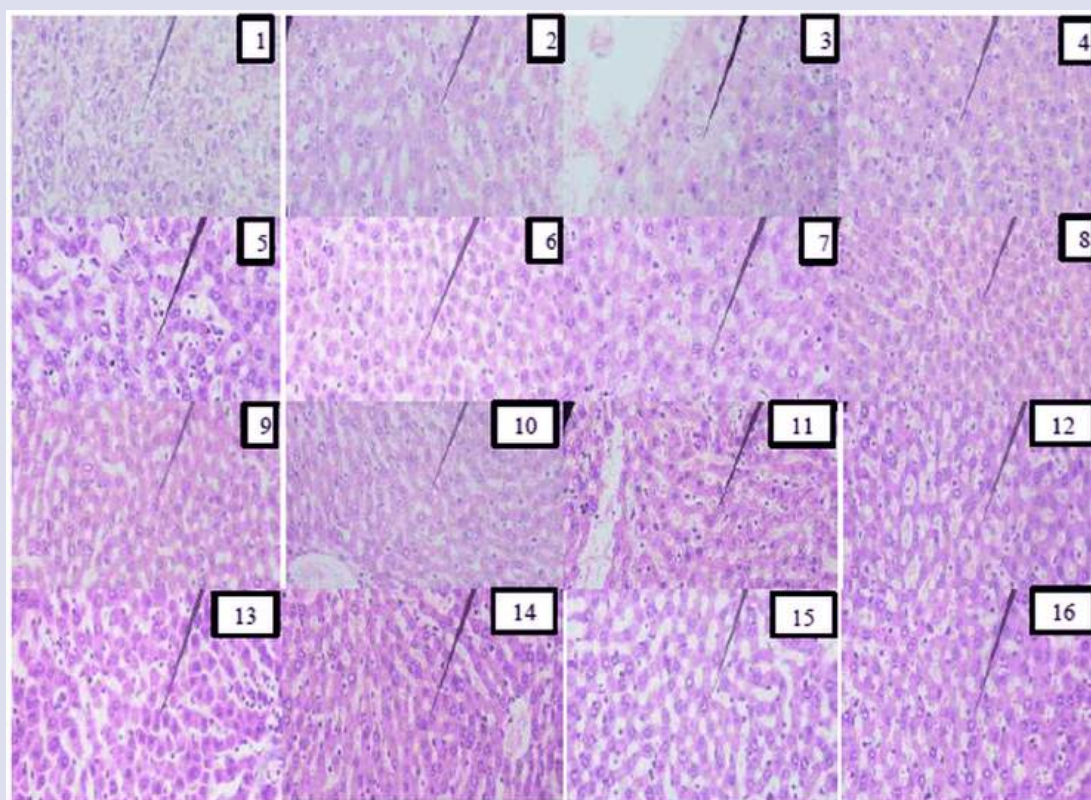
Within rice-treated groups, brown rice tended to elicit slightly higher enzyme activities compared with white and red rice, particularly at the highest dose. This observation aligns with reports that brown rice generally contains higher arsenic concentrations due to retention of the bran layer<sup>20</sup>. Nevertheless, the absence of severe biochemical derangements suggests that arsenic bioavailability and systemic toxicity may be attenuated by interactions with dietary constituents such as fiber, antioxidants, and micronutrients present in whole rice. Similar attenuation effects have been reported in dietary arsenic studies, where

food composition significantly influenced arsenic absorption and metabolic fate.

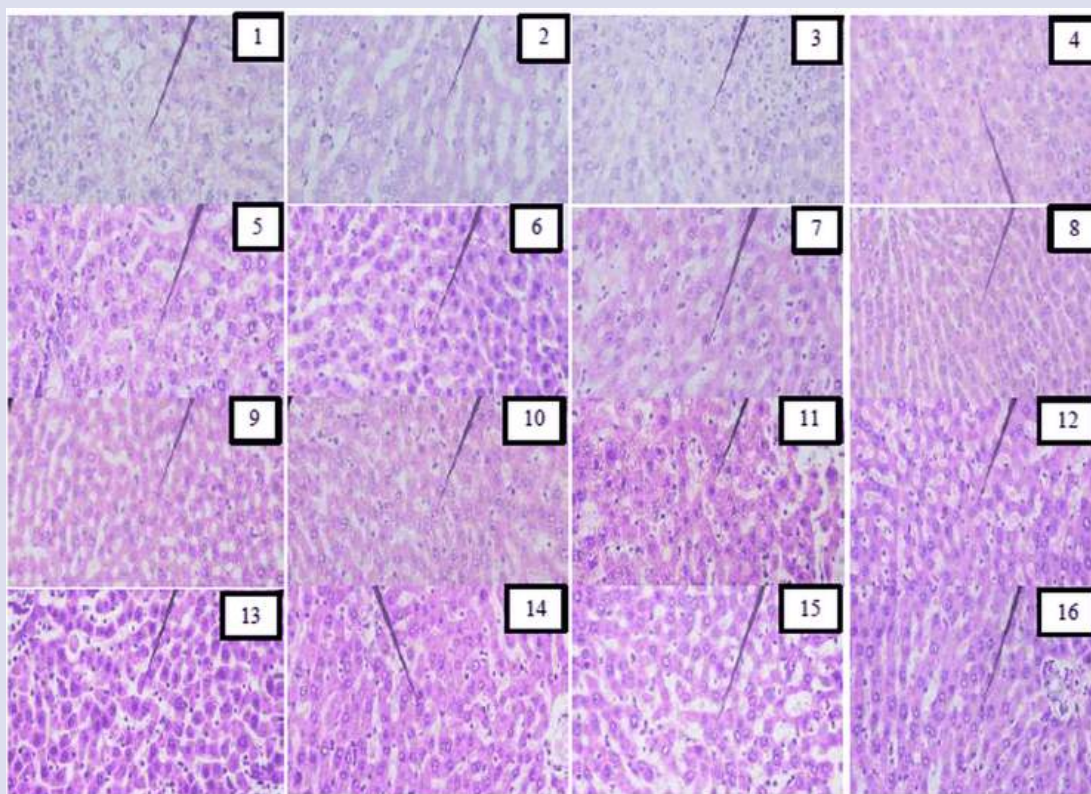
Collectively, the serum biochemical findings indicate that subchronic exposure to arsenic-contaminated rice resulted in minimal hepatic and renal functional impairment under the conditions tested. These results highlight the importance of distinguishing between biochemical adaptations and true toxic injury, and they reinforce the need to interpret liver enzyme elevations in conjunction with histopathological evidence. When considered together, the biochemical and histological data support the conclusion that dietary arsenic exposure via rice poses a lower short- to medium-term toxicological risk compared with direct inorganic arsenic exposure, while still warranting careful evaluation for potential long-term effects.

### Liver Histopathological Findings

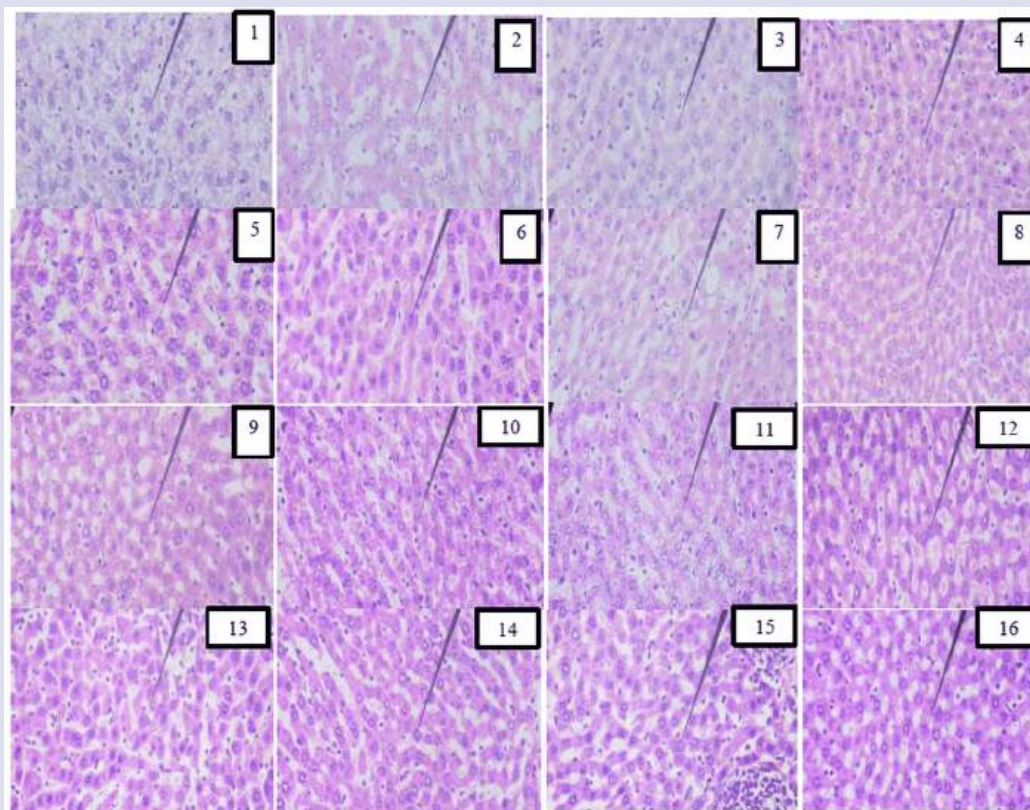
Histopathological examination of liver tissues demonstrated focal hepatocellular necrosis accompanied by early fibrotic changes in rats receiving the highest dose of brown rice. In contrast, liver sections from lower-dose groups and from rats administered white or red rice largely preserved normal hepatic architecture. Examination of kidney tissues across all experimental groups revealed intact glomerular and tubular structures, with no histological evidence of arsenic-induced nephrotoxicity. Representative histopathological findings are presented in Figures 1–5. Panel descriptions are as follows: (1) inorganic arsenic control; (2) CMC-Na control; (3) satellite inorganic arsenic control; (4) satellite CMC-Na control; (5) white rice at 8.1 g/kg body weight; (6) white rice at 16.2 g/kg body weight; (7) white rice at 24.3 g/kg body weight; (8) satellite white rice at 24.3 g/kg body weight; (9) red rice at 8.1 g/kg body weight; (10) red rice at 16.2 g/kg body weight; (11) red



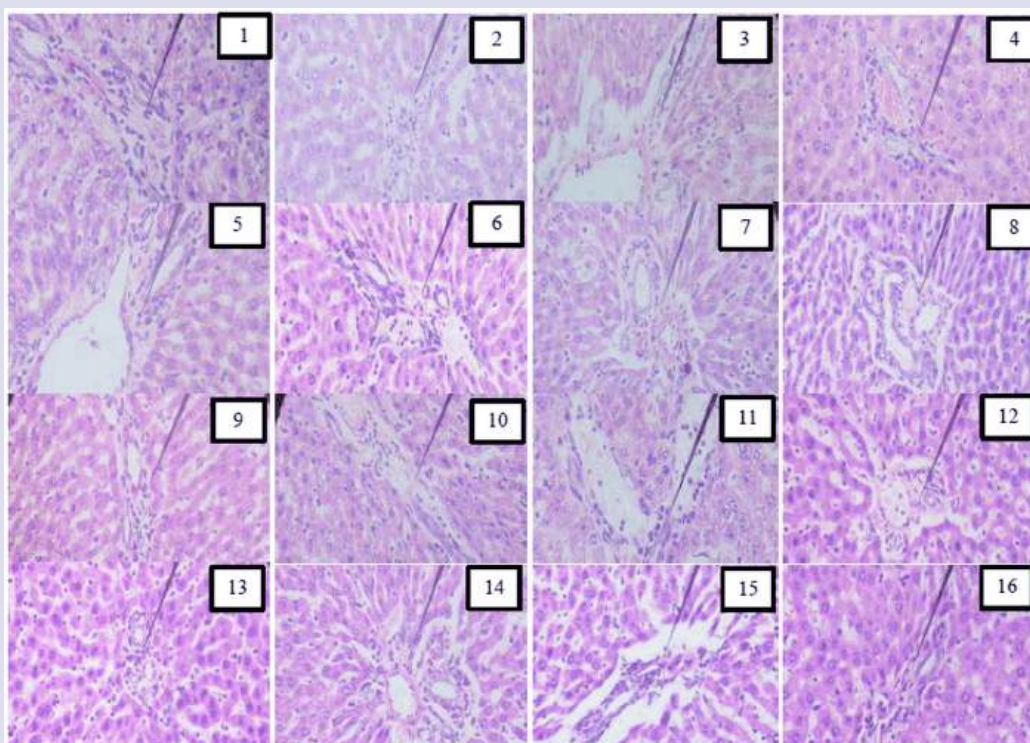
**Figure 1.** Histopathological micrographs of rat liver sections Arrows indicate areas of hepatocellular degeneration characterized by cytoplasmic vacuolization and disrupted hepatic cord architecture.



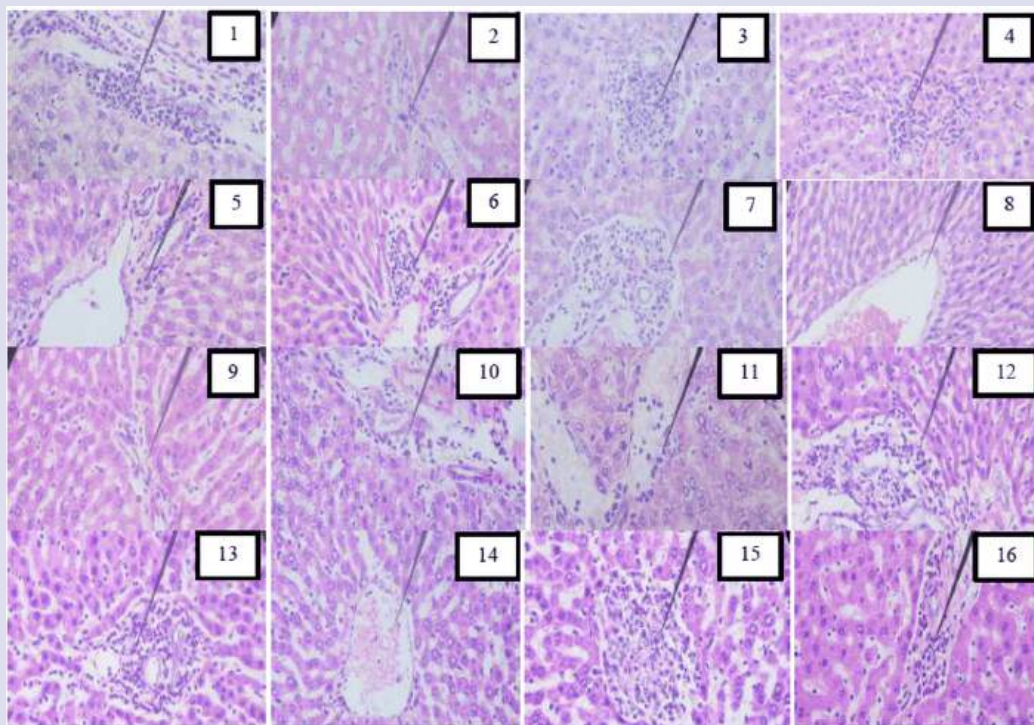
**Figure 2.** Histopathological micrographs of rat liver sections showing hepatic necrosis following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate necrotic areas characterized by loss of cellular integrity, pyknotic nuclei, and disruption of normal hepatic architecture.



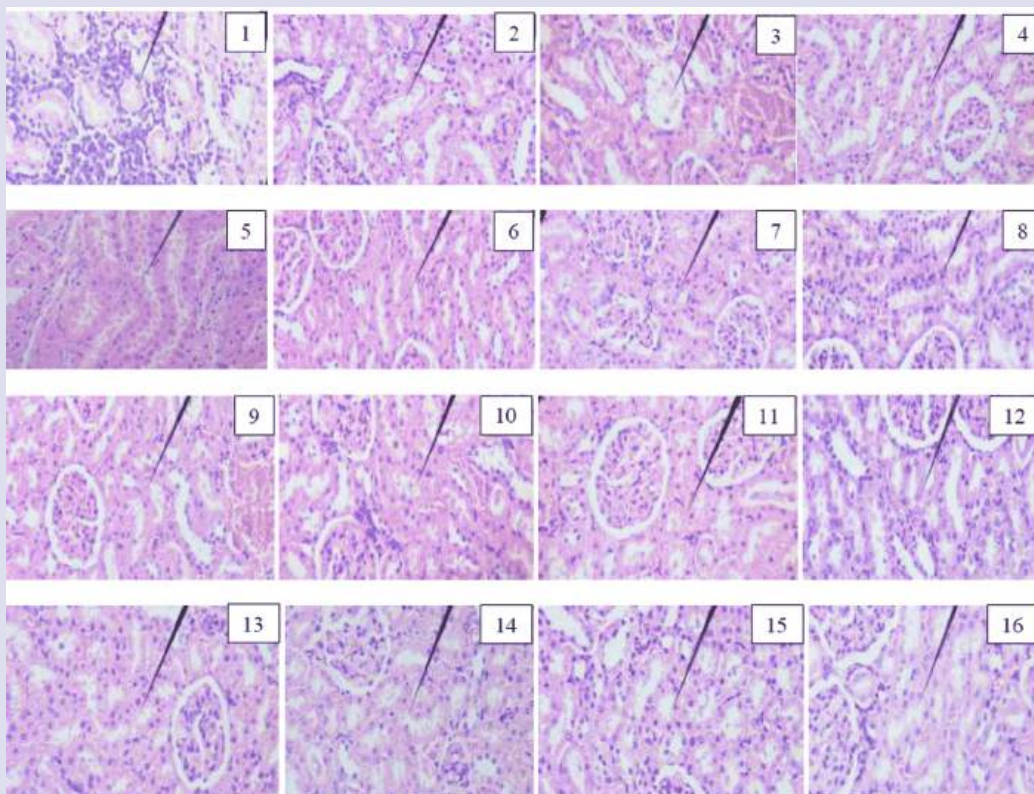
**Figure 3.** Histopathological micrographs of rat liver sections showing hepatic steatosis following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate steatotic changes characterized by cytoplasmic lipid vacuolation, hepatocyte ballooning, and displacement of nuclei.



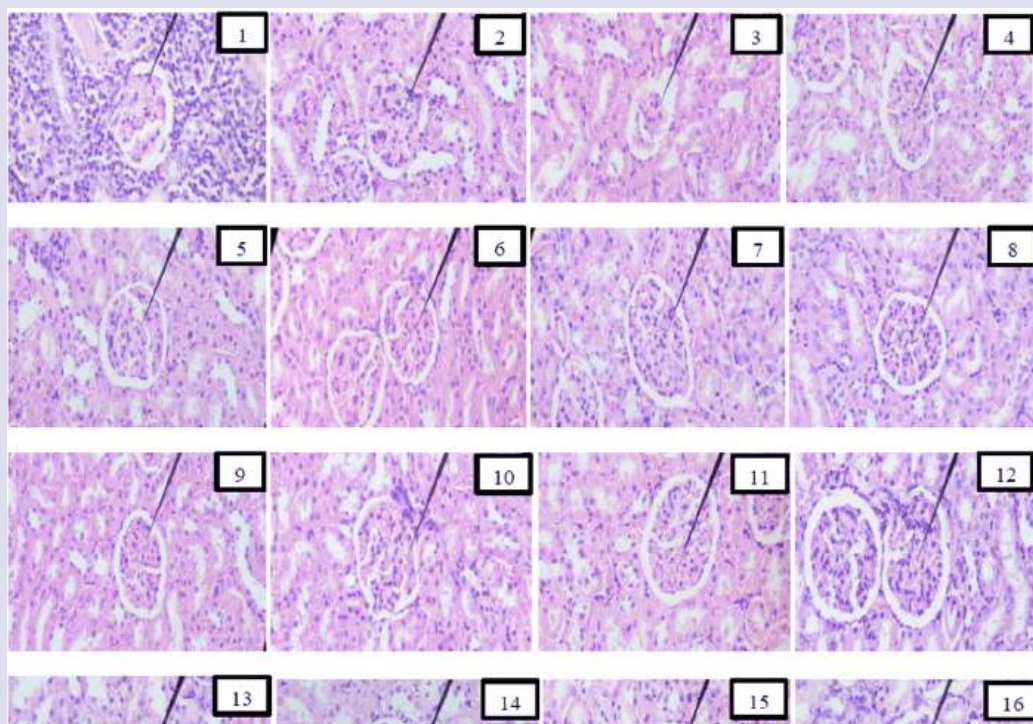
**Figure 4.** Histopathological micrographs of rat liver sections showing hepatic fibrosis following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate fibrotic changes characterized by increased collagen deposition, thickening of hepatic septa, and disruption of normal lobular architecture.



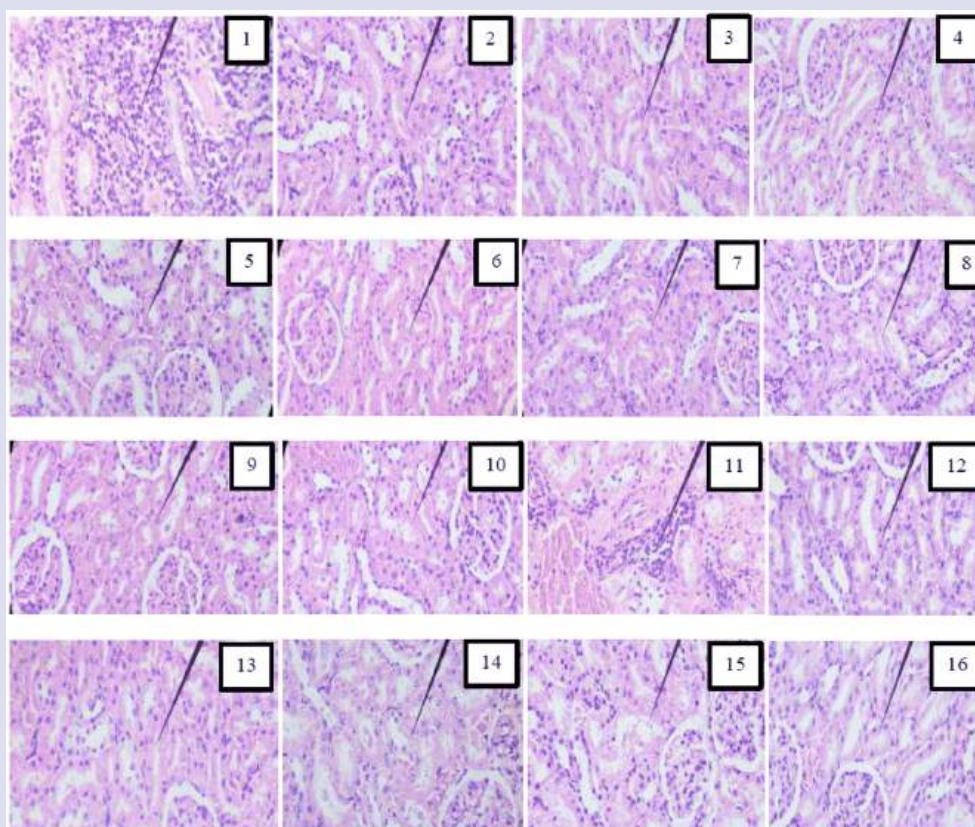
**Figure 5.** Histopathological micrographs of rat liver sections showing hepatic inflammation following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate inflammatory changes characterized by infiltration of inflammatory cells within the hepatic parenchyma and portal areas, accompanied by mild disruption of hepatic architecture.



**Figure 6.** Histopathological micrographs of rat kidney sections showing renal necrosis following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate necrotic changes characterized by tubular epithelial cell degeneration, loss of cellular integrity, and focal disruption of renal architecture.



**Figure 7.** Histopathological micrographs of rat kidney sections showing renal sclerosis following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate sclerotic changes characterized by thickening of the glomerular basement membrane, increased mesangial matrix, and partial obliteration of capillary lumens.



**Figure 8.** Histopathological micrographs of rat kidney sections showing renal inflammation following 90-day oral administration of arsenic-contaminated rice (hematoxylin–eosin staining). Arrows indicate inflammatory changes characterized by infiltration of inflammatory cells in the interstitial and periglomerular areas, accompanied by mild tubular epithelial disruption.

rice at 24.3 g/kg body weight; (12) satellite red rice at 24.3 g/kg body weight; (13) brown rice at 8.1 g/kg body weight; (14) brown rice at 16.2 g/kg body weight; (15) brown rice at 24.3 g/kg body weight; and (16) satellite brown rice at 24.3 g/kg body weight.

Liver sections from the negative control (CMC-Na) and satellite CMC-Na groups exhibited normal hepatic architecture, characterized by well-organized hepatic lobules, radially arranged hepatocyte plates, intact central veins, and narrow sinusoids lined by endothelial and Kupffer cells. These findings confirm that the vehicle did not induce structural or functional disturbances in hepatic tissue. In contrast, rats exposed to inorganic arsenic (positive control) showed pronounced histopathological damage, including hepatocellular degeneration, cytoplasmic vacuolization, nuclear pyknosis, sinusoidal dilatation, and focal necrosis (Figures 1 and 2). These alterations are consistent with the established hepatotoxic profile of inorganic arsenic, which is known to induce oxidative stress, disrupt hepatocellular metabolism, and damage cell membranes through excessive reactive oxygen species (ROS) generation<sup>21</sup>.

Satellite arsenic-treated rats demonstrated partial improvement in hepatic architecture compared with the inorganic arsenic control, with reduced severity of degeneration and necrosis. This observation reflects the high regenerative capacity of hepatocytes and suggests that cessation of arsenic exposure allows gradual structural recovery, although complete normalization was not achieved within the observation period. Such reversible changes following withdrawal of arsenic exposure have been previously reported and are considered indicative of adaptive hepatic repair mechanisms<sup>22</sup>.

Administration of white rice at all tested doses (8.1, 16.2, and 24.3 g/kg body weight) did not result in detectable histopathological abnormalities. Hepatocytes remained polygonal with centrally located nuclei and homogeneous eosinophilic cytoplasm, and no evidence of degeneration, necrosis, steatosis, fibrosis, or inflammatory infiltration was observed (Figures 1–5). These findings are in agreement with earlier reports indicating that white rice generally contains lower arsenic levels and does not exert hepatotoxic effects under typical dietary exposure scenarios.

Liver tissues from rats receiving red rice displayed a more nuanced response. At low and medium doses, hepatic architecture was largely preserved, with minimal or absent pathological changes. However, at the highest dose (24.3 g/kg body weight), mild hepatocellular swelling, cytoplasmic vacuolization, and focal necrosis were observed (Figures 1–3). These findings suggest a dose-dependent hepatic response, likely reflecting higher arsenic exposure at elevated intake levels. Nevertheless, the severity of damage remained substantially lower than that observed in the inorganic arsenic group, which may be attributed to the presence of natural antioxidants such as polyphenols and anthocyanins in red rice that confer partial hepatoprotection.

Brown rice administration did not induce significant histopathological alterations at any tested dose. Hepatic lobular organization, hepatocyte morphology, and sinusoidal structures remained intact, with no evidence of necrosis, steatosis, fibrosis, or inflammation (Figures 1–5). This preservation of hepatic integrity may be related to the combined effects of relatively lower bioavailable arsenic fractions and higher concentrations of bioactive compounds, including  $\gamma$ -oryzanol and phenolic antioxidants, which have been reported to mitigate oxidative stress and inflammatory signaling in hepatic tissue.

Evaluation of steatosis, fibrosis, and inflammatory infiltration further supported these findings. Steatotic changes were prominent only in the inorganic arsenic group, reflecting arsenic-induced disruption of lipid metabolism through mitochondrial dysfunction and impaired  $\beta$ -oxidation<sup>23,24</sup>. Fibrotic alterations, characterized by increased

connective tissue deposition in portal areas<sup>25</sup>, were likewise confined primarily to inorganic arsenic exposure, with only mild fibrosis observed in the high-dose red rice group. Hepatic inflammation followed a similar pattern, with marked inflammatory cell infiltration in arsenic controls and minimal to absent inflammatory responses in rice-treated groups.

Collectively, the histopathological findings indicate that the liver is the primary target organ affected by arsenic exposure, with severity strongly influenced by the form of arsenic and the exposure matrix. While inorganic arsenic produced clear hepatotoxic effects, arsenic exposure through rice consumption resulted in minimal to mild hepatic alterations, primarily at the highest dose of red rice. These results underscore the importance of evaluating dietary arsenic toxicity within realistic food-based exposure contexts and highlight the modulatory role of rice-derived bioactive compounds in attenuating arsenic-induced liver injury.

### Kidney Histopathological Findings

Microscopic evaluation of renal tissues is essential in subchronic toxicity studies because the kidney is a major site for arsenic filtration and elimination and is therefore vulnerable to toxicant-induced oxidative injury, particularly within the proximal tubular compartment. In this study, histopathological examination of rat kidneys stained with hematoxylin–eosin generally demonstrated preserved renal morphology in most rice-treated groups, with intact glomerular architecture (well-defined Bowman's capsule and capillary tufts) and largely maintained tubular epithelial integrity. Across the white, red, and brown rice groups at doses of 8.1, 16.2, and 24.3 g/kg body weight, there was no evidence of severe tubular necrosis, extensive cast formation, or widespread interstitial inflammatory infiltration, indicating that arsenic exposure via the rice matrix did not provoke overt nephrotoxicity under the tested conditions.

Representative renal histopathological changes are presented in Figures 6–8. Panels correspond to: (1) inorganic arsenic control; (2) CMC-Na control; (3) satellite inorganic arsenic control; (4) satellite CMC-Na control; (5) white rice 8.1 g/kg body weight; (6) white rice 16.2 g/kg body weight; (7) white rice 24.3 g/kg body weight; (8) satellite white rice 24.3 g/kg body weight; (9) red rice 8.1 g/kg body weight; (10) red rice 16.2 g/kg body weight; (11) red rice 24.3 g/kg body weight; (12) satellite red rice 24.3 g/kg body weight; (13) brown rice 8.1 g/kg body weight; (14) brown rice 16.2 g/kg body weight; (15) brown rice 24.3 g/kg body weight; and (16) satellite brown rice 24.3 g/kg body weight.

Group-specific differences were apparent when renal injury patterns were examined in relation to the inorganic arsenic control. The positive control (inorganic arsenic) exhibited the most distinct renal lesions, including tubular epithelial degeneration characterized by nuclear pyknosis, increased eosinophilia of the cytoplasm, and epithelial desquamation features consistent with arsenic-induced tubular injury mediated by excessive reactive oxygen species and mitochondrial dysfunction, which preferentially affect proximal tubules. In the satellite arsenic group, the degree of tubular damage was reduced relative to the arsenic control, supporting the interpretation that subchronic arsenic-related tubular injury may be partially reversible following cessation of exposure, likely through regeneration and repair of tubular epithelium.

In the rice-treated groups, tubular necrosis when present was generally mild and remained within an acceptable histological range for adaptive responses. White rice administration across all dose levels showed no heavy necrotic lesions, suggesting that arsenic exposure from this matrix was insufficient to elicit severe tubular destruction. Red rice groups similarly displayed low-grade renal injury overall, despite higher analytically measured arsenic levels in some samples. This pattern is biologically plausible because red rice contains anthocyanins and a

higher total polyphenol fraction, which can strengthen endogenous antioxidant defenses and reduce oxidative stress-driven tubular injury<sup>26</sup>. Brown rice groups demonstrated the least pronounced tubular injury across the tested dose range, including at 24.3 g/kg body weight, which may reflect protective contributions from bioactive constituents such as ferulic acid derivatives, tocopherols, and other antioxidant phytochemicals that stabilize cellular redox homeostasis and preserve tubular structure. Consistent with these observations, the satellite groups at the highest dose (24.3 g/kg body weight) did not exhibit progressive lesions, implying that delayed nephrotoxic effects after withdrawal were unlikely in this experimental setting.

Evaluation of glomerular sclerosis further supported the predominance of tubular rather than glomerular injury in arsenic exposure. Overall, permanent glomerulosclerosis was not a prominent feature in this study, including in the arsenic control, which aligns with the notion that arsenic-associated renal injury manifests earlier and more intensely in proximal tubular segments, whereas glomerular fibrotic remodeling typically emerges later in progressive injury. However, subtle increases in mesangial components and early microstructural glomerular alterations were suggested in some higher-dose groups particularly among rice types retaining outer grain layers indicating a potential early adaptive response to chronic burden, even when overt sclerosis is not established.

Renal inflammatory changes exhibited a similar exposure-dependent pattern. The inorganic arsenic control showed clearer signs of inflammation, including inflammatory cell infiltration and structural disturbances in glomerular and periglomerular regions, consistent with ROS-mediated activation of proinflammatory pathways and cytokine signaling<sup>27</sup>. By contrast, white and brown rice groups did not demonstrate pathological inflammation; any inflammatory cells observed were limited and within physiological boundaries. Red rice at the highest dose showed early inflammatory tendencies (interstitial widening and more apparent inflammatory infiltration) relative to lower doses, indicating that renal inflammatory responses may be both dose and rice type dependent. Importantly, these findings align with the broader toxicological principle that arsenic toxicity is influenced by speciation and bioavailability, where inorganic arsenic is substantially more toxic than arsenic forms commonly encountered in foods; consequently, dietary exposure through complex matrices often produces less severe inflammation than direct administration of inorganic arsenic.

Overall, the kidney findings indicate that inorganic arsenic produced the strongest renal injury pattern, whereas rice-based exposure generated only mild, largely non-progressive renal alterations, with minimal evidence of pathological inflammation or established glomerulosclerosis in most rice-treated groups. These outcomes suggest that the rice matrix and rice-derived antioxidant constituents may attenuate arsenic-associated oxidative injury, thereby limiting nephrotoxic expression at the tissue level under subchronic exposure conditions.

## CONCLUSION

Subchronic (90-day) oral administration of arsenic-contaminated rice to female Wistar rats produced no mortality and no overt systemic toxicity across white, red, and brown rice groups (8.1–24.3 g/kg BW/day). Body weight gain and relative organ weights remained comparable to the CMC-Na control, and hematological parameters largely stayed within physiological ranges. Serum urea and creatinine were unchanged, indicating preserved renal function, while only mild increases in hepatic serum occurred at the highest brown rice dose, suggesting limited hepatic stress. Histopathology showed largely preserved renal architecture, whereas the liver exhibited focal injury

primarily at the highest exposure level. In contrast, inorganic arsenic caused clear clinical, biochemical, and tissue toxicity.

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