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Detrimental effects of chronic arsenic exposure through daily diet on hepatic and renal health: An animal model study

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ABSTRACT

Background: Bangladesh is heavily impacted by arsenic contamination; however, in-depth research regarding the consequences of arsenic exposure through contaminated food remains in its nascent stage.
Objectives: Our study aimed to examine the effects of consuming arsenic-contaminated rice and vegetables on Wistar albino rats.
Methodology: A total of 30 Wistar albino rats were divided into control and experimental groups, receiving diets containing arsenic-contaminated rice and vegetable for 120 days. Arsenic levels in food samples were quantified using FI-HG-AAS. Hematological, biochemical, and histopathological analyses were conducted to assess hepatic and renal toxicity. Statistical analysis was performed using SPSS, ANOVA, DMART with significance set at $p < 0.05$.
Findings: In hematological studies, hemoglobin was found to be significantly reduced in exposed rats ($P < 0.05$) to 13.67 ± 0.54 gm/dl for rice diet and 13.77 ± 0.28 gm/dl for edible fern diet compared to control (14.17 ± 0.43 gm/dl for rice diet and 15.27 ± 0.13 gm/dl for edible fern diet), and similar results were also observed for RBC and WBC. Elevated levels of serum ALT, AST, urea and creatinine reflected hepatic and renal dysfunction. In renal and hepatic tissue histopathology, inflammation, degeneration, and fatty changes were observed. This study provides the first direct evidence of systemic toxicity from chronic dietary arsenic exposure in Bangladesh, identifying rice and vegetables as significant, underrecognized sources of arsenic-related health risks.
Conclusion: We conclude that eating rice and vegetables that have been exposed to arsenic over an extended period of time might be harmful to the body.

1. Introduction

Arsenic (As) is a naturally occurring element with a notorious reputation for being one of the most poisonous substances known to humankind [1]. According to the World Health Organization (WHO), around 200 million people worldwide are at risk of experiencing health effects associated with long-term exposure to high levels of arsenic in their drinking water. This risk is particularly high in regions where groundwater is the main source of drinking water, and where the groundwater is contaminated with arsenic [2]. The largest known mass

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poisoning in history, the arsenic calamity in Bangladesh, resulted from the widespread extraction of groundwater to supply the growing demand for safe drinking water and agricultural irrigation, with an estimated 80 million people exposed to arsenic-contaminated drinking water [3]. In numerous countries worldwide, continuous exposure to arsenic through drinking water has been reported where the levels exceed safe permissible limits. Specifically, in Bangladesh, the concentration of arsenic in groundwater has surpassed the permissible limit (0.2 ppm) set by the World Health Organization (WHO) in 61 out of 64 districts [4].

Asian countries play a significant role in global agriculture, accounting for around 90 % of rice and 50 % of vegetable production. Of these agricultural products, approximately 54 % are utilized locally, while the remaining 46 % are exported to various regions around the world. In this regard, groundwater serves as a significant irrigation source for agricultural production in many Asian countries [5]. The reliance on arsenic-contaminated groundwater for irrigating rice and vegetable crops in many Asian countries creates a serious risk for elevated levels of arsenic accumulation in these food products. This can lead to increased risk of arsenic toxicity from dietary intake, not only in these regions where the arsenic levels in soil or water are high but also to those where the products are exported. The potential health impacts of such exposure can be severe for the affected populations [6].

Due to the slow accumulation of arsenic in the body, the symptoms of arsenicosis may not become apparent until months or even years after starting chronic arsenic intake. This delayed onset of symptoms highlights the importance of having an accurate method to track exposure to harmful levels of arsenic [7]. Consuming foods, particularly rice and vegetables grown in arsenic-contaminated soil or irrigated with arsenic-contaminated water, can lead to arsenic accumulation in the body, causing damage to various organs and body systems. The impact of arsenic-induced toxicity in human organs after the consumption of contaminated rice and vegetables remains poorly understood. While previous studies on laboratory animals have determined the total arsenic concentration and speciation in selected organs after consuming arsenic-contaminated food, further research is needed to fully comprehend the effects of consuming such food on human health [8].

Exposure to arsenic can result in both acute and chronic toxicity, which can cause severe health problems. Long-term exposure to arsenic has been associated with various health issues, including skin cancer, diabetes, and disorders of the liver, kidneys, and central nervous system [9]. It has been reported that high concentrations of arsenic can accumulate in the liver, kidneys, and lungs. Epidemiological studies have also revealed a significant association between chronic arsenic exposure and the development of liver disease and kidney failure [2]. In a study, continued feeding of arsenic resulted in fatty liver along with elevated serum aspartate aminotransferase and alanine aminotransferase, on the other hand necrotic changes in kidney has been found in arsenic-exposed laboratory animals [10,11]. The exact relationship between chronic arsenic exposure and the development of specific target organ toxicity is not yet fully understood. To gain a better understanding of the detailed mechanisms of arsenic-mediated toxicity in mammals, it is important to investigate the organ-specific histological effects of arsenic exposure. Organ-specific histological evaluation is considered the gold standard for determining the degree of organ injury during chronic metal exposure. Interestingly, during histological degeneration, organ function markers also undergo changes [2].

Bangladesh is one of the worst arsenic-affected countries globally, and extensive research on arsenic contamination has been conducted in the country. Most studies to date have focused on quantifying arsenic levels in water, soil, and various food items, such as rice, vegetables (e.g., tomato, bottle gourd, bottle gourd leaf, and giant taro), and different fish species [12]. A previous research conducted in our laboratory studied arsenic-induced toxicity through hematological, biochemical, and histopathological analyses of rat models using sodium arsenite as the arsenic source. For that study, rice samples were confirmed to be free of background arsenic contamination before sodium arsenite was artificially introduced [5].

Rice is a major dietary contributor to arsenic (As) exposure, especially in South and Southeast Asia, where it is a staple food and a primary calorie source. The type of arsenic in rice—organic or inorganic—varies significantly depending on the rice variety and geographic location. In Bangladesh, rice primarily contains inorganic arsenic (iAs), the more toxic form, which is quickly absorbed into the bloodstream, raising serious public health concerns [13]. A recent study further validated that inorganic arsenic is the dominant species in rice, soil, and vegetables, highlighting the risks of dietary arsenic exposure [14].

A previous study identified arsenite [As(III)] as the dominant arsenic species in rice, accounting for 84 % of the total arsenic content [8]. This finding underscores the predominance of inorganic arsenic in rice and its potential dietary health risks. Consistently, another study also reported that As(III) was the most abundant arsenic species, surpassing other forms of arsenic in rice [15].

Arsenic contamination has become a significant concern as it has entered the food chain and posing potential health risks. Historically, research on arsenic in Bangladesh has predominantly focused on its presence in drinking water and its toxicological effects on human health. While studies on arsenic in rice and vegetables have been conducted, they have largely been limited to assessing its presence, concentration, and potential toxicity rather than its direct physiological impact [12,16].

Our research question was- how does naturally arsenic-contaminated food—rather than food artificially treated with arsenic in the laboratory—impacts human health? Therefore, the current study aims to investigate the histopathological alterations in hepatic and renal tissues, alongside hematological and biochemical changes, induced by the consumption of arsenic-contaminated rice and vegetable. To the best of our knowledge, this is the first study in Bangladesh to directly administer naturally arsenic-contaminated rice and vegetables to Wistar albino rats to evaluate their toxicological effects. By replicating real-world dietary exposure, this research provides a more ecologically relevant assessment of arsenic toxicity. Furthermore, increasing awareness about arsenic-related health issues was another important goal of the study. A better understanding of the effects of arsenic on target organs, with an emphasis on observing tissue architecture at critical sites, will help define the mode(s) of action for arsenic-induced toxicity in mammals and reduce uncertainty in risk assessment for this metalloid.

2. Materials and methods

2.1. Area of study

The groundwater in Chandpur, a district in southeastern Bangladesh, is severely contaminated with arsenic, leading to the area being recognized as an endemic zone for arsenic exposure [17]. It is estimated that more than 90 % of the population in this area relies on tube wells for drinking water and irrigation. However, about 80–90 % of the tube wells in this region contain arsenic levels ranging from 100 to 1318 μg/L [17,18,3]. The study area includes two highly arsenic-contaminated Upazilas (sub-districts) of Chandpur, namely Hajiganj and Shahrasti. For collecting control samples, Chattogram Hill Tracts (Bandarban) was selected as the control study area, as Hill Tracts areas are known to be arsenic-free [19] (Supplementary Data Sheet S1).

2.2. Collection of demographic data

Socio-demographic and food consumption data were collected by administering a validated questionnaire in a face-to-face context. The study sample consisted of 25 farmers from each of the two Upazilas (Sub-districts), who were purposively selected based on specific criteria. These farmers used As contaminated groundwater for drinking purposes

and consumed self-produced rice and vegetables grown in their fields irrigated with the same water source. The demographic data were collected in the year 2021 (Supplementary Data Sheet S2). The study received approval from the Ethical Committee of Biological Sciences Faculty (ECBSF) at the University of Chittagong, and all participants provided their informed consent by signing the necessary forms.

2.3. Sample collection and preparation

Composite samples were made by collecting three sub-samples of irrigation water, soils, rice, and vegetables from the same fields of the purposively selected farmers. To collect the water samples, the irrigation pump was operated for 5 minutes and 100 ml of water was collected. Immediately after collection, 5 ml of 2 M hydrochloric acid was added to the sample, and it was then transported to the laboratory while being kept on ice. Later on, 0.45-μ Millipore filters were used to filter the samples.

Soil sub-samples were collected from the upper 0–20 cm horizon of the standing crop fields using an assembled sectional auger. The collection process involved creating four holes at the corners of a 20 m^2 grid, following the recommendations outlined in IGCP 259 [20]. Once collected, the soil samples were screened to remove any stones, gravels, or other residues. The samples were then air-dried and sieved twice through mesh sizes of 2 mm and 0.149 mm. After this process, the soil samples were wrapped in Kraft paper wrappers and kept in storage until analysis.

Sub-samples of standing rice and the edible portions of vegetables were also collected directly from the same fields. The samples were stored safely in zip lock bags made of polyethylene and were quickly transported to the laboratory for analysis (Supplementary Data Sheet S1). Before analysis, the chaff was stripped from the rice grains. The vegetable samples were washed with tap water for approximately 5 minutes, then rinsed with deionized water, blotted with filter paper, and oven-dried for 24 hours at a temperature of 60°C. Prior to chemical digestion, the rice and vegetable samples were ground using a carnelian mortar. The samples were then rinsed twice with 5 ml of deionized water and methanol to remove any extraneous substances that may have been attached to the sample.

2.4. Analysis and quality control

The concentration of arsenic (As) in the water, soil, grain, and vegetable samples was determined using Flow Injection Hydride Generation Atomic Absorption Spectrophotometry (FI-HG-AAS), following the procedure described by Das et al. [21]. To determine the total As concentration in the soil, 0.25 g of soil was wetted with a few drops of deionized water in a 100-ml Erlenmeyer flask. The soil was thoroughly mixed with aqua regia, a mixture of concentrated hydrochloric acid (HCl) and concentrated nitric acid (HNO_3) in a 3:1 vol ratio, and the digestion process was carried out at a temperature of around 120°C for approximately 1.5 hours in the flask, which was covered with a small glass filter and placed on an electric heater until the reaction subsided. Following digestion, a 5 ml solution of sulfourea (50 g/L) was added to the digest in a 50-ml volumetric flask, which was then filled with double deionized water to the mark.

For analysis of As in rice grains and vegetables, a sample weighing 0.5 g (<0.5 mm) was taken into a 100-ml Erlenmeyer flask and 1 ml of concentrated perchloric acid ($HClO_4$), 1.5 ml of concentrated sulfuric acid (H_2SO_4), and 4 ml of concentrated HNO_3 were added and mixed with the sample. The mixture was left to react overnight and then digested on an electric heater by gently boiling. The resulting brown-colored solution of approximately 10 ml was further digested by adding concentrated HNO_3 (5 ml). Since the digestion process was incomplete even after two to three additional digestions with HNO_3, concentrated $HClO_4$ (2 ml) was added for further digestion. The entire digestion process took approximately 3–4 hours. After digestion, the sample was transferred to a 25-ml measuring flask, and 2.5 ml of sulfourea solution (50 g/L) was added to spike the digest. The flask was then filled with double deionized water to the mark. All reagents used were of analytical grade or higher. Samples of soil, water, rice, and vegetables from Chattogram Hill Tracts (Bandarban), which are known to be arsenic-free areas [19], were collected and used as controls.

In this study, the detection limit for arsenic in rice, water, soil, and vegetables was 0.01 ppm, ensuring high sensitivity and accuracy in quantifying arsenic contamination across diverse sample matrices. Stringent quality control measures were implemented to ensure accurate and consistent results. For the preparation of reagents, a freshly prepared 0.5 % (m/v) sodium borohydride ($NaBH_4$) solution in 0.5 % (m/v) sodium hydroxide (NaOH) was used for each analysis batch, ensuring reactivity and consistency. High-purity analytical-grade reagents were employed to minimize contamination. For hydride generation, AnalaR grade hydrochloric acid (HCl) was utilized to prepare a 50 % (v/v) HCl solution, ensuring consistent reaction conditions. Additionally, to accurately measure total inorganic arsenic, including As(V), a pre-reduction step was applied. A mixture of 10 % (m/v) potassium iodide (KI) and 10 % (m/v) L-ascorbic acid was used to convert As(V) to As(III), the required form for hydride generation. The samples were allowed to react for 30 minutes under dark conditions to prevent oxidation, ensuring precise and reliable results in the analysis of inorganic arsenic [22].

2.5. Test product preparation

In toxicity study, test (arsenic contaminated) and control (arsenic-free) products were incorporated into animal feed at a ratio of 9:6 (where 9 indicates test product and 6 indicates animal feed). The negative control group was fed with animal feed that did not contain any test material, while the positive control group was given animal feed and drinking water mixed with Sodium Arsenite.

2.6. Experimental animals

Ethical permission for conducting animal studies were obtained from the Animal Ethics Review Board, Faculty of Biological Sciences, University of Chittagong, Bangladesh (approval reference number: AERB-FBSCU-20241018-(1)). All procedures were conducted in strict compliance with the ARRIVE guidelines, the National Research Council's Guide for the Care and Use of Laboratory Animals, and the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Wistar albino rats were housed in a temperature-controlled environment (22 ± 2°C) with 40–60 % humidity, maintained on a 12-hour light/dark cycle, and kept in standard polypropylene cages with soft bedding, which was regularly changed. They were provided ad libitum access to standard laboratory chow and filtered water. To ensure animal welfare, rats were monitored daily for signs of stress, discomfort, or illness, with body weight, food intake, hydration, and activity levels recorded throughout the study. In cases of significant distress or weight loss exceeding 20 % of baseline weight, humane endpoints were applied. At the end of the study, animals were euthanized using CO_2 inhalation followed by cervical dislocation, ensuring a rapid and humane procedure in accordance with internationally accepted protocols [23].

In this study, 30 male Wistar albino rats (*Rattus norvegicus*) weighing between 150 and 180 g were obtained from the animal breeding center of Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. Sample size calculations were conducted to ensure statistical power, following the same rigorous methodology as described in our previous research on arsenic-induced toxicity [23]. To reduce variability, only male Wistar albino rats (*Rattus norvegicus*) were used in this study. The rats had an average weight of 167.35 g and were provided with adequate laboratory rodent pellet diet and water. The rats were randomly divided into six groups, with each group consisting of five rats. Group I was assigned as the normal control group and received a normal

pellet feed diet. Group II was given a normal pellet feed diet along with Sodium arsenite in distilled water (arsenic control). Group III was given a normal pellet feed diet mixed with arsenic-free rice. Group IV was given a normal pellet feed diet mixed with arsenic-contaminated rice. Group V was given a normal pellet feed diet mixed with arsenic-free edible fern. Finally, Group VI was given a normal pellet feed diet mixed with arsenic-contaminated edible fern. The duration of the study was 120 days to simulate chronic exposure. The experiments were conducted in compliance with both institutional and national guidelines.

2.7. Collection of blood and separation of serum

On the 120th day, the rats underwent euthanasia via carbon dioxide inhalation and subsequent sacrifice. Prior to sacrifice, all animals were subjected to overnight fasting. Blood samples were obtained from each rat and divided into two pre-labeled test tubes. One of the tubes contained Ethylenediaminetetraacetic acid (EDTA), an anti-coagulant, for hematological parameter examination, while the other tube was left to clot for 20 min at room temperature. After clot formation, the glass tubes were centrifuged at 3000 r.p.m for 10 minutes, and the resulting serum was collected in pre-labeled Wintrobe tubes. The collected serum was then used for biochemical testing.

2.8. Collection and preservation of liver and kidney tissue

The chest and abdomen of the rats were opened, and the liver and both kidneys were meticulously removed and cleaned. Each of these organs was weighed using an electric balance and then separately placed in pre-labeled specimen containers. A 10 % formalin-containing container was used for histopathological examination and arsenic determination, while a 100 % ethanol-containing container was used for genetic analysis. Some portions of the liver and both kidneys were preserved at $-4^{\circ}C$ for the detection of arsenic. The specimen container with ethanol was stored at $-20^{\circ}C$.

2.9. Statistical analysis

The Flow Injection Hydride Generation Atomic Absorption Spectrophotometer (FI-HG-AAS) was used to analyze the total arsenic content of the prepared soil, water, vegetable, and rice samples. The optimal HCl concentration was found to be 10 % (v/v), and 0.6 % $NaBH_4$ was determined to produce the maximum sensitivity. Three replicates were taken for each sample, and the mean values were calculated based on these replicates. The results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was carried out using a commercially available statistics software package (SPSS V.22 for Windows), and all tables and graphs were created using Microsoft Word and Microsoft Excel. The data from each control and treated group were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMART). A p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Demographic parameters

The data on demographic characteristics and food consumption of the study participants are provided in Supplementary Data Sheet S2. Nearly two-thirds of the respondents in our study were young to middle-aged, completed primary school education, and were local residents. Forty percent of the participants had low to medium-income, while 39 % belonged to the high to very high-income category, mainly derived from agriculture cultivation in small to medium-sized farm holdings. Approximately 70 % of the farmers had small to medium family sizes, and with the exception of a few, all respondents were asymptomatic regarding arsenicosis-related ailments.

Regarding dietary habits, all respondents used safe water. Of them, 79 % consumed at least 1.0 kg of vegetables per week, which is almost similar with the national consumption rate of 1.1 kg per week [24]. The rice consumption rate was a minimum of 3.0 kg per week for 73 % of the respondents, while the rest consumed less than 3.0 kg rice per week.

3.2. Arsenic in irrigation water and soils

The study found that irrigation water from Digchail, Kangaish, and Nijmeher had significantly higher levels of arsenic than irrigation water from Randhanimura, Phulchua, Olipur, Uaruk, Suchipara, and Narenpur. The lowest and highest arsenic concentrations were found in irrigation water from Randhanimura and Olipur, respectively. Groundwater used for irrigation had arsenic concentrations ranging from 0.025 to 0.373 mg/L, with the highest concentration found in Kangaish and the lowest in Narenpur. The mean concentration of arsenic in groundwater samples was 0.201 mg/L for Kangaish, 0.210 mg/L for Randhanimura, 0.235 mg/L for Digchail, 0.173 mg/L for Phulchua, 0.10 mg/L for Olipur, 0.120 mg/L for Uaruk, 0.185 mg/L for Nijmeher, 0.136 mg/L for Suchipara, and 0.081 mg/L for Narenpur. The arsenic concentrations in irrigation water samples from As-affected areas of Chandpur were significantly higher when they originated from groundwater sources compared to surface water sources. The detected groundwater arsenic content in Chandpur was much higher than the allowable limits for irrigation proposed by FAO (0.1 mg/L) and WHO (0.01 mg/L). The higher arsenic concentrations in irrigation water at nine different locations are comparable with the findings of other studies in Chandpur, i.e. Williams et al. [25]. Although Ahmed et al. reported higher arsenic concentrations in irrigation water compared to our findings, their overall results are consistent with ours [26]. Another study documented groundwater arsenic concentrations exceeding those measured in our study [27].

The study also found that soil As content varied significantly ($p \leq 0.05$) across the study locations, with the highest and lowest values found in Digchail (28.67 mg/kg dry weight) and Uaruk (5.52 mg/kg dry weight), respectively. Soil As concentrations ranged from 5.52 to 32.51 mg/kg dry weight, with the highest mean concentration found in Nijmeher (21.51 ± 9.51 mg/kg dry weight) and the lowest in Uaruk (13.03 ± 5.93 mg/kg dry weight). The mean soil As content (16.72 ± 5.26 mg/kg dry weight) was higher than the global average soil As level (10 mg/kg), but below the FAO recommended permissible limit for agricultural soils (50 mg/kg). These values are much higher than the previous study of Meharg and Rahman [28] in Chandpur, which found As concentrations in soil ranging from 6.8 to 18.4 mg/kg dry weight. A recent study reported comparable findings [29]. The study suggests that the extensive use of As-contaminated groundwater for irrigation, in addition to natural sediment deposition and diagenesis processes, has contributed to the high As levels in surface soils in Chandpur.

3.3. Arsenic accumulation in rice

The As concentrations in our study were measured in milligrams per kilogram (mg/kg). We found that rice grains from three areas - Digchail, Randhanimura, and Nijmeher - contained significantly higher levels of As than grains from other areas ($p \leq 0.001$). The highest concentration of As was recorded in Digchail (0.66 mg/kg), and the lowest was recorded in Phulchua (0.073 mg/kg).

The mean concentration of As in rice grains was 0.37 ± 0.28 mg/kg for Kangaish, 0.38 ± 0.16 mg/kg for Randhanimura, 0.46 ± 0.23 mg/kg for Digchail, 0.17 ± 0.12 mg/kg for Phulchua, 0.31 ± 0.14 mg/kg for Olipur, 0.24 ± 0.12 mg/kg for Uaruk, 0.41 ± 0.27 mg/kg for Nijmeher, 0.28 ± 0.21 mg/kg for Suchipara, and 0.35 ± 0.25 mg/kg for Narenpur. These values indicate the average concentration of As in the rice grains from each area, with the standard deviation (SD) representing the variation in the concentrations. The concentrations ranged from 0.05 to

0.66 mg/kg for Kangaish, 0.09–0.49 mg/kg for Randhanimura, 0.18–0.79 mg/kg for Digchail, 0.07–0.39 mg/kg for Phulchua, 0.13–0.52 mg/kg for Olipur, 0.09–0.44 mg/kg for Uaruk, 0.1–0.71 mg/kg for Nijmeher, 0.08–0.55 mg/kg for Suchipara, and 0.073–0.63 mg/kg for Narenpur.

The study also compared the As concentrations in rice grains to food hygiene concentration limits set by the World Health Organization (WHO) and proposed by China. The safe limit of As in rice for south Asia proposed by China is 0.15 mg/kg, while the food hygiene concentration limit set by the WHO is 0.37 mg/kg. The study found that the mean grain As content from Randhanimura, Digchail, and Nijmeher exceeded the WHO limit, while grains from other areas were within the safe limit. The study also found that 50 % of sampled grains from Kangaish, Nijmeher, and Suchipara, 17 % from Phulchua, Olipur, and Uaruk, 67 % from Digchail, and 83 % from Randhanimura exceeded the safe limit proposed by China.

Finally, the study notes that the maximum concentration of As found in this study (0.91 mg/kg) is comparable to a previous study by Williams et al. [25] that reported 0.91 mg As per kg grains in Chandpur. Multiple research group reported arsenic accumulation in rice, with findings consistent with our study [8,30]. Their analyses further substantiate the widespread presence of arsenic across different rice matrices, reinforcing the patterns observed in our research. However, the study also found that the As content in rice from all areas was below 1 mg/kg, consistent with earlier studies in Bangladesh.

3.4. Arsenic accumulation in vegetables

We found that the arsenic content in vegetables (edible fern, bottle gourd, bottle gourd leave, giant taro) was significantly lower ($p \leq 0.01$) in Olipur and Narenpur, compared to the other seven locations. The mean ± SD of vegetable As concentration was 0.76 ± 0.48, 0.53 ± 0.57, 1.47 ± 0.78, 0.43 ± 0.35, 0.25 ± 0.37, 0.67 ± 0.31, 1.29 ± 0.67, 1.33 ± 0.58, and 0.22 ± 0.19 mg/kg for Kangaish, Randhanimura, Digchail, Phulchua, Olipur, Uaruk, Nijmeher, Suchipara, and Narenpur, respectively. These values ranged from 0.08 to 2.33 mg/kg, with 40–80 % of samples having levels greater than 1 mg/kg, except for two locations where no samples were above the 1 mg/kg level. We found maximum As concentration 1.93 mg/kg in edible fern in our study. The arsenic concentrations detected in our vegetable samples were largely within a comparable range; however, certain samples exhibited elevated levels. Overall, our findings demonstrate a substantial concordance with the reported data [30].

The maximum permissible limits (MPL) of As in vegetables vary between countries. For example, the MPL value in China is 0.5 mg/kg [31], while in the United Kingdom, Ireland, and Singapore, it is 1.0 mg/kg [32-34]. Bangladesh lacks legislation in this regard, and some researchers consider the maximum food safety value, 1 mg/kg, as the MPL of As for fresh vegetables in Bangladesh. The mean As content in vegetables at three locations exceeded this safe limit for the food safety limit in Bangladesh.

Vegetables account for around 16 % of the overall diet in Bangladesh [24], and the current study revealed that the daily As intake from consuming vegetables ranged from 0.43 to 9.87 μg, with an average intake of 5.6 μg/day. The average per capita consumption of leafy and non-leafy vegetables in the village of Samta in Bangladesh is 130 g for adults [35], which is less than the result of the current study and the average intake of 205 g in Basantapur and Chiladi villages in Noakhali district [36]. However, daily vegetable consumption by Bangladeshi adults ranging from 126 to 195 g [37].

Considering the average daily vegetable consumption of 130 g, Williams et al. [25] found that As intake ranged from 0.9 to 16.9 μg/day, while Alam et al. [35] calculated an average intake of 5.6 μg As/person/day in Samta village. Rahman et al. [36] estimated much less (2.3 μg only) As intake in Basantapur and Chiladi villages. In contrast, Karim et al. [38] reported very high (105 μg/day) As ingestion in Feni, Bangladesh.

Arsenic contamination remains a significant environmental and public health concern in Bangladesh, with rice and vegetables serving as primary dietary sources of exposure. Regulatory guidelines predominantly consider total arsenic concentration rather than distinguishing between its chemical species [30], which may lead to an underestimation of the actual health risks. However, the toxicological consequences of arsenic exposure are largely dictated by its chemical speciation, with inorganic arsenic (iAs), particularly arsenite, posing a greater toxicological burden [8]. This study sought to assess arsenic levels in soil, water, rice, and vegetables to elucidate potential dietary exposure risks.

Previous studies have established that inorganic arsenite [As(III)] is the predominant arsenic species in rice (approximately 84 %) [8]. While we did not perform arsenic speciation analysis in the present study, we infer that a similar distribution pattern should be present in our samples. However, the absence of direct speciation analysis remains a study limitation, precluding definitive conclusions on the specific arsenic species present. Despite this constraint, the presence of arsenic in staple food sources raises profound public health concerns, given the well-documented associations between chronic arsenic exposure and carcinogenesis, metabolic dysregulation, and epigenetic modifications.

Although this study represents an initial step in assessing the toxicity of naturally arsenic-contaminated rice and vegetables, our previous research has demonstrated arsenic-induced molecular perturbations, including telomere length modulation, mitochondrial DNA (mtDNA) copy number variations, mtDNA deletions, aberrant DNA methylation, and cardiovascular disease susceptibility linked to genetic polymorphisms considering drinking water as the source of exposure [23, 39-41].

3.5. Arsenic-contaminated rice and vegetable induced toxicity analysis

The study found that both the control group and the group treated with arsenic had normal activities and no mortality was observed during the 120-day study period. However, the reduction in body weight is an indicator of the deterioration of the general health status of rats. In addition to monitoring body weight, the researchers also monitored changes in food and water intake between the control and experimental groups. The study found that the rats treated with arsenic had a reduced body weight gain and decreased water and food intake compared to the control group. These findings are consistent with previous reports that rats exposed to arsenic show a decrease in water and food intake, retardation in growth rate, and alterations in organ body weights [42].

Throughout the study period, close attention was paid to the dietary and water consumption patterns, as well as the body weight, of the animals under investigation. As a metric of animal well-being, any fluctuation in body weight was closely monitored. Our observations revealed a statistically significant alteration ($P < 0.05$) in the food and water intake of the subjects following exposure to arsenic (Table 1).

The study involved six groups of animals that were fed different diets over a 120-day period. Group I was given a normal diet, Group II received a normal diet along with sodium arsenite in their drinking water, Group III was given a normal diet mixed with arsenic-free rice, Group IV received a normal diet mixed with arsenic-contaminated rice, Group V was given a normal diet mixed with arsenic-free edible fern, and Group VI received a normal diet mixed with arsenic-contaminated vegetable.

At the beginning of the study, the mean initial body weight for each group was recorded. Group I had a mean initial body weight of 175.5 ± 0.3 gm, Group II had a mean initial body weight of 173.2 ± 0.7 gm, Group III had a mean initial body weight of 169.33 ± 1.62 gm, Group IV had a mean initial body weight of 163.54 ± 0.6 gm, Group V had a mean initial body weight of 172.12 ± 1.27 gm, and Group VI had a mean initial body weight of 167.5 ± 1.64 gm.

After 120 days, the mean final body weight was recorded for each

Table 1
Effect of arsenic contaminated rice and vegetable on daily food and water consumption of albino rats. Arsenic control rats consumed significantly ($p < 0.05$) less food and water compared to normal control group. Whereas, in Group IV and VI food and water consumption declined significantly ($p < 0.05$) after arsenic contaminated rice and vegetable treatment compared to arsenic free rice and vegetable exposed rats of group III and V.

Groups	Water intake (ml/rat/day)	Food intake (gm/rat/day)
Group-I (Normal Control)	15.08 ± 0.12	25.43 ± 0.08
Group-II (Arsenic Control)	8.55 ± 0.10	18.52 ± 0.12
Group-III (Control Rice)	12.15 ± 0.14	25.03 ± 0.12
Group-IV (Test Rice)	9.65 ± 0.15	19.13 ± 0.14
Group-V (Control Edible Fern)	13.54 ± 0.18	24.56 ± 0.18
Group-VI (Test Edible Fern)	9.49 ± 0.34	19.71 ± 0.18

group. Group I had a mean final body weight of 211.46 ± 0.26 gm, Group II had a mean final body weight of 195.62 ± 0.98 gm, Group III had a mean final body weight of 210.43 ± 0.17 gm, Group IV had a mean final body weight of 203.97 ± 1.15 gm, Group V had a mean final body weight of 205.25 ± 0.11 gm, and Group VI had a mean final body weight of 194.7 ± 0.94 gm.

Comparing the mean final body weight of Group II to Group I, it was observed that the mean final body weight of Group II significantly ($P < 0.05$) decreased. Additionally, the mean final body weight of Group IV was found to be significantly lower than Group III, while the mean final body weight of Group VI was significantly lower than Group V.

3.6. Hematological and biochemical parameters

Table 2 shows the results of the study that examined the effects of edta administration of rice and vegetable with pellet food on hematological parameters, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), and platelets (PLT). The table presents the values obtained for each parameter and group, allowing for the comparison of the results between groups. This is also depicted in Fig. 1 for better understanding.

The results showed a decrease in mean RBC count, mean WBC count, and Hb in all three arsenic-treated groups. However, the platelet count remained within the normal range. The absence of significant changes in platelet counts may be attributed to multiple physiological and cellular mechanisms. A dynamic compensatory response, potentially mediated by thrombopoietin (TPO) signaling, likely maintains hematological homeostasis despite arsenic-induced stress [43]. Megakaryocytes exhibit a remarkable resilience to arsenic toxicity, preserving thrombopoiesis even under exposure to environmental stressors. Additionally, the inherently short lifespan and high turnover rate of platelets facilitate continuous replenishment, effectively counteracting potential cytotoxic effects. Moreover, the anucleate nature of platelets confers relative resistance to genotoxic insults and oxidative damage, further contributing to their stability in the systemic circulation [44]. The mean Hb dropped to 12.83 ± 0.8 gm/dl for sodium arsenite treatment, 13.67 ± 0.54 gm/dl for rice diet and 13.77 ± 0.28 gm/dl for edible fern diet compared to control. Similarly, the mean RBC declined to 4.57 ± 0.3 for sodium arsenite treatment, 4.83 ± 0.2 m/ul for rice diet and 4.9 ± 0.4 m/ul for edible fern diet compared to control. The mean WBC reduced to 7933 ± 478.8 cmm for sodium arsenite treatment, 7633 ± 367.17 cmm for rice diet and 7700 ± 569.6 cmm for edible fern diet compared to control. The negative control, rice diet, and edible fern diet showed Hb levels of 14.53 ± 0.25, 14.17 ± 0.43 gm/dl, and 15.27 ± 0.13 gm/dl, respectively. The corresponding RBC levels were 5.17 ± 0.08 m/ul, 5.33 ± 0.08 m/ul, and 5.4 ± 0.07 m/ul, while the WBC levels were 9900 ± 284.8 cmm, 8600 ± 264.58 cmm, and 8333 ± 346.9 cmm, respectively.

The present study found that exposure to sodium arsenite and arsenic-contaminated rice and vegetable resulted in an anaemic condition in Wistar albino rats, as evidenced by a lowered Hb level. This decrease in Hb level may be due to arsenic's action on the RBC membrane, inhibiting erythropoiesis. Previous studies have reported a decrease in the number of RBCs in animal models and human populations due to toxicant exposure, which is consistent with our findings [45]. Additionally, the decrease in WBC count in the treated groups observed in our investigation may be associated with a decrease in nonspecific immunity due to arsenic exposure, as hindered maturation of white blood cells could be a possible cause. This finding is also in agreement with previous studies [11,45].

The reduction in hemoglobin (Hb) concentration and total red blood cell (RBC) count may be attributed to the high binding affinity of arsenic (As) to Hb, leading to the inhibition of the heme biosynthesis pathway [5]. Arsenic exposure has been identified as a contributing factor to bone marrow suppression, leading to hematological dysfunction and abnormalities. Another important reason behind the decline in blood cell count is arsenic's detrimental effect on hematopoietic stem cells and the bone marrow microenvironment [39]. Additionally, the observed decrease in total white blood cell (WBC) count could result from the apoptotic effects of arsenic on plasma cells, as previously reported by Rousselot et al. [46]. Similar hematological alterations were also documented by Ferzand et al. [10].

Table 3 displays the values for various biochemical parameters

Table 2
Effect of arsenic contaminated rice and vegetable supplementation on hematological markers. Hematological parameters were decreased after arsenic exposure in Group II compared to normal control rats. After treatment with arsenic contaminated rice and vegetable respectively in Group IV and VI, hematological parameters reduced compared to arsenic free rice and vegetable exposed groups. There was no observable difference in platelet count.

Groups	Hb (gm/dl)	RBC (m/ul)	Platelet ($\times 10^3$ cm m)	WBC (cm m)
Group-I (Normal Control)	14.53 ± 0.25	5.17 ± 0.08	270 ± 3.15	9900 ± 284.8
Group-II (Arsenic Control)	12.83 ± 0.80 * ** *	4.57 ± 0.3 * ** *	265 ± 3.12	7933 ± 478.8 * ** *
Group-III (Control Rice)	14.17 ± 0.43	5.33 ± 0.08	265 ± 4.02	8600 ± 264.58
Group-IV (Test Rice)	13.67 ± 0.54 *	4.83 ± 0.2 *	263 ± 3.14	7633 ± 367.17 * ** *
Group-V (Control Edible Fern)	15.27 ± 0.13	5.4 ± 0.07	260 ± 3.11	8333 ± 346.9
Group-VI (Test Edible Fern)	13.77 ± 0.28 * ** *	4.9 ± 0.11 * *	260 ± 2.99	7700 ± 569.6 * ** *

Significance level: *P < 0.05; * *P < 0.01; * **P < 0.001; * ** *P < 0.0001.

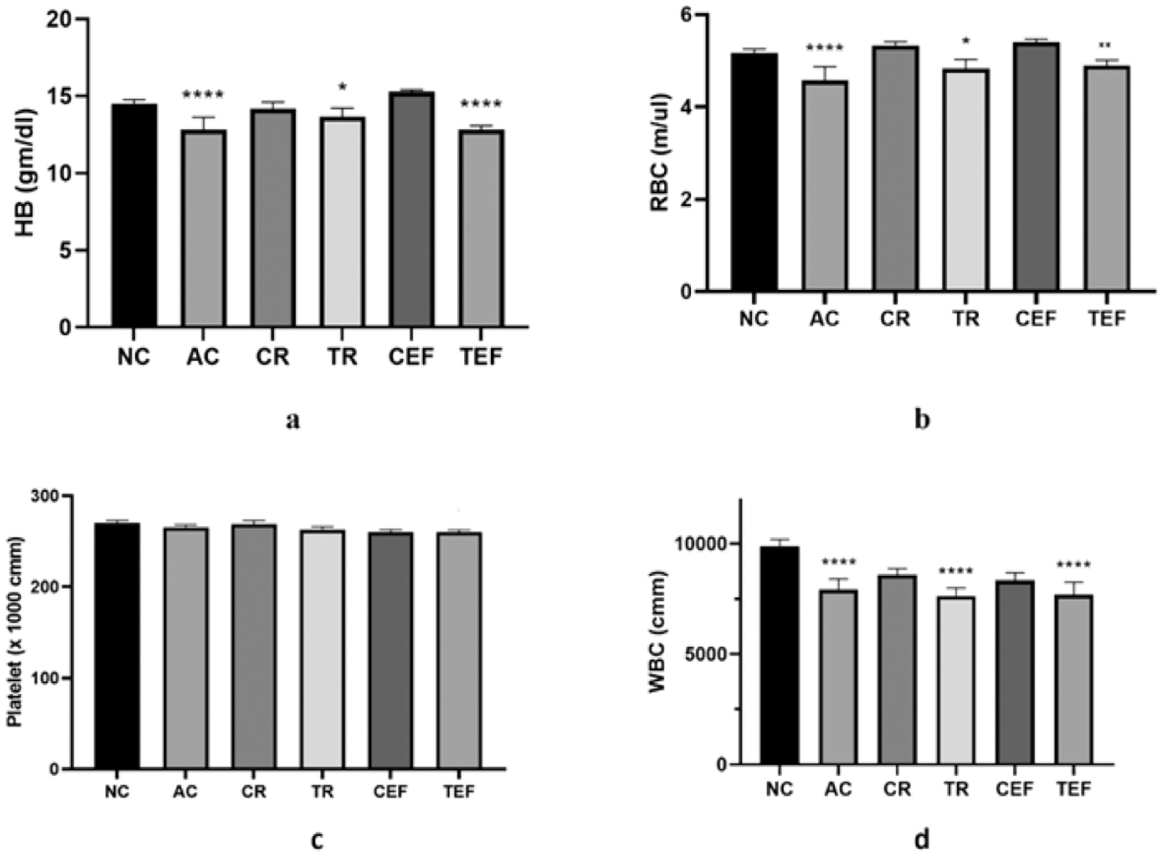

Fig. 1. Level of hematological biomarkers in the experimental groups. a. Hemoglobin (HB), b. Red Blood Cell (RBC), c. Platelet and d. White Blood Cell (WBC). Data were analyzed using one-way ANOVA followed by Dunnett's Multiple Comparisons Tests and reported as mean SD (n = 5). Here, the different groups are denoted by NC - Normal control, AC - Arsenic control, CR - Control rice, TR - Test rice, CED – Control edible fern, TED – Test edible fern. When compared to the Normal Control (NC) group, values are statistically significant at *P < 0.05; * *P < 0.01; * **P < 0.001, * ** *P < 0.0001.

Table 3
Effect of arsenic contaminated rice and vegetable supplementation on biochemical markers. Biochemical parameters were elevated after arsenic exposure in Group II compared to normal control rats. After treatment with arsenic contaminated rice and vegetable respectively in Group IV and VI, biochemical parameters reduced compared to arsenic free rice and vegetable exposed groups.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
Group-I (Normal Control)	55.33 ± 3.5	115.0 ± 9.3	120.67 ± 7.6	13.37 ± 0.9	0.61 ± 0.01
Group-II (Arsenic Control)	71.0 ± 5.2 * *	155.33 ± 4.0 * ** *	149.67 ± 5.8 * ** *	14.33 ± 0.5 * **	0.72 ± 0.03 * *
Group-III (Control Rice)	56.67 ± 7.0	114.0 ± 15.2	130.33 ± 4.6	14.4 ± 0.8	0.59 ± 0.006
Group-IV (Test Rice)	67.0 ± 4.8 *	133.33 ± 7.8 *	143.33 ± 4.2 * ** *	15.3 ± 0.6 * **	0.63 ± 0.03 *
Group-V (Control Edible Fern)	60.33 ± 9.1	110.0 ± 12.0	114.67 ± 5.2	15.33 ± 0.5	0.587 ± 0.1
Group-VI (Test Edible Fern)	69.33 ± 4.67 * *	147.67 ± 6.8 * **	140.67 ± 10.7 * **	16.2 ± 0.5 * ** *	0.623 ± 0.03 *

Significance level: *P < 0.05; * *P < 0.01; * **P < 0.001; * ** *P < 0.0001.

including creatinine (CRN), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The mean serum levels of ALT, AST, ALP, CRN, and BUN were found to be increased in the groups exposed to sodium arsenite and As-contaminated rice and vegetable (Fig. 2).

The biochemical parameters creatinine (CRN), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured in the study. The mean levels of ALT, AST, and ALP were found to be increased in the groups exposed to sodium arsenite and As-contaminated rice and vegetables compared to the negative control group. Specifically, the mean ALT level was elevated to 71.0 ± 5.2 u/l for sodium arsenite treatment, which was significantly higher than the negative control value of 55.33 ± 3.5 u/l. The mean ALT level was also found to be elevated in the rice and edible fern diets compared to their respective control groups. The mean AST level was also increased to 155.33 ± 4.0 u/l for sodium arsenite treatment, which was significantly higher than the negative control value of 115.0 ± 9.3 u/l. Similarly, the mean AST levels were elevated in the rice and edible fern diets compared to their respective control groups. The mean ALP level was slightly increased to 149.67 ± 5.8 u/l for sodium arsenite treatment compared to negative control (120.67 ± 7.6 u/l), and was also found to be elevated in the rice and

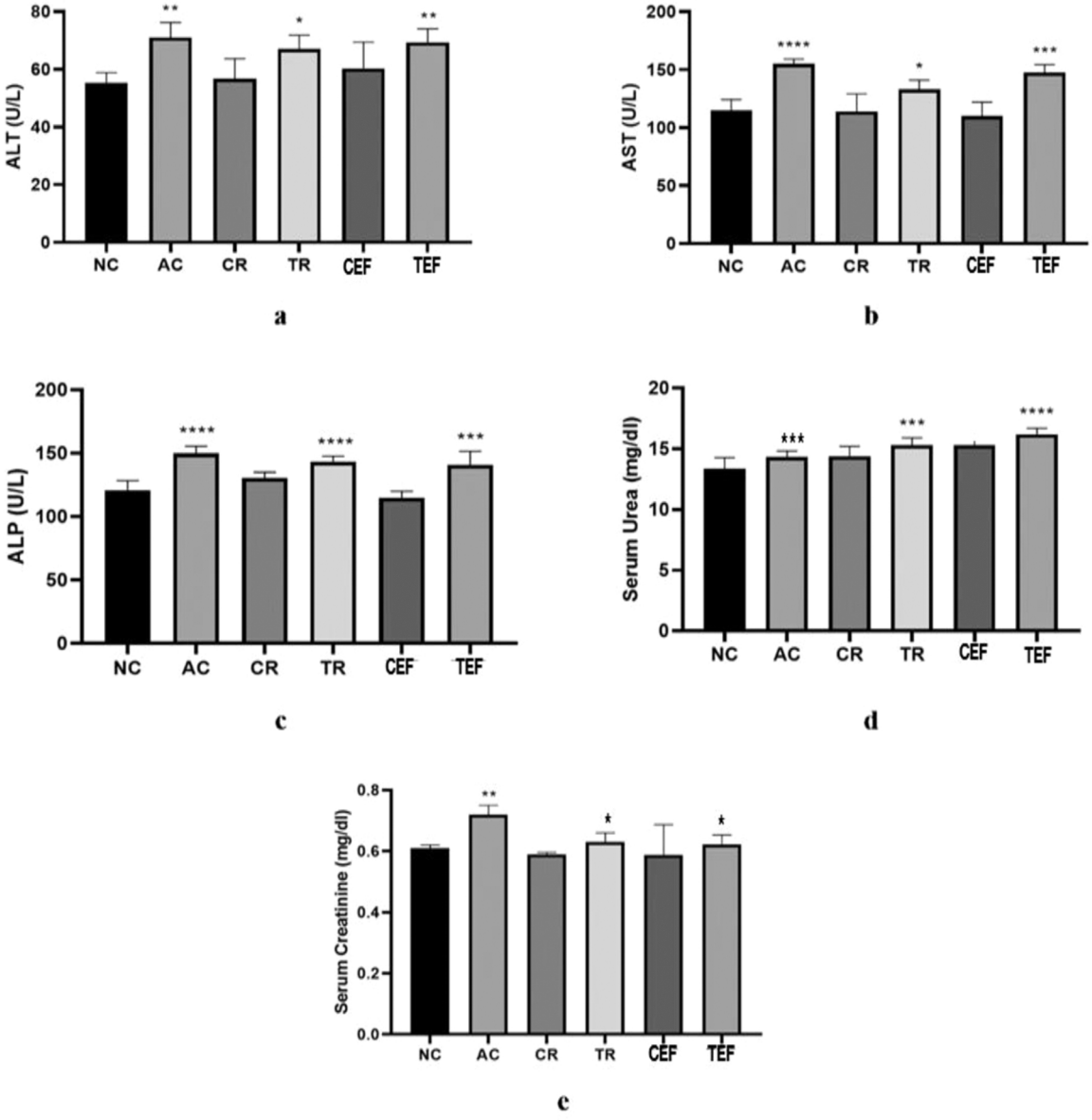

Fig. 2. Level of biochemical biomarkers in the experimental groups. a. Alanine Transaminase (ALT), b. Aspartate Aminotransferase (AST), c. Alkaline Phosphatase (ALP), d. Serum Urea and e. Serum Creatinine. Data were analyzed using one-way ANOVA followed by Dunnett's Multiple Comparisons Tests and reported as mean SD (n = 5). Here, the different groups are denoted by NC - Normal control, AC - Arsenic control, CR - Control rice, TR - Test rice, CED – Control edible fern, TED – Test edible fern. When compared to the Normal Control (NC) group, values are statistically significant at *P < 0.05; * *P < 0.01; * **P < 0.001, * ** *P < 0.0001.

edible fern diets compared to their respective control groups.

The liver is an important organ in the body that plays a crucial role in various metabolic processes. AST and ALT are two enzymes that are found in liver cells and are used as indicators of liver damage or disease. In this study, it was observed that exposure to arsenic through drinking water and food consumption led to an increase in the levels of AST and ALT in the subjects. This increase is indicative of cellular leakage and failure of functional integrity of liver cell membranes, which suggests that the liver is not functioning properly [47]. Furthermore, ALP is another important enzyme that plays a vital role in biological processes, such as detoxification, metabolism, and biosynthesis of energetic macromolecules. An interference in the function of this enzyme can lead to impaired liver function, which has been described in previous studies [47,48].

In addition to liver function, the study also looked at kidney function by measuring levels of serum urea and creatinine. An increase in serum urea levels compared to normal is a hallmark of nephrotoxicity and indicates that there is a problem in the kidney's ability to excrete metabolic waste products. However, in this study, only slight changes in creatinine levels were observed.

Arsenic has been shown to induce oxidative stress by generating reactive oxygen species (ROS), which initiate lipid peroxidation by targeting polyunsaturated fatty acids in cellular membranes. This process triggers a chain reaction of free radical propagation, ultimately leading to membrane destabilization, loss of structural integrity, and leakage of microsomal enzymes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are key hepatic biomarkers, primarily localized in the cytoplasm, and their release into circulation occurs as a consequence of hepatocellular damage [39,40,5]. Increased serum levels of urea and creatinine serve as key biomarkers of arsenic-induced nephrotoxicity, indicating impaired renal function [39]. Therefore, elevated serum levels of these enzymes serve as a critical indicator of hepatic injury.

Our research group has been actively investigating arsenic toxicity

for over a decade. Previously, we reported arsenic-induced alterations in hepatic and renal biomarkers, as well as hematological parameters. This study follows a similar trend in biomarker fluctuations. While our earlier research focused on arsenic exposure through contaminated drinking water, we extended our analysis to evaluate whether exposure through naturally arsenic-contaminated food induces comparable toxicological effects. As expected, our findings demonstrated a consistent pattern of toxicity [39,40].

Overall, the study suggests that exposure to arsenic-containing foods can have negative effects on liver and kidney function, as indicated by the changes in the levels of various enzymes and biomarkers.

3.7. Histopathological parameters

To investigate the effects of arsenic exposure on tissue structure, we conducted a histopathological analysis of two types of tissue. Specifically, we examined the kidney tissues of rats that were exposed to arsenic, and compared it to the kidney tissues of control rats. Our results revealed significant changes in the kidney tissues of sodium arsenite and arsenic-contaminated food treated Wistar albino rats, including the presence of fat bodies, fatty degeneration, mononucleated inflammatory cell infiltration, and cytoplasmic vacuoles. These alterations were not observed in the control kidney tissue, which maintained its normal tissue architecture and glomerular structure. These findings suggest that arsenic exposure can cause substantial tissue damage and disruption, particularly in the kidney tissue of mammals.

Heavy metals such as arsenic are known to cause damage to various tissues, especially the liver. In our study, we observed that exposure to sodium arsenite and arsenic-contaminated food caused damage to the liver tissue, including hepatocyte degeneration, mononucleated inflammatory cell infiltration, and steatosis. In contrast, the control liver tissue exhibited intact hepatic lobules (Fig. 3). These findings suggest that exposure to arsenic can cause significant damage to liver tissue, potentially leading to impaired liver function and other health issues.

Increased oxidative stress in tissue due to arsenic exposure is seemed to be the major cause for arsenic-induced toxicity in rat. Arsenic mediated oxidative stresses are indicated for changing the organ degeneration during exposure. Liver has long been identified as a target organ of arsenic exposure. Because of its unique metabolic functions and related to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics. The sections of liver in Sodium arsenite and As-contaminated rice and vegetable treated groups showed mild to moderate degeneration, mononucleated inflammatory cell infiltration with mild to moderate fatty change where control groups did not reveal any lesions of pathological significance (Fig. 4). Exposure of rat to arsenic in drinking water causes elevation of liver enzymes in plasma. Like other toxic elements Sodium arsenite and As-contaminated rice and vegetable primarily increased the generation of free radical species and cause an imbalance between pro-oxidation and antioxidant homeostasis in liver system as a result causes hepatic degeneration.

Arsenic concentrates in the kidney during its urinary elimination that affects the function of proximal convoluted tubules [49]. We have observed the degenerative changes in kidney tissue in arsenic treated rat (Fig. 4). Further we have observed the elevated level of BUN in arsenic exposed rat sample. BUN test is primarily used to evaluate kidney function in a wide range of circumstances, to monitor people with acute or chronic kidney dysfunction [50]. We therefore interlinked between kidney tissue degeneration and elevated level of BUN, we have observed in our study.

Recognized as one of the most extensive and impactful environmental disasters in Bangladesh's history, we are conducting comprehensive investigations to elucidate the mechanistic underpinnings of this crisis and identify potential remedial strategies. Histopathological examinations have provided irrefutable evidence of arsenic-induced cellular and tissue damage, consistent with the pathophysiological manifestations of arsenic toxicity. These observations are in alignment with our prior research on arsenic-mediated molecular pathogenesis, which demonstrated disruptions in cellular homeostasis, epigenetic modifications, and oxidative stress pathways. Such findings further validate the profound adverse effects of arsenic exposure at both the cellular and molecular levels, underscoring the urgency for targeted interventions [23,39,40].

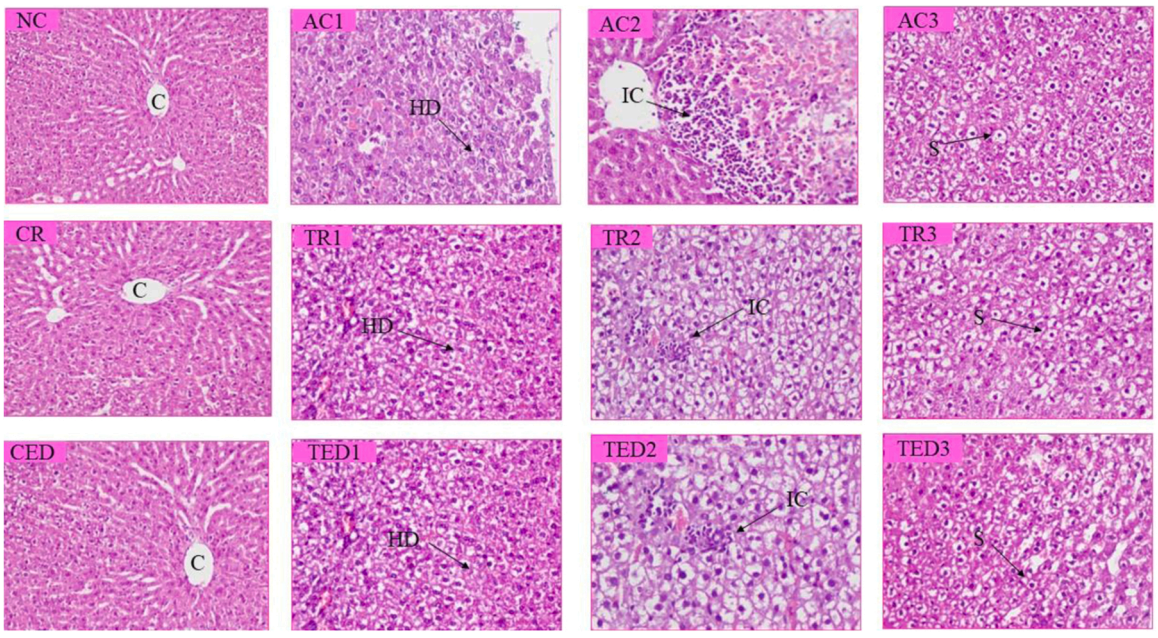

Fig. 3. Histopathological interpretation of liver tissue sections from different groups of experimental arsenic treated animals. Light microscopic image of hematoxylin and eosin-stained rat liver (microscopic resolution: 20X and 40X). Here, the different groups are denoted by NC - Normal control, AC - Arsenic control, CR - Control rice, TR - Test rice, CED – Control edible fern, TED – Test edible fern. The arrow indicates HD – Hepatocyte degeneration, IC – Inflammatory cells, S – Steatosis.

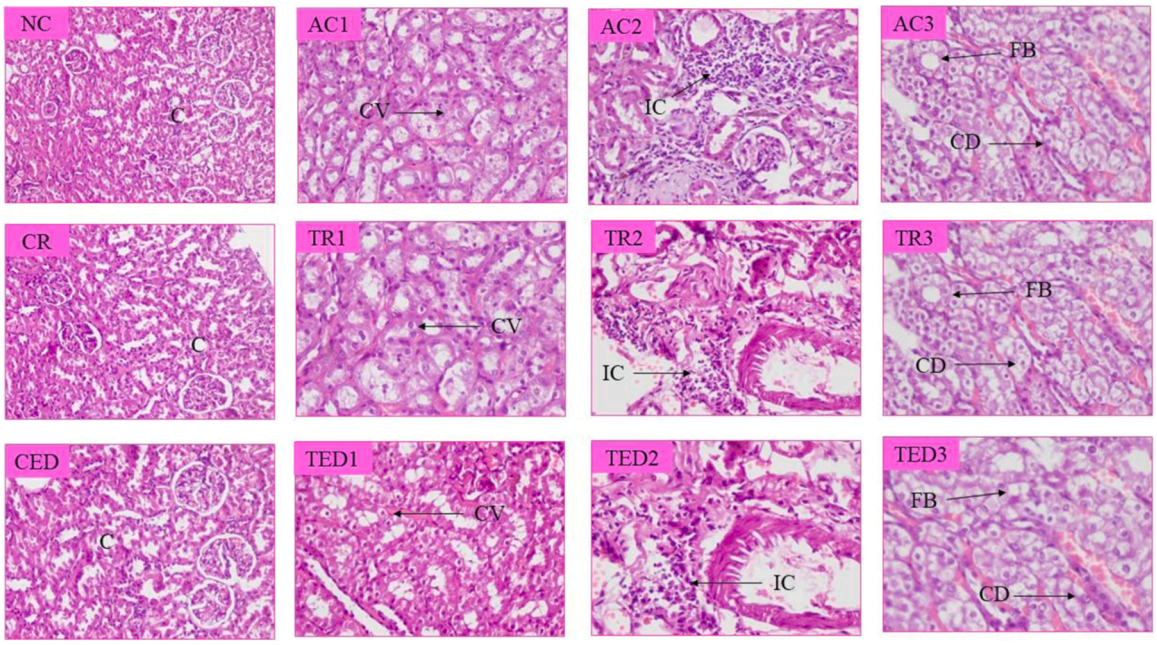

Fig. 4. Histopathological interpretation of liver tissue sections from different groups of experimental arsenic treated animals. Light microscopic image of hematoxylin and eosin-stained rat liver (microscopic resolution: 20X and 40X). Here, the different groups are denoted by NC - Normal control, AC - Arsenic control, CR - Control rice, TR - Test rice, CED – Control edible fern, TED – Test edible fern. The arrow indicates that CV – Cytoplasmic vacuoles, IC – Inflammatory cells, FB – Fat bodies, CD – Cellular degeneration.

3.8. Correlations

The results of the study showed a notable and positive correlation between the concentration of arsenic in groundwater and that in soil, grains, and vegetables. This correlation was observed at all nine sampling locations studied. Additionally, the concentration of arsenic in soil was also found to have a positive correlation with the concentration of arsenic in grains and vegetables. These findings suggest that the contamination of groundwater and soil by arsenic can significantly impact the concentration of arsenic in food crops, which may pose a health risk to individuals consuming those crops. Therefore, it is important to monitor the levels of arsenic in soil, water, and food crops to ensure public health and safety.

We found that when combining data from all nine locations, there was a significant positive correlation between the As content in irrigation water and As content in soil (with a p-value of ≤ 0.001). Additionally, we found significant positive correlation between As content in irrigation water and As content in both grains and vegetables (with a p-value of ≤ 0.05 for grains and ≤ 0.001 for vegetables). Furthermore, a significant positive correlation was also observed between As content in soil and As content in both rice and vegetables, with p-values of ≤ 0.05 and ≤ 0.001, respectively.

The presence of arsenic in irrigation water has been consistently shown to result in higher levels of arsenic in soils and plants, including grains and vegetables [51]. This is supported by previous studies which found that elevated groundwater irrigation with arsenic can lead to significant accumulation of arsenic in rice fields [52]. Additionally, even low concentrations of arsenic in soil can result in increased arsenic content in rice grains [25]. The correlation between irrigation water and grain arsenic content has been demonstrated in several studies. The relationship between irrigation water, soils, and rice grain arsenic content has also been observed in Bangladesh [28]. As a result of long-term consumption of arsenic-containing rice and vegetables, we found significant negative impacts on the health of rats, including changes in various blood, biochemical, and histopathological parameters. These findings emphasize the importance of understanding and mitigating the risks associated with arsenic contamination in irrigation water and its impact on human and animal health.

The primary limitations of this study stem from the relatively small sample size and shorter study duration, both constrained by funding limitations. While the controlled experimental design ensured methodological rigor and minimized variability, a larger cohort and extended study period would enhance statistical power, improve effect size estimation, and strengthen the reproducibility of the findings. Nevertheless, we implemented stringent methodological controls, including standardized dietary regimens, tightly regulated environmental conditions, and randomized subject allocation, to mitigate potential sources of bias. Given that all experimental groups were treated identically except for the key variables under investigation, we attribute the observed physiological and biochemical alterations primarily to arsenic exposure and dietary interventions.

Potential confounding factors, such as genetic polymorphisms affecting arsenic metabolism, environmental toxin co-exposure, and pre-existing health conditions, may influence arsenic toxicity outcomes. However, our study aimed to minimize these confounders by ensuring homogeneous baseline characteristics among experimental groups and maintaining uniform environmental conditions throughout the study. While complete elimination of confounding effects is challenging, the observed differences are most likely driven by the experimental conditions rather than extraneous variables.

The controlled experimental setup enhances internal validity but may not fully account for variations in arsenic exposure, dietary patterns, and genetic heterogeneity in broader populations. However, our results align with previous studies investigating arsenic-induced biochemical and physiological disruptions, suggesting the relevance of these findings beyond this specific experimental context.

4. Conclusion

While our previous research has demonstrated the detrimental health effects of arsenic-induced toxicity, we were unable to definitively establish food as a major route of arsenic exposure. Based on the findings of this study, it can be concluded that the consumption of As-contaminated rice and vegetables poses a significant health risk to the

residents of the naturally As-contaminated regions. The observed adverse effects in the albino rats, including anemia, immunosuppression, altered serum liver enzyme level, kidney markers, and histological damages in liver and kidney are likely to be associated with chronic dietary exposure to arsenic. Often, the dietary routes of arsenic exposure are neglected as drinking water is considered the most prominent source of arsenic intoxication. But our findings suggest that chronic exposure to arsenic contaminated food may also lead to systemic disorders in exposed populations.

The study also revealed a strong positive correlation between the presence of arsenic in irrigation water, soil, grains, and vegetables. This indicates that the more arsenic present in irrigation water, the more likely it is to be deposited in soils and absorbed by plants, resulting in higher levels of arsenic in food. This is an alarming situation considering the fact that global climate change has worsened the arsenic contamination of groundwater. Furthermore, even low levels of arsenic in soil can increase arsenic content in rice grains, and arsenic concentration in irrigation water has been significantly correlated with grain As in various studies. These findings suggest that reducing the presence of arsenic in irrigation water and soil could help to reduce the levels of arsenic in food and mitigate the health risks associated with its consumption.

Overall, this study will help broaden our understanding of arsenic exposure pathways by establishing food as a critical source of arsenic toxicity, alongside drinking water. This necessitates a more holistic approach when assessing arsenic-related health risks, considering the cumulative effects of dietary arsenic intake over time. Any future discourse, policy-making, or public health intervention on arsenic poisoning must acknowledge food as a significant source of exposure.

Furthermore, these findings highlight the urgent need for advanced research to unravel the molecular and physiological mechanisms of arsenic toxicity and to develop effective mitigation strategies. Future studies should focus on identifying genetic and epigenetic biomarkers of arsenic toxicity, elucidating its impact on cellular pathways, and exploring agricultural interventions to reduce arsenic uptake in crops. A multidisciplinary approach—integrating environmental science, toxicology, public health, and agricultural innovations—will be essential to developing sustainable solutions for mitigating arsenic contamination and its far-reaching consequences on human health.

In future, we aim to address the current limitations by incorporating comprehensive arsenic speciation analysis, expanding sample sizes, more diverse populations, extending study durations to provide a more precise characterization of arsenic exposure and real-world environmental settings are necessary to validate and extend the applicability of these findings.

Institutional review board statement

Ethical permission for conducting animal studies were obtained from the Animal Ethics Review Board, Faculty of Biological Sciences, University of Chittagong, Bangladesh [approval reference number: AERB-FBSCU-20241018-(1)]. All animal experiments were performed in compliance with the ARRIVE guidelines, National Research Council's Guide for the Care and Use of Laboratory Animals, and the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

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CRediT authorship contribution statement

Khaleda Laila: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Alam Md. Mazharul:** Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Data curation. **Tasnim Zarin:** Writing – original draft, Visualization, Project administration, Formal analysis, Data curation. **Ezaj Md. Muzahid Ahmed:** Writing – original draft, Formal analysis, Data curation. **Apu Md. Abdur Rahman:** Visualization, Formal analysis. **Akter Rasheda:** Methodology, Conceptualization. **Bakar Md. Abu:** Methodology, Conceptualization. **Alam Md. Jibran:** Methodology, Conceptualization. **Chowdhury Rahee Hasan:** Writing – review & editing, Methodology, Conceptualization. **Datta Amit:** Methodology, Conceptualization. **Shawon Inzamamul Ismail:** Methodology, Conceptualization. **Rahman Md. Zillur:** Methodology, Funding acquisition, Conceptualization. **Al-Forkan Mohammad:** Writing – review & editing, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2025.101993.

Data Availability

Data will be made available on request.

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