Spirulina (*Arthrospora platensis*) in The Diet Reduces Sodium Arsenates’ Impacts on Kidney Enzyme Activities, Histopathology, and Arsenic Accumulation in Rats Models

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**ABSTRACT**

The study aimed to investigate the effects of arsenate (As) exposure on body weight, and kidney enzymes activities, assess histopathological changes in this tissue, and explore the potential protective role of spirulina (Sp) in reducing the harmful impacts of (As) on kidney health. The research utilized forty-eight female Wistar rats and divided them into six groups, including a control group that fed a normal diet and distilled water, the second group the rat treated with a 5mg/kg body weight of sodium arsenate-only group, the group three and four the rats treated with spirulina at different doses (300mg and 600mg), and the last group treated with combined (As) and (Sp) treatments. After four weeks of the experiment, the kidney was collected for enzyme analysis and histological examination. The results showed that As exposure decreased body weight (BW) compared to the control group, while (Sp) supplementation partially improved body weight. Arsenate exposure significantly increased the levels of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) enzymes in kidney tissues. However, spirulina supplementation at different doses reduced the enzyme levels, albeit statistically not significantly. The histological examination revealed As exposure resulted in necrosis, congestion, leukocyte infiltration, glomerular atrophy, and fibrosis, while Sp supplementation showed limited effects on reducing these changes. Overall, this study contributes to the understanding of the toxic effects of As and suggests that Sp supplementation may offer some protection against its harmful impacts on kidney health.

**INTRODUCTION**

Due to the rise in anthropogenic activity, heavy metal pollution has been rising (Gouva et al., 2020). Among the many toxicants, arsenic stands out as a common and epidemiologically metalloid that contaminates tens of millions of citizens every year (Hughes et al., 2012). Arsenic (As, atomic weight 74.9216, atom number 33), the 20th most prevalent substance within the crust of the earth (National Research Council 1977), is a harmful metalloid that's found in ecosystems (Cullen, & Reimer, 1989). The bioavailability and toxicity of arsenic can be influenced by its various chemical forms and states of oxidation. Despite variations between the effects of arsenite (AsIII) and arsenate (AsV), inorganic chemicals are more poisonous than those that are organic (Ventura et al., 2009).
Numerous investigations have established links between As exposure and a wide range of adverse health consequences, including neurological damage, diabetes, dermatitis, and harmful effects on the liver, kidney, spleen, and circulatory system (Chen et al., 2009). According to investigations, Reactive oxygen species (ROS) are another mechanism by which As causes toxicity (Ramos et al., 1995) which include free radicals (as superoxide \( \text{O}_2^- \), hydroxyl \( \text{OH} \), and peroxyl \( \text{ROO} \) radicals) and hydrogen peroxide, among other chemical compounds (Peng et al., 2000). The most effective and safe choices for healthcare derive from different organic chemicals produced by plants (AL Sulivany, 2023). Bluegreen algae known as spirulina, which can grow in both salt and fresh water, have recently been the subject of more research (Venkataraman, 1997). Due to its high protein content, amino acids, and vitamins, spirulina has been used as a food supplement (Guldas, & Irkin, 2010). With phenolic composition and polyunsaturated fatty acids of spirulina is a natural substance with excellent antioxidant properties (Gulldas et al., 2021). This study aims to determine the effect of arsenate on plasma and liver enzyme activities, examine the histopathological changes in kidney tissue, and investigate the potential protective role of spirulina in the presence of arsenate exposure.

2. Experimental Design:
For this investigation, forty-eight (48) female rats have been chosen at random and subdivided into six groups, and treated once a day for four weeks, as shown below.
Group 1: Distilled water.
Group 2: sodium arsenate oral intubations at 5mg/kg body weight.
Group 3: spirulina oral intubations at 300 mg/kg body weight.
Group 4: spirulina oral intubations at 600 mg/kg body weight.
Group 5: Oral intubations containing 5mg/kg sodium arsenate and 300 mg/kg of spirulina.
Group 6: Oral intubations containing 5mg/kg sodium arsenate and 600 mg/kg of spirulina.

3. Preparation of Kidneys Tissue:
After four weeks of experiment, rats were sacrificed by using Diethyl ether, the abdominal and thoracic cavities were promptly opened, the kidneys were removed and flushed with distilled water and the activities of enzymes in the kidneys were measured by using slide kits. For histological review, the remainder of the kidney tissue had been embedded in 10% neutral-buffered formalin.

4. Determination of Enzyme Activities in The Kidney:
Pieces of kidneys were flushed in icy cold saline solution before being blotted dry and weighed in 0.15 M Tris-Hcl Buffer (PH 7.4). In 0.15 M Tris-Hcl Buffer, a 10% (w/v) tissue homogenate was produced and processed for assessing AST, ALT, and LDH (Saeed and Al-Habbib, 1990). A portion of this tissue was also washed with cold saline before being treated with 0.2 M of Carbonate-Bicarbonate Buffer (PH 10.5) for ALP assessment in the kidneys. In 0.2 M Carbonate-Bicarbonate Buffer, 10% (w/v) kidney homogenate was obtained (Saeed and Al-Habbib, 1990). The supernatant for all enzyme activities was...
measured by using FUJIFILM (DRI_CHEM NX500- Czech Republic) according to the manufacturer's instructions for the slide reagent kits.

5. **Histological Studies:**
The samples of tissue from the kidneys were fixed in formalin 10% with a neutral buffer. The processing of the tissue was done behind the scenes, with pieces cut at 5 µm thickness with a microtome. The subsections have been stained with hematoxylin and eosin (H&E) and captured under a light microscope (Ramandi et al., 2017).

6. **Statistical Analysis:**
GraphPad Prism viewer mode 9 for Windows was used to assess the experimental outcomes' data, and One-way analysis of variance (ANOVA) was performed for comparing the experimental groups with the control group. Tukey’s test for multiple comparisons after (ANOVA) was used for determining the significance. Statistics were deemed significant at p-values ≤ 0.05 values.

**RESULTS**

1. **Effect of Arsenate on Body Weight:**
According to the obtained result, it is evident from the results that exposure to As at a dosage of 5mg/kg for twenty-eight (28) days caused a reduction in the rate of body weight compared to the control animals (Fig. 1). The control animals had a rate of weight measured at 204.8±7.37g, while the rats were given (As) experienced a decrease in the rate of weight to 176.5±3.3g. When the rats were orally intubated with 300mg and 600mg of (Sp), there was a slight elevation in the rate of weight compared to the rats given (As) alone. However, this increase was statistically insignificant, with a p-value greater than 0.05.

![Fig. 1: Effect of arsenate on body weight of rats in the presence and absence of 300mg and 600mg of spirulina supplementations (n=8 per group) during four weeks of feeding.](image-url)

2. **Kidney Enzyme Activities**
In Figure 2, the results demonstrate a significant increase (p<0.001) in the levels of AST, ALT, ALP, and LDH in the kidneys of rats fed a diet containing (As) compared to those fed normal pellets. Conversely, the administration of different doses of Sp (300mg and 600mg) led to an inhibition in the enzyme levels, but the differences were not statistically significant when compared to the rats fed normal pellets. When (As) was present along with both doses of (Sp) (300mg and 600mg), the enzyme levels remained higher, although at a lower level of significance (p<0.05-0.01). Specifically, the enzyme levels were reduced to (33.33±3.51U/l) and (33.83±3.91 U/l) in AST, (27.1±2.3 U/l) and (19.67±2.7 U/l) in ALT, (85.5±6.04 U/l) and (94.83±4.72 U/l) in ALP, and (184.3±11.43 U/l) and (149.3±6.91 U/l) in LDH.
Fig. 2: Exploring the kidney activity of (a) Aspartate Aminotransferase (AST), (b) Alanine Transaminase (ALT), (c) Alkaline Phosphatase (ALP), and (d) Lactate Dehydrogenase (LDH) in rats treated with 5mg/kg of As, with and without 300mg and 600mg of Spirulina (n=8 per group).

3. Histopathology of Kidney:
When the kidneys of the normal rats were examined (Fig. 3a), the renal glomeruli were found to have normal mesangial cells, normal capsules, and Bowman's space, as well as normal distal and proximal convoluted tubules. Arsenate treatment resulted in the following effects on the kidneys (Fig. 3b): necrosis of the collecting tubules, congestion of the renal tubules, infiltration of leukocytes between the tubules, atrophy of the glomeruli, and fibrosis of Bowman's membrane. On the co-treatment with (As) and (Sp), a rise in angiogenesis between the tubules (Fig. 3e), severe infiltration between the renal tubules and around the blood vessels, and a cloudy degeneration of the renal tubules (Fig. 3f) was noted. While the impact of Sp was restricted to mild to severe congestion (Fig. 3c) and simple cloudy degradation of renal tubules (Fig. 3d).
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Fig. 3: (a): normal structure of the kidney, glomerulus, distal and proximal convoluted tubules (brown arrow→); (b): As group showed glomerular atrophy (light blue circle), Bowman’s capsule fibrosis (orange arrow→), collecting tubes necrosis (yellow circle) and epithelial cells sloughing (grey arrow→); (c): Sp-300mg group showed, moderate congestion (green arrow→) and bowman’s space wide, (d): Sp-600mg group showed mild interstitial congestion (green arrow→) and simple cloud degeneration (blue circle); (e): As-Sp-300mg group showed renal inter-tubules vacuolation, increasing angiogenesis (black circle) among tubules and focal tubular pyknotic nuclei; (f): As-Sp-600mg group showed high infiltration (red circle) among tubules and around tubules, mild congestion and cloudy degeneration of collecting tubules.

DISCUSSION
Arsenic, a well-known toxic substance, can be found in both natural and artificial environments. When there is a contamination of (As) in groundwater and feed, it leads to detrimental effects on both humans and animals. The impact of this contamination extends to wildlife as well (Zubair et al., 2022). One crucial indicator of an animal’s growth is weight gain, which can be hindered by the consumption of hazardous substances. Arsenic exposure has been shown to cause a decrease in feed intake, resulting in weight loss and, in severe cases, even death among rat species. These harmful effects impede various metabolic processes and contribute to delays in growth and development (Kozul-Horvath et al., 2012; Sayed et al., 2015). Oral exposure to As for 28 days causes a slight decrease in body weight in rats compared to control rats. This contradicts Zubair et al. (2022) findings, which showed enhanced development by substituting standard feed pellets with reduced levels of As. The discrepancy suggests differences in outcomes based on factors like dosage, duration, or animal species.

The weight gain of rats orally exposed to 300 and 600 mg of (Sp) showed a minor improvement compared to the control group. This suggests that the ingestion of (Sp) at these doses had a positive effect on the rats’ weight gain. The results indicate that supplementation with Sp may have a beneficial impact on weight gain in rats. Our findings align with the study conducted by Rabeh et al. (2021), where rats were provided with supplements of Sp at different levels of 2.5%, 5%, and 7%. They demonstrated
that as the percentage of (Sp) supplementation in the diet increased, there was a corresponding increase in body weight gain, feed intake, and feed efficiency ratio. When comparing the group that received only (As) treatment to the group that received a combination of (As) and (Sp) supplementation (at doses of 300mg and 600mg), it was clear that the rats supplemented with Sp experienced substantial protection against weight loss. Notably, the rats treated with (As) and 600mg of (Sp) showed even greater improvement compared to those receiving 300mg of (Sp). This suggests that a higher dose of Sp may have a more pronounced effect in preventing weight loss induced by arsenic exposure in rats (Rabe et al., 2021). The findings of this study are also consistent with those reported by Korany et al. (2019), who investigated the effects of incorporating 300 mg/kg of Sp into the rats' diet. The researchers indicated that the observed improvement in weight gain could be attributed to the unique nutrients present in Spirulina, such as B-complex vitamins, minerals, proteins, γ-linolenic acid, and antioxidants like β-carotene and vitamin E (Holman & Malau-Aduli, 2012). Another explanation for this could be the fact that spirulina has more metabolizable energy per serving and a complete protein with all nine necessary amino acids than most vegetable components. Furthermore, the amino acids in spirulina protein are very easily absorbed. Additionally, the beneficial effects of spirulina on growth performance may be related to the physiological functions of bioactive components such as phenolic compounds, carotenoids, vitamins, minerals, and others found in spirulina (Abdel-Moneim et al., 2022).

The kidney is highly susceptible to the toxic effects of As. Environmental exposure to as is strongly linked to chronic kidney disease (Jayasumana et al., 2015). The results of the current inquiry showed that levels of AST, ALT, ALP, and LDH in the kidney of As-treated rats were higher. In 2004, Ahmed reported substantial rises in AST and ALT activity in arsenic-treated rabbits. Islam et al., (2010) observed that arsenic exposure raised AST and ALT activities markedly. Sharma et al., (2007) have also found the improved activity of AST, ALT, ALP, and ACP in arsenic-treated mice. Our findings supported the prior scientists' decisions about ALP and ALT activities. However, this is consistent with the results obtained by Nozohour and Jalilzadeh-Amin (2019), who discovered that the state of oxidation and chemical structure of (As) have a significant impact on its toxicity. By interacting with vital cysteinyl residues in many kinds of enzymes, arsenic affects enzyme activity, such as enzymes involved in protein ubiquitination. Prolonged arsenic exposure raises the chance of fibrosis in the kidneys via nephron epithelial cell epithelial-mesenchymal conversion (Lindberg, 2008). Treatment rates with oral gavage Sp at levels (300 and 600 mg) resulted in a substantial drop in the ratio of AST, ALT, ALP, and LDH in the kidneys. It was discovered that Sp lowered lipid peroxidation more successfully than pharmaceutical antioxidants such as -tocopherol and -carotene (Miranda et al., 1998). Since, the presence of Phycocyanin (C-phycocyanin and allophycocyanin) may contribute to its antioxidant protective activity (Bhat & Madyastha, 2001).

Most of the previous studies indicate that heavy metals have many negative side effects on mammalian organs and animals (Noman et al., 2015). Arsenic raises the number of free radicals in the body, generating an imbalance between them and antioxidants in the organ, resulting in tissue degeneration. One of the most toxic heavy metals is arsenate causes damage to the DNA by hypo-methylation or hyper-methylation both actions lead to dawn regulation of DNA and leads to cell death (Gopisetty et al., 2006), then leads to histopathological alternations (Noman et al., 2015). It is unclear how arsenic induces nephrotoxicity on a
fundamental level. However, some data point to ROS as a significant arsenic nephropathy mediator. As a result of arsenic metabolism in cells, ROS such as superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1O$_2$), and peroxyl radicals are produced. These ROS are toxic and can damage cellular macromolecules such as DNA, proteins, and lipids by disrupting the antioxidant defense system (Rizwan et al., 2014). This is consistent with the current findings when Noman and his colleagues (2015) documented that 5mg/kg BW daily delivered caused renal tubular degeneration and intratubular necrosis. In addition, while using spirulina as an amelioration, arsenic affects organ tissues in current outcomes comes agreement with Khan et al., (2005) state that spirulina has antioxidant properties, and decreases liver, and kidney toxicities. It was shown that feeding spirulina to mice causes a considerable increase in spleen cells and the production of immunoglobulins of type IgM, while other animal models generate globulins of type IgA and IgE (Khan et al., 2005). Spirulina also stimulates antiapoptotic genes to express bcl-2 (Liu & Zhang 2002), and it contains gamma-linolenic acid, which has antioxidant properties and reduces heavy metal toxicity in the liver and kidney (Saxena and Kumar 2004), as well as stabilizes membrane functions in various tissues (Upasani and Balaraman 2003). This protective effect is attributed to its antioxidant and anti-inflammatory activities. β Carotene, C-phycocyanins, vitamins, and minerals, among other antioxidant chemical compounds found in SP, are crucial in protecting renal tissues from damage caused by As (Abdelkhalek et al., 2017).

**CONCLUSION**

In conclusion, this study demonstrated that arsenate (As) exposure lowered body weight and raised the activity of enzymes in the renal system. Spirulina (Sp) dietary supplements, on the other hand, suppressed weight reduction and reduced enzyme levels. The histopathological study indicated that (As) exposure induced unfavorable alterations in kidney tissues, with only minimal relief provided by Sp supplementation. Overall, this investigation contributes to our understanding of (As) toxicity and implies that Sp may offer some protection against its negative effects on renal function.

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**REFERENCES**


Bangladesh Agri. University, Bangladesh.


Jayasumana, C., Gunatilake, S. & Siribaddana, S. (2015). Simultaneous exposure to multiple heavy metals and...


Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels. *Environmental Health Perspectives* 103,85–88 (1995). DOI: 10.1289/ehp.95103s185


