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Protective Effect of *Spirodela polyrhiza* on Various Organs of Arsenic-induced Wistar Albino Rats

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Abstract

Our study was undertaken to assess the protective effect of *Spirodela polyrhiza* against sodium arsenite-induced toxicity in experimental model. Animals were divided into six groups. The first group was used as control group while the other groups were treated with *S. polyrhiza*, sodium arsenite (10 ppm), 10 ppm As plus *S. polyrhiza*, sodium arsenite (30 ppm), and 30 ppm As plus *S. polyrhiza* respectively. Organs (kidney, spleen and heart) were collected at the end of the experiment for conducting histopathological analysis and measuring the amount of deposited arsenic. Tubular epithelium necrosis was seen affecting less than 50% of all renal specimens, on the other hand slightly enlarged white pulp region in splenic tissue were found in the groups of Wistar rat where *S. polyrhiza* and arsenic was administered concomitantly. Moreover, the observed moderate tissue changes (edema and muscle necrosis) in cardiac specimens of arsenic treated groups were found to be mild in the *S. polyrhiza* +arsenic treated groups. It is noteworthy to mention that *S. polyrhiza* not only reversed the arsenic-induced toxicity but also reduced the tissue arsenic load in Wistar rats to a considerable extent. Overall, the results indicated that *S. polyrhiza* might become useful in mitigating the toxic effects of arsenic.

Keywords: Arsenic; Histopathology; *Spirodela polyrhiza*; Wistar rat

Introduction

From ancient times, arsenic (As) - a ubiquitous element present in air, soil, and water, has been recognized as deadly human poison. Continuous exposure of human as well as animals to arsenic contaminated environment can be identified by its elevated levels in nail, hair, hoof, and urine [1,2]. Arsenic compounds known as arsenicals, basically exist in two forms: inorganic arsenic and organic arsenic. Between the two forms, the inorganic ones are considered as a potential threat in developing arsenicosis. In underground water, trivalent inorganic arsenicals (arsenite) are the prime reason of arsenicosis which affects more than 140 million people around the world [2,3]. After consuming arsenic-contaminated drinking water, arsenites enter into the gastrointestinal tract and then distributed into different organs [4]. However, inhalation and dermal absorption could be the other possible routes for arsenic to enter into the body. Arsenic takes part in cellular redox events following bioaccumulation which leads to production of excessive reactive oxygen species (ROS). Arsenic-induced apoptotic events are triggered by increasing oxidative stress and depletion of endogenous antioxidant system. Oxidative damage of DNA, per-oxidative damage of membrane lipids, and carbonylation of proteins are also caused by oxidative stress [5]. By deploying various other mechanisms, arsenic affects almost all vital organs and tissues like kidneys, liver, lungs, heart, testes and brain. In spite of being a global problem, still there is no reliable, specific and safe management strategy of arsenicosis.

Chelation therapy has been remained to be the primary treatment in arsenicosis. Various synthetic chelating agents such as meso-2,3-dimercaptosuccinic acid, 2,3-dimercaprol, and 2,3-dimercaptopropane-1-sulfonate has been used so far in patients suffering from arsenicosis [6]. However, elimination of essential metals and redistribution of As within tissues limited their clinical values to a large extent [7]. On the contrary, dietary antioxidants are familiar for their efficacy against oxidative stress related complications without generating remarkable toxic manifestations. Researchers are now exploiting dietary antioxidants in arsenicosis by taking into account the interconnection between As toxicity and oxidative stress.

Lately a variety of plant products and their vital constituents have been utilized to combat arsenic induced toxicity [8-10]. Chinese and Indian traditional system of medicine has shown that fruit extract of Emblica officinalis and its active constituents has anti-oxidative, anti-cancer and immune modulatory properties [11-13]. A study has shown that sequestered yellow pigment from turmeric containing antioxidant potentials mitigates untoward effects of arsenic in mice [14]. Similarly another study was conducted with aqueous extract prepared from Psidium guajava leaves where it was found to reverse the histopathologic changes in kidney and liver of arsenic-intoxicated rats to some extent by re-establishing various oxidative stress markers [15]. Co-administration of different plant extracts like Hippophae rhamnoides, Centella asiatica, and Aloe barbadensis also provided positive outcome in experimental animals [16-18]. Previous experiments also demonstrated the protective effects of Oscimum sanctum, Spirulina fusiformis, and Lycopersicon esculentum against heavy metal (lead, mercury, cadmium and arsenic) toxicity [19-21].

Spirodela polyrhiza is a perennial aquatic plant which is recognized by the common names like common duckweed, greater duckweed, and duck meat which are basically freshwater habitant. By growing in dense colonies it forms a mat on the water surface [22]. This macrophyte is non-toxic and nutritionally rich in protein and antioxidants [23-25]. According to a study in contaminated water bodies or paddy soils, *S. polyrhiza* has shown its potentiality in As phytofiltration [26,27]. However, in order to confront arsenic toxicity in an animal model the

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potentiality of *S. polyrhiza* has never been explored before. Hence, our study was designed with a view to assess whether *S. polyrhiza* plant powder could minimize or halt arsenic-mediated untoward health effects in animal model.

Method

Collection of plant materials

Whole part of fresh water habitant, *Spirodela polyrhiza* was collected from different local ponds of Hathazari zone, Chittagong.

Preparation of *Spirodela polyrhiza* plant powder

Chlorine containing water and distilled water was used for washing the collected plant thoroughly, and then they were dried in sun-light and grinded to produce the powder form of the collected plant. After that, plant powder (4% wt/wt) was mixed well with respective pelleted diet of rat.

Animals

Wister albino rates weighing 160-180 gm were procured from the animal facility of BCSIR laboratories, Chittagong. Albino rats were maintained following the ethical protocol of experimental animal which was set by the experimental animal ethics committee of Faculty of Biological Science, University of Chittagong. Before commencing the experiments Wistar rats were acclimatized in environmental condition of the laboratory (room temperature 23 ± 5°C, humidity 60 ± 70%, 12 : 12 h light : dark cycle). For measuring protective effect of Spirodela, six different groups of albino rats were taken each containing 6 rats. The control group (Group-I) received distilled water for 90 days. Group-II received *S. polyrhiza* (4% wt/wt) via their feed. On the other hand, Group III and Group V received 10 ppm and 30 ppm sodium arsenite respectively for 90 days. Group IV and Group-VI received both distilled water and *S. polyrhiza* via their feed (4% wt/wt) for similar days like control groups. After 90 days, rats were starved overnight and euthanized by light diethyl ether anesthesia in the next morning. Using 21G needle mounted on 5 mL syringe, blood samples were obtained by cardiac puncture and organs (kidney, spleen and heart samples) were carefully collected and weighed.

Histopathology of kidney, spleen and heart

10% neutral formalin was used for preservation of collected organ from sacrificed rat. Then, one block from each tissue was processed by an automatic tissue processor and embedded in paraffin. Six micron thick sections were cut, stained with hematoxylin and eosin and examined under light microscope (Olympus, Japan) [28].

Kidney

A semi-quantitative evaluation of renal tissue was utilized to score the degree of damage severity according to previously published criteria [29]. The injury score system for kidney tissue is shown in Table 1.

Spleen

A semi-quantitative scoring system was adopted for analyzing spleen histology. Segments of spleen was scored for the enlargement of B- and T-lymphocyte areas in red and white pulps (absent=0; slight=1; moderate=2; and pronounced=3) and for the increased number of apoptotic cells, macrophages, necrotic cells and presence of pigments (absent=0; and present=1) [30].

Heart

Pathology of heart was graded based on the presence and severity of edema, leukocytic infiltration, muscle necrosis, chronic inflammation, and fibrosis. Grading for each component was performed by using a semi-quantitative scale where 0 was normal and 1–4 represented mild through severe abnormalities [31].

Quantification of arsenic

A mixture of $HClO_4$-HNO_3 solution (ratio 1:3 v/v) was used for digestion of a portion of each organ (0.25 g) for 2 days at 130°C [32]. The digested samples were diluted with deionized water after evaporation of HNO_3 and then analyzed for arsenic by Flow Injection Hydride Generator Atomic Absorption Spectrophotometer (FI-HG-AAS) (ICE 3000).

Statistical Calculations

A commercially available statistics software package (SPSS, Chicago, IL, and V.22) was used for statistical analysis. All of the data had been expressed as the mean ± standard errors of mean (SEM). Besides, data from control and each treated group were analyzed by employing one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMART) with a p-value less than 0.05 was considered to be statistically significant.

Results and Discussion

Arsenic contaminated drinking water, usage of As-contaminated water in irrigation of food crops and in food preparation, various industrial processes, intake of As-contaminated food and smoking tobacco are the plausible routes of elevated levels of arsenic exposure. Skin lesions and skin cancer are the most characteristic effects among various adverse health outcomes of chronic arsenic poisoning [33,34].

Kidney, the excretory organ of human body, continually excretes arsenic in urine. Residual arsenic form complex with intracellular proteins, lipids or DNA molecules and produce free radicals which is responsible for the ultimate architectural changes of kidney. The kidney of control rats showed regular glomerular structure (Figures 1A and

Score	Criteria
0	Normal
1	Areas of focal granulovacuolar epithelial cell degeneration and granular debris in the tubular lumen with or without evidence of desquamation in small foci (less than 10% of total tubule population involved by desquamation)
2	Obvious tubular epithelial necrosis and desquamation but involving less than 50% cortical tubules
3	Necrosis and desquamation in more than 50% of the proximal tubules, but intact tubules easily identified
4	Complete or almost complete proximal tubular necrosis

Table 1: Injury scoring system for kidney tissue of Wistar rats.

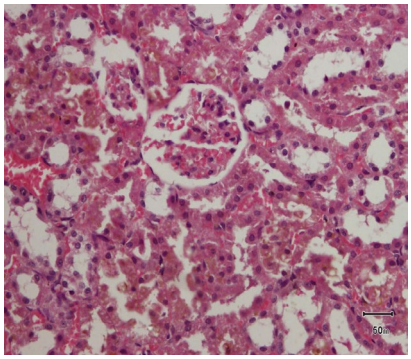

Figure 1A: Photomicrograph of rat kidney structure from Group-I (Control) revealing normal architecture (H&E stain, 200x).

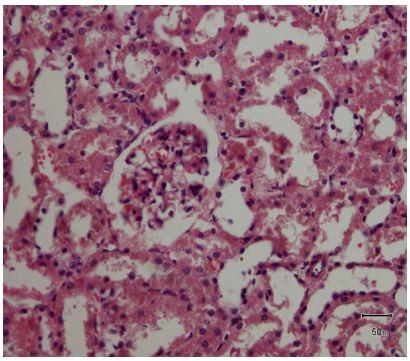

Figure 1B: Section of rat kidney structure from Group-II (S. polyrhiza) showing normal architecture (H&E stain, 400x).

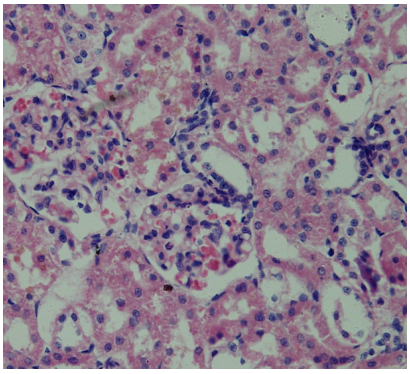

Figure 1C: Section of rat kidney structure from Group-IV (10 ppm As+*S. polyrhiza*) showing mild necrosis (H&E stain, 400x).

Histologic Injury Score	Group-I (Control)	Group-II (*S. polyrhiza*)	Group-III (10 ppm As)	Group-IV (10 ppm+*S. polyrhiza*)	Group-III (30 ppm As)	Group-IV (30 ppm+*S. polyrhiza*)
Kidney	1 ± 0	1 ± 0	2.67 ± 0.21**	1.67 ± 0.21##	2.5 ± 0.22**	2 ± 0.37##
Spleen	1 ± 0	1 ± 0	5 ± 0.26**	3.67 ± 0.50##	5.67 ± 0.21**	3.33 ± 0.42##
Heart	0 ± 0	0 ± 0	4.33 ± 0.21**	1.67 ± 0.54##	2.33 ± 0.21**	1 ± 0.45##

Table 2: Protective effect of *S. polyrhiza* on histologic injury score of albino rats. Here, values of hepatic injury score are expressed as MEAN ± SEM. **Control versus 10 ppm/30 ppm ($p<0.05$.) ##10 ppm vs. 10 ppm+ *S. polyrhiza*/30 ppm vs. 30 ppm+*S. polyrhiza* ($p<0.05$).

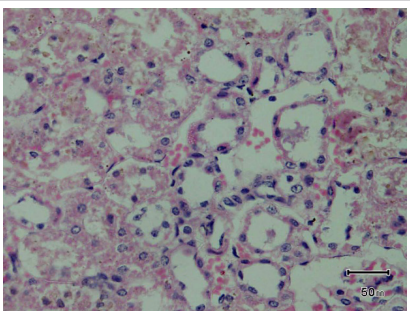

Figure 1D: Section of rat kidney structure from Group-VI (30 ppm As+*S. polyrhiza*) showing mild necrosis (H&E stain, 400x).

1B). In arsenic-exposed groups (Group-III and Group-V) necrosis was found to affect greater than 50% of all tissues (Figure 1C). Moreover, increased number of inflammatory cells was present in specimens of both arsenic-exposed groups. The histological injury scoring of kidney tissues was significantly higher ($p<0.05$) in these arsenic-exposed groups compared to that of the control group. In Group-IV (10 ppm As+ *S. polyrhiza*) and Group-VI (30 ppm As+ *S. polyrhiza*), the tissue injury score was found to significantly decrease ($p<0.05$) while compared to their counter arsenic-exposed groups (Table 2). Furthermore, it was seen that the tubular epithelium necrosis was seen affecting less than 50% of all specimens from the groups treated with *S. polyrhiza* in addition to sodium arsenite (Figure 1D).

The structure of spleen from Group-I (Control Group) and Group-II (*S. polyrhiza*) revealed the regular structure constituting of white and red pulps enclosed by a capsule of dense connective tissue (Figure 2). In previous studies, spleenocytosis and mild fatty degeneration as reported to be found in animal model [35]. In the arsenic-treated groups, there was seen moderate to pronounced enlargement of white pulp due to increased hematopoietic support and increased numbers of macrophages. Therefore, increased effete erythrocytes count caused by phagocytosis is the consequences of these changes. Sometimes it results in production of brown pigments named haemosiderin in the splenic parenchyma. Haemosiderin is produced as a result of destruction of red blood cell carried out by macrophages and swollen reticuloendothelial cells in the spleen [36]. Haemosiderin was found in all arsenic-exposed groups. When rats were administered arsenic for 90 days, the white pulp region enlarged due to cellular proliferation. Most of the cells were darkly stained and the spaces between cells were widened. This disorganization was due to hyperplasia of the lymphoid tissue. The number of apoptotic cells, macrophages and pigments was increased in both arsenic-treated groups. The white pulp region in *S. polyrhiza* and arsenic-treated groups was found to be slightly enlarged. The injury score in Group-III (10 ppm As) and Group-V (30 ppm As) was increased significantly ($p<0.05$) compared to Group-I (Control Group) while the injury scoring for Group-IV (10 ppm As + *S. polyrhiza*) and Group-VI (30 ppm As + *S. polyrhiza*) was found to be decreased significantly while compared to control group (Table 2).

Cardiac tissue from control (Group-I) and *S. polyrhiza* supplemented groups (Group-II) showed regular cardiac histology (Figures 3A and 3B). In cardiac tissues of rats from Group-III (10 ppm As) moderate edema and leukocytic infiltration was found (Figure 3C). Furthermore, moderate muscle necrosis was observed in both Group-III (10 ppm As) and Group-V (30 ppm As) (Figure 3D). The observed moderate tissue changes (edema and muscle necrosis) in arsenic treated groups was found to be mild in the groups of albino rats where *S. polyrhiza* was given as feed supplement along with the supply of sodium arsenic via drinking water (Figure 3E). After calculating the histological injury scoring, it was found that the score was comparatively higher in Group-

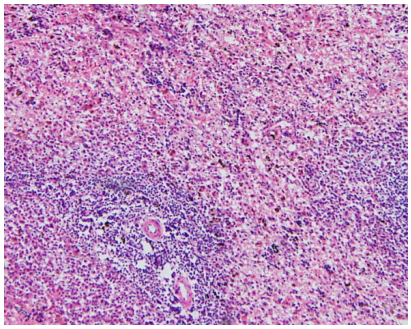

Figure 2: Photomicrograph of rat spleen structure from Group-I (Control) revealing normal architecture (H&E stain, 400x).

III (10 ppm As) and Group-V (30 ppm As) than control group. On the other hand, the injury score was significantly decreased ($p<0.05$) in Group-IV (10 ppm As+ *S. polyrhiza*) and Group-VI (30 ppm As+ *S. polyrhiza*) (Table 2).

The solubility of arsenic compounds, the matrix or tissue in which arsenic is being ingested, and the interaction with other nutrients that are present in the gastrointestinal tract are the main factors on which the bioavailability of ingested inorganic arsenic depends [37]. Kidney is the major route of excretion of arsenic compounds which filter into the urine and hence it accumulates arsenic after repeated exposure [38]. In arsenic exposed individuals, erythrocyte lysis leads to reduction of the life span of circulating erythrocytes. Filtration of blood and the storage of blood are the two functions of spleen which play crucial roles in splenic arsenic deposition. Accumulation of arsenic in spleen may be increased by trapping of erythrocytes [39]. On the hand, having shorter half-life in blood, heart tends to accumulate less arsenic than other organs. We have found significant renal, splenic and cardiac accumulation of arsenic in arsenic exposed albino rats. The accumulated arsenic level was mitigated noticeably in *S. polyrhiza* supplemented groups which indicates that this macrophyte has the potentiality to diminish the adverse effects caused by arsenic (Table 3).

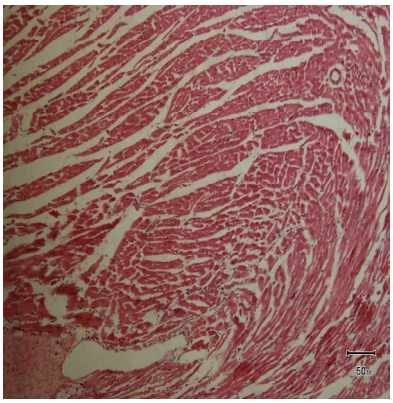

Figure 3A: Photomicrograph of rat heart structure from Group-I (Control) revealing normal architecture (H&E stain, 200x).

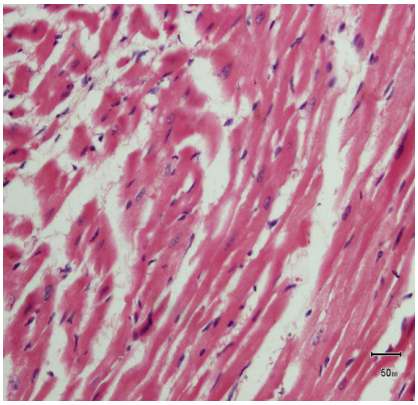

Figure 3B: Rat heart architecture from Group-II (*S. polyrhiza*) showing normal structure (H&E stain, 400x).

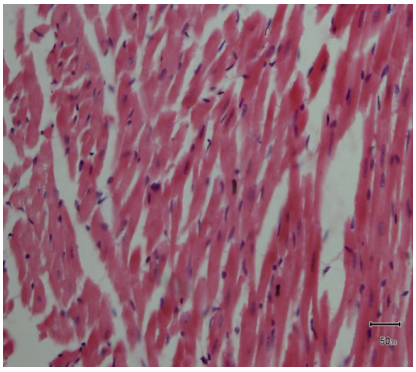

Figure 3C: Photomicrograph of rat heart structure from Group-IV showing moderate muscle necrosis (10 ppm As+*S. polyrhiza*) (H&E stain, 400x).

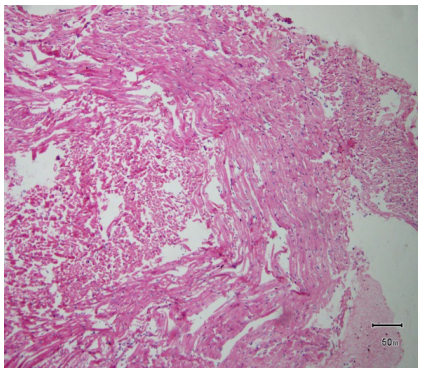

Figure 3D: Photomicrograph of rat heart structure from Group-V (30 ppm As) showing mild muscle necrosis (H&E stain, 200x).

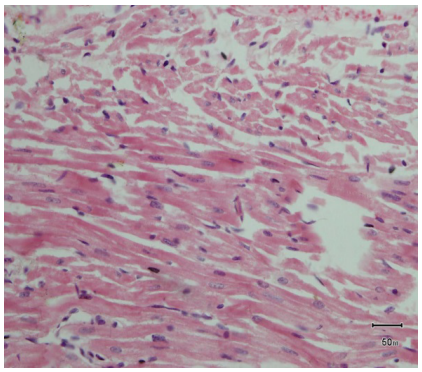

Figure 3E: Photomicrograph of rat heart structure from Group-VI (30 ppm As+*S. polyrhiza*) showing moderate muscle necrosis (H&E stain, 200x).

Arsenic toxicity varies due to its oxidation state and chemical composition. Arsenite binds to cysteinyl residues of a variety of enzymes and thereby affect enzyme activities. Moreover, arsenic also interacts with cellular antioxidant mechanisms. It decreases glutathione levels and hamper the DNA repair systems, which contribute to the oxidative damage in cells [40,41]. The distinct mechanism behind these positive effects of *S. polyrhiza* remains still obscure; however, the antioxidants occurring in *S. polyrhiza* might play roles in neutralizing free radicals generated by arsenic. This hypothesis is endorsed by a study where tea extracts manifested effectiveness against arsenic- and lead-induced toxicity in laboratory animals due to the antioxidant properties of the polyphenols [42,43].

Conclusion

Arsenic-induced rats had experienced organ damage as evidenced by numerous significant histological damages and marked accumulation

Accumulated Arsenic in Organs	Group-I (Control)	Group-II (*S. polyrhiza*)	Group-III (10 ppm As)	Group-IV (10 ppm+*S. polyrhiza*)	Group-III (30 ppm As)	Group-IV (30 ppm+*S. polyrhiza*)
Kidney	0.42 ± 0.005	0.43 ± 0.004	12.10 ± 0.486**	6.81 ± 0.486##	8.64 ± 0.268**	4.97 ± 0.360##
Spleen	0.51 ± 0.008	0.50 ± 0.009	20.27 ± 1.465**	12.13 ± 0.536##	18.60 ± 0.620**	11.44 ± 0.392##
Heart	0.44 ± 0.007	0.42 ± 0.006	6.77 ± 0.469**	4.10 ± 0.325##	5.60 ± 0.302**	4.44 ± 0.235##

Table 3: Protective effect of *S. polyrhiza* on deposition pattern of arsenic (µ/g tissue) in different organs of Wistar albino rats. Here, values are expressed as MEAN ± SEM. **Control versus 10 ppm/30 ppm ($p<0.05$.) ##10 ppm vs. 10 ppm+*S. polyrhiza*/30 ppm vs. 30 ppm+*S. polyrhiza* ($p<0.05$).

of arsenic in various tissues. On the other hand, histological damages were minimal in *S. polyrhiza* supplemented groups in comparison with the arsenic-exposed rats. It was found in *S. polyrhiza* supplemented groups that histological lesions like focal necrosis, cellular edema, and inflammatory cell infiltration were reduced in considerable amount. Therefore, our study revealed the potentiality of *S. polyrhiza* to attenuate arsenic mediated toxicity in Wistar albino rats. However, further exploration is still required to elucidate the ameliorative mechanism of *S. polyrhiza* in reducing arsenic induced toxicity.

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