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PROTECTIVE EFFECTS OF *BUTEA MONOSPERMA* AGAINST ARSENIC CONTAMINATED RICE INDUCED TOXICITY

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ABSTRACT

A pot experiment was conducted with As amended irrigation water (0.0, 25.0, 50.0 and 75.0 mg/L As) to investigate the As accumulation in different parts of rice. A significant ($p \leq 0.05$) increase of As accumulation was found with the increase of As concentrations in irrigation water and the trend of accumulation was found as root > straw > husk> grain. The animals were fed As contaminated rice exhibited a significant ($p<0.05$) alterations in haematological parameters, liver marker enzymes, kidney function test along with alterations in lipid profile. Supplementation of *B. monosperma* with As contaminated rice significantly ($p<0.05$) restored these parameters towards the normal values. As deposition pattern on different organs and histological studies on the ultra structural changes of liver, kidneys, spleen and heart also supported the protective role of *B. monosperma*. In fine, it can be concluded that *B. monosperma* has significant role in protecting animals from As contaminated rice induced toxicity.

Keywords: *Butea monosperma*, Arsenic, rice, accumulation, protective.

INTRODUCTION

Among the plethora of toxicants, arsenic (As), the king of poisons, is one of the most important global environmental toxicants, currently poisoning tens of millions of people worldwide (Hughes *et al*., 2011; Smeester *et al*., 2011). Because of abundance of As in the environment, humans experience daily exposure through drinking water, inhalation and skin absorption (Shi *et al*., 2004). Besides, animals can be exposed to As through soil-crop-food transfer (Alam *et al*., 2003). Bangladesh is an agricultural country and here, cultivations are largely dependent on groundwater irrigation (Dey *et al*., 1996). Unfortunately, groundwater in most part of the country is heavily contaminated with dissolved As. This ultimately results in the accumulation of As in the food crops cultivated on these lands. Rice is considered as the staple food of our country and groundwater irrigation-based farming practices has led to high accumulation of As in rice as other food crops (Meharg and Rahman, 2003; Dittmar *et al*., 2010; Roberts *et al*., 2010; Spanu *et al*., 2012). By consuming the rice grown in As contaminated area, peoples are suffering from different adverse health effects. A recent cohort study in West Bengal, India revealed that high concentrations of As in rice are associated with elevated genotoxic effects in humans (Banerjee *et al*., 2013). Another study has conducted in Narayangonj, Dhaka, which utilized samples provided by 18,470 volunteers living in an As contaminated area and showed that those who ate large amounts of rice had higher levels of As in their urine than those who ate little rice (Melkonian *et al*., 2013). These findings clearly indicate the adverse effects of As contaminated rice on human body. Since As is correlated with hepato-renal toxicity, cardiovascular diseases and different types of cancer, so we had an increasing interest to assess the toxicity created by As contaminated rice and find out its possible remediation. That is why, this research work was carried out.

Though various hypotheses have been put forward indicating the mechanism of As toxicity but the exact mechanism is still obscure. Plethora of scientific reports demonstrated that, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during As metabolism are the main components to induce As pathogenicity (Wiseman and Halliwell, 1996; Imlay *et al*., 1998). Although As toxicity is considered as one of the serious problems worldwide but regrettably, there is still no specific, reliable and safe treatment for it. The

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treatment option for As toxicity is mainly restricted to some sulfhydryl having chelating agents i.e., 2,3-dimercaptopropane-1-sulfonate or 2,3-dimercaprol, meso-2,3-dimercaptosuccinic acid (Aposhian and Aposhian, 1990; Gupta *et al*., 2005), some antioxidants like Vitamin C, Vitamin E, n-acetyl cysteine (Ramnathan *et al*., 2002; Flora, 1999) and few micronutrients such as zinc and selenium (Modi *et al*., 2005). Besides, most of the traditional antioxidants and metal chelating agents are not biologically safe (Shi *et al*., 2004) which has increased our interest of using medicinal plants that possess free radical scavenging property to combat against free radical-mediated As toxicity (Manna *et al*., 2008).

As contamination in drinking and ground water is particularly common in the Ganges delta and adjacent plains of Bangladesh. *Butea monosperma* (Lam.), usually known as flame of the forest, is one of the prominent medicinal plants of As contaminated areas of Bangladesh. It is widely used in traditional medicine and among its different parts, flower is the one which is associated to several pharmaceutical effects (Burlia and Khadeb, 2007; Choedon *et al*., 2010). The flowers of *B. monosperma* have been reported to possess hepatoprotective, nephroprotective, chemopreventive, anti-inflammatory, antidiabetic, and free radical scavenging activities (Sharma and Shukla, 2011; Rajeswari *et al*., 2013; Choedon *et al*., 2010; Talubmook and Buddhakala, 2012; Lavhale and Mishra, 2007). Furthermore, phytochemical screening revealed that *B. monosperma* flowers contain different bioactive compounds such as flavonoids, phytosterols, glycosides, saponins, and phenolics (Kokate *et al*., 1996; Kasture *et al*., 2002; Lavhale and Mishra, 2007), which encouraged us to study the effect of *B. monosperma* against As contaminated rice induced toxicity.

MATERIALS AND METHODS

Pot experiment to investigate the As accumulation in rice

To investigate the As accumulation in different parts of rice, a pot experiment was conducted at University of Chittagong campus using a popular rice variety BR-29 and four treatments of 0.0, 25.0, 50.0 and 75.0 mg/L As containing irrigation water. The experimental site had subtropical and humid climate with adequate sunshine. From the seedbed, seedlings of 35 days old were uprooted carefully in the morning and on the same day, 5 seedlings were transplanted on each plastic pot (having no leakage) with 3 replications. The seedlings which died within first week of transplantation were discarded and replaced with new seedlings. Bio-fertilizers were applied in appropriate amount to provide the necessary nutrients. Throughout the growth period, 3-4 cm water above soil level was maintained in each treatment and the irrigation was continued before 10 days of harvest. At the maturity stage, the full-grown rice plants were carefully uprooted and the rice grain was harvested. Thereafter, the collected root, straw, husk and grain samples were washed thoroughly with As-free tap water followed by several rinsing with de-ionized water to remove soil and other contaminants. After drying the washed samples in the hot air oven at 60^{o}C for 72 h, the samples were stored at room temperature in airtight polyethylene bags having proper labeling. Finally, the samples were digested separately according to heating block digestion procedure (Rahman *et al*., 2007) and As concentrations were measured by Flow Injection Hydride Generation Atomic Absorption Spectrophotometer, FI-HG-AAS (iCE 3300 AA system, Thermo scientific, China) at BCSIR Laboratory, Chittagong.

Animals and treatment

Twenty female Wistar albino rats, weighing between 160-170 g were collected from animal house of Jahangirnagar University, Dhaka and were allowed free access to food (commercially available laboratory rodent diet) and water *ad-libitum* throughout the experimental period. Our institutional and national guidelines for the care and use of laboratory animals were followed in handling these animals throughout the experimental process. For the experimental treatment, the animals were randomly divided into four groups (I, II, III and IV) containing five rats in each group. The control group I, were fed with normal pellets while group II, III, and IV received As contaminated rice, *B. monosperma* flower powder (4%), As contaminated rice plus *B. monosperma* flower powder (4%) respectively, for a period of 150 days alongside the normal feed pellet and water.

Preparation of *B. monosperma* flower powder

Fresh *B. monosperma* flowers were collected from University of Chittagong campus and then identified by Dr. Sheikh Bokhtear Uddin, a taxonomist (Department of Botany, University of Chittagong, Bangladesh). The petals were separated from the whole flower, washed thoroughly with distilled water, sun-dried and then powdered by grinding. After that, *B. monosperma* flower powder (4% wt/wt) were mixed with respective pelleted diet of rat and used throughout the experiment.

Preparation of As contaminated rice powder

BR-28 rice was collected from the local market and tested for background As concentration by FI-HG-AAS according to Rahman *et al*. (2007). No As was found in that rice sample. Then, the rice was soaked in 200 mg/kg sodium arsenite solution for 36 hours and again tested for As concentration, and the amount of As accumulated in rice grain was found 46.33 ± 0.01 mg/kg. The As contaminated rice was dried, blended and mixed with the respective pelleted diet and used throughout the experiment to induce As toxicity.

Collection of blood and separation of serum

On the 150th day, rats were starved overnight and sacrificed next morning by light ether anesthesia. Blood was collected through cardiac puncture. For each rat, half of the blood was taken in a heparinated tube for haematological examination and the remaining blood was collected in another test tube and allowed to clot formation at room temperature for 20 minutes. Then, the tubes were centrifuged at 3000 rpm for 10 minutes. After centrifugation, serum were pipetted out & collected into pre-labeled wintrobe tubes. From collected blood and serum samples, haematological and biochemical analysis were carried out.

Collection and preservation of different organs

Chest and abdomen of the rats were opened. The liver, heart, spleen and both kidneys of each rat were carefully removed, washed in normal saline and then immersed separately into pre-labeled 10% formalin containing specimen container for histopathological examination. Some portion of liver, spleen and kidneys were preserved at -20°C for detection of As.

Haemato-biochemical assay

Using Auto-Haematology Analyzer (Beckmann, USA), different haematological indices such as total white blood cell (WBC) count, total red blood cell (RBC) count, haemoglobin (Hb) concentration and platelet count were estimated. In addition, different serum indices such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, total protein, total cholesterol (TC), high density lipoprotein (HDL) and triglycerides (TG) were measured by using the kits from Human GmbH (Germany) and the analyzer (CHEM-5V3, Erba, Mannheim, Germany). To calculate the mean values, all the samples were analyzed in triplicate.

Histopathological study

At first, gross section of liver, kidney, spleen and heart (preserved in 10% formalin containing specimen container) tissues were taken. Then the tissues were cut in longitudinal and transverse pieces, passed through ascending series of ethanol baths, cleared in toluene and embedded in paraffin. Tissues were sectioned at 5 µm and stained with Haematoxylin and Eosin (H&E). Stained sections were then mounted on glass slides with DPX and covered with a cover slip. Finally, histopathological changes were examined by light microscope and photographed using a digital camera.

Estimation of As in different tissues

The concentration of As in different organs (liver, kidney, spleen, heart) was measured using FI-HG-AAS method (Hirano, 1994). From each organ, 0.25 g sample was weighed and taken in beaker. The sample was digested with a mixture of $HClO_4$-HNO_3 solution (ratio 1:3 v/v) at 130^0C. After removal of HNO_3 by evaporation, the digested samples were diluted with deionized water up to 100 ml. The concentrations of As in digested samples were measured at 193.7 nm wave length and 10mA current using Atomic Absorption Spectrophotometer equipped with As lamp. Vapour generation accessory (VGA) was used to produce hydride vapours using 0.6% sodium borohydride and 10 Mm HCl.

Statistical analysis

Statistical analysis was performed with SPSS V.22 for Windows. All data were analyzed by using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMART) with a p-value < 0.05 considered to be statistically significant. All the values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Accumulation of As in different parts of rice

In this study, we found no plant to survive at the treatment of 75 mg/L As containing irrigation water. As accumulation in all parts of rice were high for 50 mg/L and low for control (Table 1). This indicates As accumulation in different parts of rice increased significantly with the increase of As concentration in irrigated water. Accumulation of As were found in high amounts in root followed by straw, husk and grain. In the absolute control condition (0 mg/L), some As accumulation were found, that could perhaps be due to the background As in the soil. All of our findings are affirmative with the findings of Abedin *et al*. (2002) and Imamul Huq (2011). From this study, we noticed that As accumulation in different parts of rice were significantly higher than the permissible limits of WHO.

Table 1. Accumulation of As in different parts of BR-29.

As added in water (mg/L)	As in root (mg/kg)	As in straw (mg/kg)	As in husk (mg/kg)	As in grain (mg/kg)
0	2.80 ± 0.02a	0.90 ± 0.03a	0.60 ± 0.03a	0.30 ± 0.03a
25	38.80 ± 0.03b	23.60 ± 0.03b	11.10 ± 0.01b	5.73 ± 0.01b
50	45.66 ± 0.01c	37.84 ± 0.01c	16.51 ± 0.01c	10.70 ± 0.03c

Mean in a column followed by uncommon letter differed significantly at $p<0.05$

Effect of B. monosperma on different haemato-biochemical parameters

The haematological profile (Table 2) of the present study revealed that overall mean values of Hb, total RBC count and total WBC count were significantly ($p<0.05$) decreased in Group-II in comparison to other groups. Reduction in Hb and total RBC count might be due to binding ability of As to Hb that leads to inhibition of heme synthesis pathway (Gupta and Flora, 2006). Decreased level of WBC count might be due to apoptotic effect of As on plasma cells as also studied by Rousselot *et al*. (2004). We found no significant differences in platelet count among the groups in our study. Similar findings were also observed by Ferzand *et al*. (2008). The ameliorative group, Group-IV, showed significant improvement when compared to the Group-II but lower values than Group-I and Group-III. This confirms the beneficial roles of *B. monosperma* in restoring haematological parameters.

As is known to produce disturbances in liver function. In the present study, the extent of hepatic damage was assessed by measuring the liver marker enzymes such as ALT and AST, which are cytoplasmic in origin and are released into the circulation after cellular damage (Lin *et al*., 2000). We observed that these enzyme activities were significantly ($p<0.05$) higher in Group-II than all other groups (Table 3). A significant ($p<0.05$) lower levels of these altered enzymatic activities were observed in Group-IV which suggests the hepatoprotective role of *B. monosperma* flower. This might be due to the presence of butrin and isobutrin in *B. monosperma* flower that possess antihepatotoxic properties which have been confirmed by the studies of Sharma and Shukla (2011).

The kidney plays essential roles in maintaining a number of vital body functions. Kidney dysfunction is one of the major health effects of chronic As exposure, and elevated levels of serum urea have been reported to be associated with renal dysfunction (Wang *et al*., 2009). In the present study, we have found significant ($p<0.05$) elevation of serum urea levels in Group-II compared with control

Table 2. One way ANOVA of haematological parameters.

Parameters	Group-I	Group-II	Group-III	Group-IV
Haemoglobin (g/dl)	13.8 ± 0.12	** 13.3 ± 0.20	NS 13.96 ± 0.04	## 13.8 ± 0.10
Total RBC (x 10^6/cmm)	5.16 ± 0.10	** 4.68 ± 0.16	NS 5.12 ± 0.10	## 5.04 ± 0.11
Total WBC (/cmm)	6300 ± 122.50	** 4860 ± 97.98	NS 6200 ±339.12	## 6160 ± 381.58
Platelet (x 10^3/cmm)	180 ± 3.20	NS 170 ± 5.50	NS 178 ± 3.74	NS 172 ± 3.74

Values are expressed as mean ± SEM. NS denotes non-significant; **denotes significantly different from control at $p<0.05$; ## denotes significantly different from the arsenic-treated group at $p<0.05$.

Table 3. One way ANOVA of biochemical parameters.

Parameters	Group-I	Group-II	Group-III	Group-IV
AST (U/L)	80.6 ± 3.12	** 120.4 ± 6.45	NS 82.6 ±5.39	## 69.4 ± 5.85
ALT (U/L)	64.8 ± 6.84	** 89.0 ± 6.55	NS 64.2 ± 4.82	## 59.8 ± 4.65
Urea (mg/dl)	49.00 ± 2.41	** 63.00 ± 2.24	NS 49.60 ± 1.50	## 53.20 ± 2.44
Total Protein (g/dl)	6.02 ± 0.25	** 4.92 ± 0.11	NS 6.02 ± 0.10	## 6.00 ± 0.17
Total Cholesterol (mg/dl)	79.22 ± 1.52	** 88.35 ± 1.61	NS 78.38 ± 0.96	## 79.86 ± 0.90
HDL (mg/dl)	32.33 ± 1.47	** 27.4 ± 1.89	NS 33.75 ± 1.30	## 32.94 ± 1.03
LDL (mg/dl)	30.41 ± 2.58	** 43.23 ± 1.61	NS 28.00 ± 1.58	## 30.45 ± 1.75
Triglycerides (mg/dl)	82.91 ± 0.96	** 88.6 ± 1.81	NS 83.04 ± 1.10	## 82.34 ± 1.01

Values are expressed as mean ± SEM. NS denotes non-significant; **denotes significantly different from control at $p<0.05$; ## denotes significantly different from the arsenic-treated group at $p<0.05$.

group (Table 3). Supplementation of *B. monosperma* flower with As contaminated rice significantly ($p<0.05$) reduced the As induced increase in serum urea level which might be due to the antioxidant properties of this plant. *B. monosperma* flower is a good source of alkaloids, flavonoids and phenolic contents, all of which are responsible for antioxidant activity. By the virtue of antioxidant activity, *B. monosperma* demonstrated nephroprotective activity. Our findings were affirmative to the findings of Rajeswari *et al*. (2013).

A significant ($p<0.05$) decrease was observed in the level of serum total protein in Group-II after As intoxication when compared with the control group (Table 3). Our findings were consistent with the results showed by Mehta and Hundal (2013). This reduction might be attributed to reduced protein synthesis or increased proteolytic activity or destruction of hepatic protein synthesizing sub-cellular structures. It is also possible that severe nephrotoxic lesions caused drainage of protein through the urine, resulting to hypoproteinaemia. Interestingly, supplementation of *B. monosperma* with As contaminated rice significantly ($p<0.05$) reversed the serum protein level towards control. One possibility for increasing serum protein level might be due to stimulatory effect of *B. monosperma* in insulin secretion as mentioned in umpteen reports, and insulin might help the incorporation of amino acids into protein (Talubmook and Buddhakala, 2012).

Cardiovascular disease is one of the major causes of As related mortality (Chen *et al*., 1996). In this study, we observed that serum TC, TG and LDL (low density lipoprotein) cholesterol levels elevated significantly ($p<0.05$) in Group-II as compared to control group, whereas HDL cholesterol reduced significantly in Group-II (Table 3). Alteration in lipid profiles and increased levels of oxidative stress have been implicated in the cardiotoxicity induced by As (Bhattacharjee *et al*., 2014; Muthumani and Milton, 2013). Our findings are in full agreement with these findings. Interestingly, supplementation of *B. monosperma* flower with As contaminated rice was found to restore the altered lipid profile. This might be due to the presence of phytosterols in the *B. monosperma* flower, which have lipid lowering effects on hyperlipidemia as mentioned in previous reports (Parveen *et al*., 2011).

Histopathological observation

To gather more evidence to support the protective behavior of *B. monosperma* against As contaminated rice induced toxicity, histopathological studies, using light microscope were undertaken. Histologically mild to marked venous congestion, sinusoidal dilation, multiple foci of mononuclear cell infiltration, focal haemorrhages, varied degree of necrosis and degenerative changes in the hepatocytes were observed in the liver tissues of Group-II (Fig.1a) Similar findings were noticed in liver treated with As by Tanju and Madhuri (2013). Hepatic necrosis might be due to oxidative stress induced by As that further involved in the cellular protein degradation. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells. Sections of liver of Group-IV revealed mild sinusoidal congestion and dilation, no necrosis, lesser degree of focal haemorrhage and almost intact lobular structure (Fig.1b). This suggests the reparative quality and maintenance of structural integrity of hepatocytic cell membrane of damaged liver cells by the *B. monosperma* flower. Our findings are consistent with the results of Sharma and Shukla (2011). Light microscopic observations on kidneys of As contaminated rice treated rat showed glomerulonephritis, proximal tubular necrosis, epithelial damage and loss of nuclei (Fig.1c). It could be due to increased glomerular filtration and capillary permeability by As toxicity as a result of which leakage of proteins occurs that cause tubular necrosis as also observed by Cullen *et al*. (1995). Supplementation of *B. monosperma* flower with As contaminated rice restored the normal kidney architecture which indicates its nephroprotective role (Fig.1d). From the spleen section of Group-II, increased number of apoptotic cells, necrotic cells and macrophages were observed which indicate the disturbances of spleen functional activity (Fig.1e), whereas in Group-IV, these perturbations were not pronounced (Fig.1f). It might be due to the antioxidant properties of the *B. monosperma*. The effect of As was not pronounced on heart as compared to other organs like liver and kidney. The cardiac histology of Group-II revealed mild cellular edema and lukocytic infiltration (Fig.1g) while rest of the groups showed normal cardiac architecture (Fig.1h).

Effect of B. monosperma on As deposition pattern in different organs of rat

In cases of chronic ingestion, As is known to accumulate in the liver, kidneys, heart, lungs, muscles and spleen (Benramdane *et al*., 1999; Ratnaike, 2003). FI-HG-AAS analysis of the organ samples in our study also showed deposition of As in liver, kidney, spleen and heart. In all As treated groups, we found high amount of As accumulation in spleen followed by kidney, heart and liver. But, Group-IV deposited significantly ($p<0.05$) lesser amount of As than Group-II (Fig. 2). This might be due to the presence of different phytochemicals i.e, flavonoids, alkaloids, saponins, phytosterols etc. having antioxidant properties in the *B. monosperma* flower, by means of which it might reduce the tissue As burden. Our findings are in agreement to the findings of Nasir *et al*. (2004). From the above mentioned results, it is clear that the present investigation provides a well validated supportive literature about counteracting As contaminated rice induced toxicity with edible *B. monosperma* flower of As prone zone.

CONCLUSION

Conclusively, data and information obtained from this study are indication of *B. monosperma* potential to counteract As contaminated rice induced toxicity. However, the exact mechanism of *B. monosperma* action in neutralizing As-induced toxic effects *in vivo* is still unclear. Therefore, further molecular and biochemical investigations are needed to explain the mode of its action to explore the use of *B. monosperma* as potential

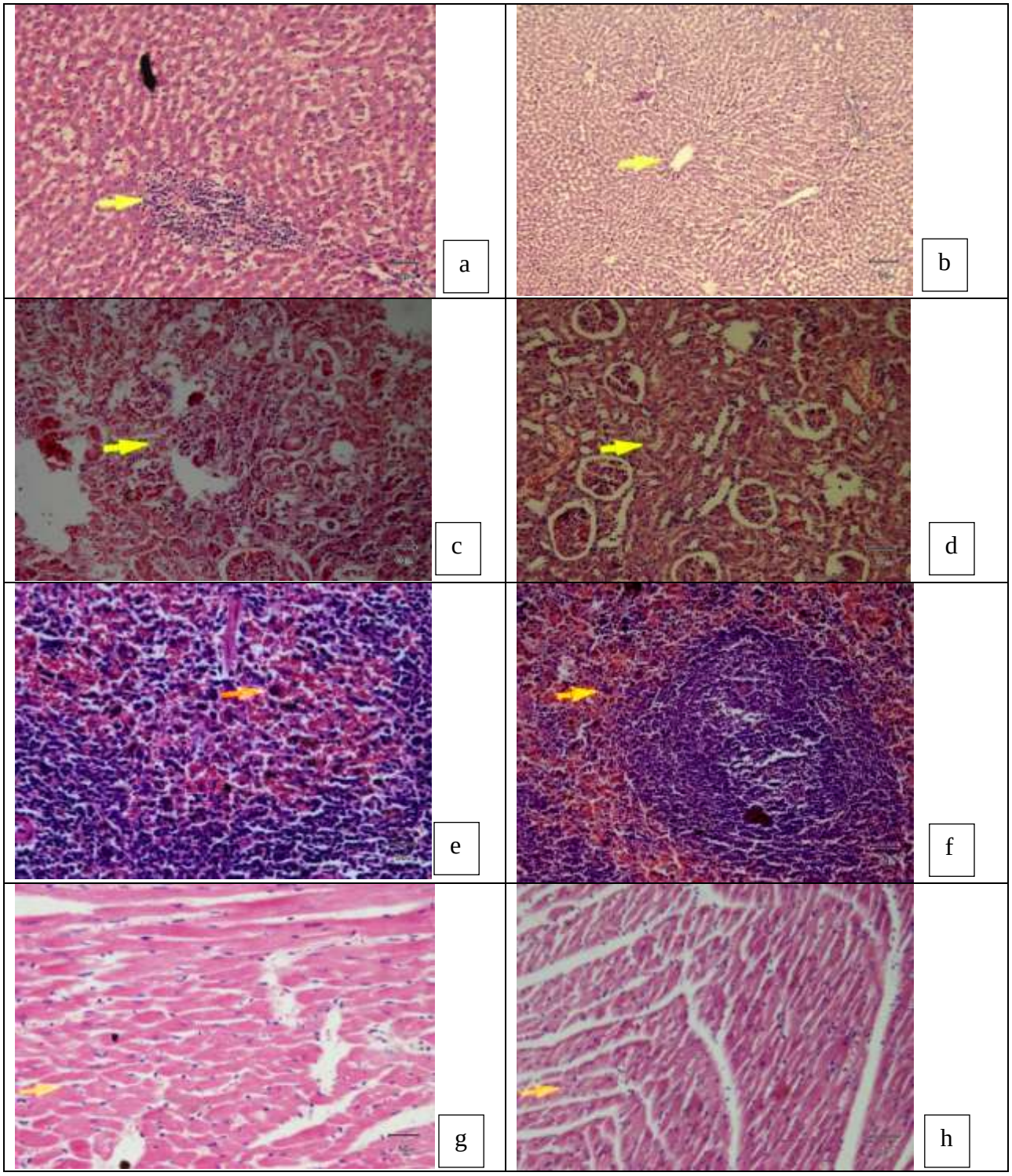

Fig. 1. Liver (a, b), kidney (c, d), spleen (e, f) and heart (g, h) sections from Group-II (left side) and Group-IV (right side), respectively.

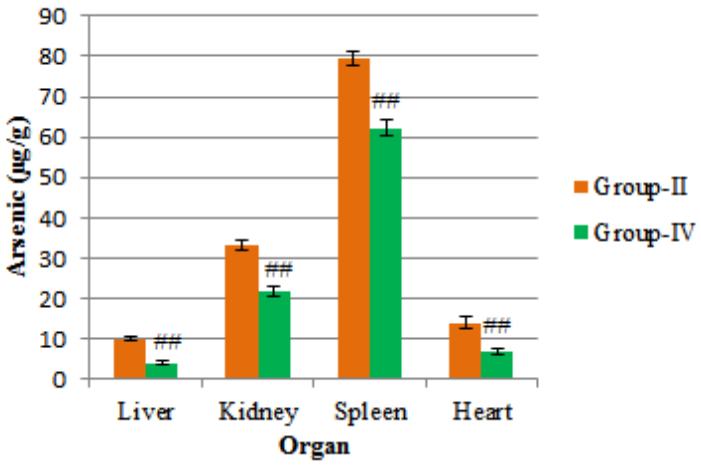

Fig. 2. Accumulation of As in different organs of rat. (Here, values are expressed as MEAN ± SEM. ## denotes highly significant ($p<0.05$) compared to Group-II).

candidate to remediate As toxicity.

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