Original Article

Reduction of Sodium Arsenite-Mediated Adverse Effects in Mice using Dietary Supplementation of Water Hyacinth (*Eichornia crassipes*) Root Powder

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Abstract

Background: In this study, we evaluated the protective effects of water Hyacinth Root Powder (HRP) on arsenic-mediated toxic effects in mice.

Methods: Swiss albino mice, used in this study, were divided into four different groups (for each group n=5). The control group was supplied with normal feed and water, Arsenic group (As-group) was supplied with normal feed plus arsenic (sodium arsenite)-containing water, and arsenic+hyacinth group (As+Hy group) was supplied with feed supplemented with HRP plus arsenic water. The remaining Hy-group was supplied with feed supplemented with HRP plus normal water.

Results: Oral administration of arsenic reduced the normal growth of the mice as evidenced by weight loss. Interestingly, tip of the tails of these mice developed wound that caused gradual reduction of the tail length. Supplementation of HRP in feed significantly prevented mice growth retardation and tail wounding in As+Hy group mice. However, the growth pattern in Hy-group mice was observed to be almost similar to that of the control group indicating that HRP itself has no toxic or negative effect in mice. Ingested arsenic also distorted the shape of the blood cells and elevated the serum enzymes such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and serum glutamic pyruvic transaminase (SGPT). Importantly, elevation of these enzymes and distortion of blood cell shape were partially reduced in mice belong to As+Hy group, indicating HRP-mediated reduction of arsenic toxicity.

Conclusion: Therefore, the preventive effect of hyacinth root on arsenic-poisoned mice suggested the future application of hyacinth to reduce arsenic toxicity in animal and human.

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Keywords: Arsenic, Blood cell morphology, Growth retardation, Mice, Water hyacinth

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Introduction

Arsenic is a metalloid present in our natural environment. Arsenic toxicity created a great health concern by affecting millions of people in the world including Bangladesh, where people are mainly exposed to arsenic through drinking water [1-3]. Long time exposure to arsenic results in its accumulation in hair, nails, muscles and skin [4]. This leads to various complications and diseases manifested by cancer [4], diabetes [5], hypertension [6], hepatic

damage [7], peripheral neuropathy and multiple vascular diseases, weight loss [1] and miscarriage [8]. Arsenic is also known to affect various tissues/organs including liver and heart of human and animal causing increase in the level of various enzymes including LDH, ALT and SGPT [7,9-11]. Arsenic toxicity is thought to be caused by the signals generated due to its reaction with sulfhydryl groups of various enzymes and proteins followed by their cross-linking. This cross-linking of various proteins may activate potential intracellular signaling pathways that ultimately lead to arsenic-mediated adverse effects [12,13].

The current available treatments for arsenicosis patients are thiol containing chelating agents such as meso 2,3-dimercaptosuccinic acid (DMSA), 2,3 dimercaptopropane-1-sulfonate (DMPS) or British Anti Lewisite (BAL; 2,3-dimercaprol) [14]. However, use of these drugs is still limited due to various accompanying side effects such as hepatotoxicity, renal toxicity, headache, nausea, vomiting, high blood pressure, pain in stomach and chest, anxiety, *etc* [15].

In contrast to chemical drugs, plants are usually less toxic and mostly free from adverse side effects [16]. It has been reported that seed powder of *M.oleifera* reduced uptake of arsenic in kidney, liver and brain [17]. Garlic extracts is also reported to revert high level of Reactive Oxygen Species (ROS) in hepatic tissue generated by arsenic [18].

In our experiment we used water Hyacinth *(Eichhornia crassipes)* Root Powder (HRP) for possible remediation of arsenic-mediated effects in mice. Water hyacinth is a free floating weed and obtains nutrients from the water by its fibrous roots. The root possesses some mechanisms through which it can chelate various heavy metals [19]. It is one of the most studied aquatic plants that can accumulate pollutants especially heavy metals [20-22]. A previous report demonstrated the removal of more than 90% of arsenic from a solution (200 *μg/L* arsenic) within one hour of administrating non-living dried HRP [23]. In addition, intraperitoneal injection of ethanolic extract of hyacinth root in arsenic-exposed rat has been shown to reduce arsenic accumulation in various organs [24]. The present study has been used to investigate whether HRP could reduce or neutralize arsenic-mediated toxic effects in mice model. Our data have demonstrated that HRP might be a good alternative for remediation of adverse health effects of arsenic.

Materials and Methods

Animal maintenance

Swiss albino male mice (6 weeks of age) of average body weight were purchased from Animal Division of International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The mice were randomly selected and kept in plastic cages with wood-cobe bedding (5 mice/cage). After five days of acclimation, mice were divided into four groups namely control, hyacinth (Hy), arsenic (As) and arsenic-hyacinth (As+Hy). Control mice were maintained with available supply of distilled water and normal mice feed. Hy was provided with HRP containing feed (8% *wt/wt*) and distilled water. Arsenic group was given normal feed and sodium arsenite ($NaAsO_2$) containing water (0.2 *g/L*). The fourth group, As+Hy group was provided with HRP containing feed and arsenic containing water. These different groups of mice were maintained for 8 weeks. All these procedures and experiments using mice were undertaken following the ethical issues set by the Faculty of Biological Sciences, University of Dhaka, Bangladesh.

Preparation of HRP

Water hyacinths were collected from local lakes around Dhaka, Bangladesh and then the root parts were taken. The roots were washed several times with clean water, and finally degerminated through washing with chlorine containing water. The roots were then cut into smaller pieces and sun dried for a week. HRP were obtained by grinding the dried roots. This powder (8% *wt/wt*) was mixed with mice feed purchased from ICDDR, B.

Determination of body weight and tail length of mice

Each mouse of a group was weighed every 2 weeks after starting the respective diet and recorded accordingly. A scale in *cm* was used to measure the tail length.

Blood collection and assay for blood cell morphology, LDH, ALP and SGPT

Surgical blade (size 11) was pinched sharply between ear and eye of mice. Blood came out as drops and collected in test tubes. Blood films were made by placing a drop of blood on one end of a slide, and using a spreader slide blood drop was dispersed over the slide's length. The slide was left to air dry, and fixed immersing it in methanol for 3 to 5 *min*. After fixation, the slide was stained with Leishman's stain (a mixture based on methylene blue and eosin) to distinguish the cells from each other. Blood cell morphology was then observed under inverted microscope (Kruss Optronic, D22297, Germany). Serum was prepared from collected blood, and LDH, ALP and SGPT were measured using comercially available assay kit (DiaSys Diagnostic Systems, Turkey; Biosystems S.A., Spain; and Human Diagnostic, Germany).

Statistical analysis

Data were statistically analyzed using Student's *t*-test with GraphPad Prism 5.

Results

HRP rescued arsenic-mediated loss of body weight and tail wounding

Some previous studies reported that arsenic reduced normal growth and caused loss of body weight and tail wounding in experimental mice [25,26]. Here, we investigated whether HRP could rescue the loss of body weight and other physical damage in mice caused by arsenic. At the beginning of the experiment, the average mice weights of control, hyacinth (Hy group), arsenic (As group) and arsenic-hyacinth (As+Hy group) were 30.12± 0.58, 30.3±0.57, 30.82±1.17 and 30.65±0.73*g*, respectively.

Weight of the mice in each groups were taken up to 8 weeks at every 2 weeks interval. After 8 weeks, the average body weight of control, Hy, As and As+Hy group were found 43.4±1.03, 42±1.84, 24.09±1.19 and 30.7± 1.12 *g*, respectively (Figures 1 and 2). The average body weight of the control mice was increased from 30.12 to 43.4 *g* over 8 weeks. This normal increase of mice body weight was retarded after drinking arsenic containing water. A gradual weight loss in As group of mice was observed during those 8 weeks indicating arsenic-mediated growth retardation. Interestingly, HRP partially rescued growth retardation in arsenic exposed mice when supplemented in feed. Body weight of As+Hy mice was significantly higher at each 2 weeks interval (**p<0.05) (Figure 1). The growth pattern of control and Hy groups were found to be similar indicating that HRP alone had no visible effects on the growth of the mice.

Mice of each group were also investigated frequently to observe other physical changes caused by arsenic. Arsenic exposed mice were found relatively restless compared with control mice. After four weeks, As-group mice developed wound at their tail tips. The tails became gradually shorter due to cell death at the tail tip (Figure 3). As-Hy group of mice also showed tail reduction, however, this reduction was significantly less than that of As-group mice. This indicated that HRP might have some effects to neutralize arsenic toxicity that caused tail wounding.

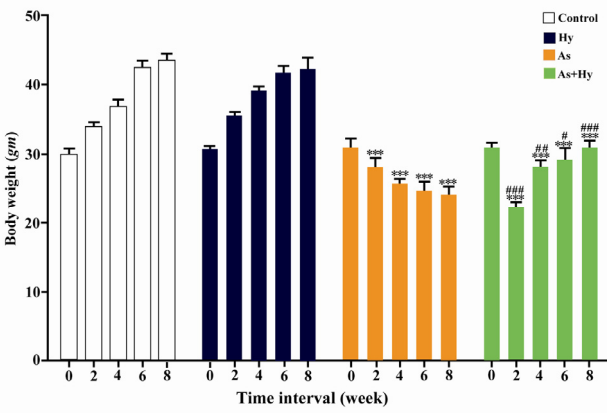

Figure 1. Protective effect of HRP on arsenic-mediated weight loss in mice. Body weight was taken at every two weeks up to 8 weeks. Data shown as mean±SD (n=5 per group). ***p<0.05 control *vs*. As or As+Hy. #p<0.05, ##p<0.05, ###p<0.05 As+Hy *vs*. As.

Figure 2. A representative photograph of one from each group (as indicated) after 8 weeks of arsenic exposure

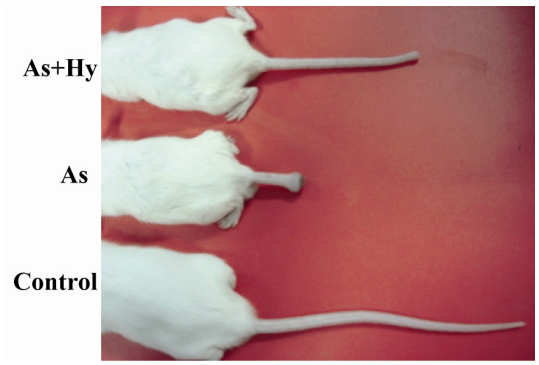

Figure 3. Protective effect of HRP on arsenic-mediated tail wounding in mice. Photograph of a representative mouse from each group is shown

HRP blocked distortion of arsenic-mediated blood cell morphology

The blood cells were then observed under microscope to see the effect of arsenic on cellular morphology. The blood cells of control group were found spherical in shape (Figure 4). However, this normal spherical shape of the cells was distorted in blood collected from arsenic exposed mice. Surprisingly, this distortion of cellular morphology was almost blocked in As-Hy mice. This result suggested that HRP could reduce arsenic-mediated stress causing distortion of blood cell morphology.

Arsenic-induced serum elevation of LDH, ALP and SGPT was reduced by HRP

Some previous reports showed that arsenic could affect heart and liver tissues and increase the level of LDH, ALP and SGPT in serum [7,9-11,27,28]. We next investigated whether HRP could reduce the elevation of these serum enzymes in mice caused by arsenic. In

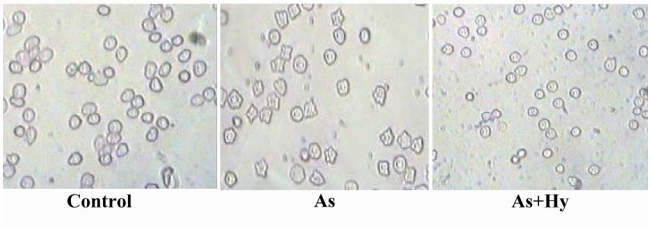

Figure 4. Arsenic induces morphological change of RBC. Mice blood was collected and slides were prepared and observed under microscope. Photographs were taken at 40X resolution

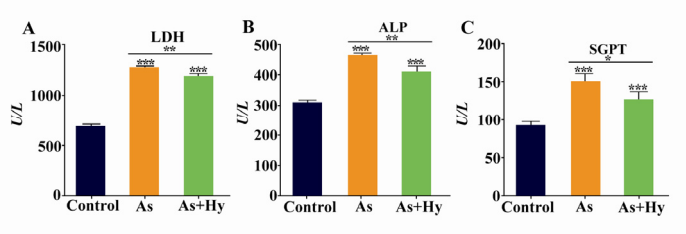

Figure 5. Hyacinth root powder (HRP) partially rescue arsenic-mediated elevation of serum, A) LDH, B) ALP and C) SGPT. Blood samples were taken after 8 weeks of arsenic exposure and treatment with HRP. Data shown as mean±SD (n=5 per group). **p<0.05 control *vs*. As or As+Hy

arsenic-exposed mice, serum LDH level was found almost double (1268.15±18.98 *U/L*) when compared with control mice (686.5± 17.04 *U/L*) (Figure 5). This result indicated possibilities of heart and other tissue damage in arsenic-exposed mice that might elevate the level of LDH in the serum. However, this serum LDH elevation was partially blocked (1189.88±22.84 *U/L*) in As-Hy group mice. The reduction of the enzyme level was found significant (**p<0.05) compared to arsenic-exposed mice (Figure 5).

As-mice also showed a significant increase (***p<0.05) in serum ALP (458.5±11.6 *U/L*) and SGPT (152.02±10.43 *U/L*) levels compared to control (305.6±11.0 and 92.14±5.11 *U/L*, respectively). Increased levels of ALP and SGPT refer that arsenic might cause liver damage resulting in release of these enzymes into the serum. Again, in case of As-Hy mice, HRP was found to partially reduce the elevation of serum ALP (409.25±19.15 *U/L*) and SGPT (126.75±10.14 *U/L*) values.

Discussion

Epidemiological studies from several arsenic endemic regions have shown that chron-

ic exposure to arsenic can lead to a range of adverse health effects in human [1,2,29,30]. Arsenic can affect different organs including heart, liver, kidney, brain leading to various related diseases [31-33]. The effect of arsenic in animal model studies has therefore created immense importance to find out mechanism of toxicity and ways of remediation.

In the present study, decrease in body weight was found in arsenic-exposed mice. Arsenic is known to inhibit growth by interfering with various metabolic processes [34]. Moreover, arsenic is also shown to induce death of various cells *in vitro* through activation of apoptotic pathway [13,35]. In our study, we also found that the arsenic-exposed mice developed wound at the tail tips. This tail wounding might be caused by activation of similar apoptotic pathways in mice.

Water hyacinth has been used as sustaining feed source for livestock especially ruminant animal [36-38]. More importantly, it has also been shown that water hyacinth leaf protein concentrate is nontoxic and nutritionally available for applications in food and feed, such as biscuits or seasonings industries [39]. Hyacinth root is known to adsorb soluble arsenic in solution [22] and this phenomenon has been used for arsenic removal from contaminated water [20]. However, the potentiality of hyacinth for managing arsenic toxicity in an animal model has never been explored before.

We, for the first time, found that HRP prevented growth retardation and tail wounding of mice exposed to arsenic. In our experiment, it was also evident that the HRP has itself no toxic or growth retardation effect in mice. Some earlier studies have shown that arsenic can bind to animal and human hemoglobin and other blood cell proteins [40-43]. This binding might cause distortion of blood cell shape/ morphology observed in our study (Figure 4). Importantly, HRP supplementation in diet almost restored the normal spherical shape of blood cells in As+Hy group mice. Not only that, HRP also caused partial reduction of arsenic-mediated increased levels of serum LDH, ALP and SGPT.

Conclusion

This study, therefore, indicated the efficacy of HRP in protecting arsenic-induced toxicity in mice. The exact mechanism of HRP action in neutralizing arsenic-induced toxic effects *in vivo* is still unclear, however, HRP might adsorb ingested arsenic in a way that might mimic arsenic removal from water shown earlier in *in vitro* studies [22,23,44]. Further investigation is needed to explain the mode of HRP action to explore its use as a potential candidate to remediate arsenic effect.

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References

1. Khan MMH, Aklimunnessa K, Ahsan N, Kabir M, Mori M. Case-control study of arsenicosis in some arsenic contaminated villages of Bangladesh. Sap Med J 2006;75(4-6):51-61.
2. Chowdhury UK, Rahman MM, Mondal BK, Paul K, Lodh D, Biswas BK, et al. Groundwater arsenic contamination and human suffering in West Bengal, India and Bangladesh. Environ Sci 2001;8(5): 393-415.
3. Mazumder DNG, Ghoshal UC, Saha J, Santra A, De BK, Chatterjee A, et al. Randomizedplacebo-controlled trial of 2, 3- dimercaptosuccinic acid in therapy of chronic arsenicosis due to drinking arsenic-contaminated subsoil water. J Toxicol Clin Toxicol 1998;36(7):683-690.
4. Kitchin KT, Kirk T. Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites. Toxicol App Pharmacol 2001;172(3):249-261.
5. Longnecker MP, Daniels JL. Environmental contaminants as etiologic factors for diabetes. Environ Health Perpect 2001;109(Suppl 6):871-876.
6. Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, et al. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 1995;25:53-60.
7. Santra A, Maiti A, Das S, Lahiri S, Charkaborty SK, Mazumder DNG. Hepatic damage caused by chronic arsenic toxicity in experimental animals. J Toxicol Clin Toxicol 2000;38(4):395-405.

8. Rahman A, Vahter M, Ekström EC, Rahman M, Golam Mustafa AH, Wahed MA, et al. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. Am J Epidemiol 2007;165(12):1389-1396.
9. Islam K, Haque A, Karim R, Fajol A, Hossain E, Salam KA, et al. Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. Environ Health 2011;10:64.
10. Mazumder DNG. Effect of chronic intake of arsenic contaminated water on liver. Toxicol Appl Pharmacol 2005;206(2):169-175.
11. Bharti VK, Srivastava RS, Sharma B, Malik JK. Buffalo (Bubalus bubalis) epiphyseal proteins counteract arsenic-induced oxidative stress in brain, heart, and liver of female rats. Biol Trace Elem Res 2012;146(2):224-229.
12. Akhand AA, Du J, Liu W, Hossain K, Miyata T, Nagase F, et al. Redox-linked cell surface-oriented signaling for T-cell death. Antioxid Redox Signal 2004;4(3):445-454.
13. Hossain K, Akhand AA, Kato M, Du J, Takeda K, Wu J et al. Arsenite induces apoptosis of murine T lymphocytes through membrane raft-linked signaling for activation of c-Jun amino-terminal kinase. J Immunol 2000;165(8):4290-4297.
14. Flora SJ, Kaila K, Narula GD, Kannan GM. Effects of combined administration of captopril and DMSA on arsenite induced oxidative stress and blood and tissue arsenic concentration in rats. Comp Biochem Physiol C Toxicol Pharmacol 2007;144(4):372-379.
15. Bosque MA, Domingo JL, Corbella J, Jones MM, Singh PK. Developmental toxicity evaluation of monoisoamyl meso-2,3-dimercaptosuccinate in mice. J Toxicol Environ Health 1994;42(4):443-450.
16. Valiathan MS. Healing plants. Curr Sci 1998;75 (11):1122-1126.
17. Gupta R, Dubey DK, Kannan GM, Flora SJS. Concomitant administration of Moringa oleifera seed powder in the remediation of arsenic induced oxidative stress in mouse. Cell Biol Int 2007;31(1): 44-56.
18. Flora SJ, Mehta A, Gupta R. Prevention of arsenic-induced hepatic apoptosis by concomitant administration of garlic extracts in mice. Chem Biol Interact 2009;177(3):227-233.
19. Ingole NW, Bhole AG. Comparative study of production of biogas from water hyacinth by single phasic and diphesic digestion process. J Indian Water Work Assoc 2000;32(2):137-140.
20. Rahman MA, Hasegawa H. Aquatic arsenic: phytoremediation using floating macrophytes. Chemosphere 2011;83(5):633-646.
21. Prakash O, Mehrotra I, Kumar P. Removal of cadmium from water by water hyacinth. J Environ Eng 1987;113(2):352-365.
22. Misbahuddin M, Fariduddin A. Water hyacinth removes arsenic from arsenic-contaminated drinking water. Arch Environ Health 2002;57(6):516-518.
23. Rmalli SW, Harrington CF, Ayub M, Haris PI. A biomaterial based approach for arsenic removal from water. J Environ Monit 2005;7(4):279-282.
24. Quayum SL. Effect of water hyacinth root extract on arsenic level in different organs of arsenic treated rat. Bangladesh J Pharmacol 2007;2(2):73-80.
25. Verma RJ, Vasu A, Saiyed AA. Arsenic toxicity in mice and its possible amelioration. J Environ Sci 2004;16(3):447-453.
26. Karim MR, Haque A, Islam K, Ali N, Salam KA, Saud ZA, et al. Protective effects of the dietary supplementation of turmeric (Curcuma longa L.) on sodium arsenite-induced biochemical perturbation in mice. Bangladesh Med Res Counc Bull 2010;36 (3):82-88.
27. Mazumder DNG. Arsenic and liver disease. J Indian Med Assoc 2001;99(6):314-315.
28. Meliker JR, Wahl RL, Cameron LL, Nriagu JO. Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: A standardized mortality ratio analysis. Environ Health 2007;6:4.
29. Grissom RE, Abernathy CO, Susten AS, Donohue JM. Estimating total arsenic exposure in the United States. In: Chapell WR, Abernathy CO, Calderon RL, editors. Arsenic exposure and health effects. Oxford: Elsevier Science Ltd; 1999, 51-60.
30. Chakraborti D, Rahman MM, Paul K, Chowdhury UK, Sengupta MK, Lodh D, et al. Arsenic calamity in the Indian subcontinents: What lessons have been learned. Talanta 2002;58(1):3-22.
31. Chen CJ, Chen CW, Wu MM, Kuo TT. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer 1992;66:888-892.
32. Fengyuan P, Ning M, Yusuke H, Mariko M, Shinji O, Fanyin C, et al. Oxidative DNA damage in relation to neurotoxicity in the brain of mice exposed to arsenic at environmentally relevant levels. J Occup Health 2005;47(5):445-449.
33. Rahman M, Tondel M, Ahmad SA, Axelson O. Diabetes mellitus associated with arsenic exposure

in Bangladesh. Am J Epidemiol 1998;148(2):198-203.

34. Cong Tu, Lena Q Ma. Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. J Environ Qual 2002;31: 641-647.
35. Dong Z. The molecular mechanisms of arsenic-induced cell transformation and apoptosis. Environ Health Perspect 2002;110(Suppl 5):757-759.
36. Mako AA, Babayemi OJ, Akinsoyinu AO. An evaluation of nutritive value of water hyacinth (Eichhornia crassipes Mart. Solms-Laubach) harvested from different water sources as animal feed. Livestock Res Rural Devel 2011;23:23-25.
37. Khan MJ, Razzaque MA, Tareque AMM. Effect of feeding water hyacinth in combination on the growth of bullocks. Bangladesh J Agri Sci 1981;6 (1):16-22.
38. Khan MJ, Steingass H, Drochner W. Nutrition evaluation of some aquatic plants for animal feeding. Bangladesh J Agri Sci 2002;29(2):317-324.
39. Wu W, Sun Y. Dietary safety evaluation of water hyacinth leaf protein concentrate. Hum Exp Toxicol 2011;30(10):1514-1520.
40. Biswas D, Banerjee M, Sen G, Das JK, Banerjee A, Sau TJ, et al. Mechanisms of erythrocyte death in human population exposed to arsenic through drinking water. Toxicol Appl Pharmacol 2008;230 (1):57-66.
41. Ferzand R, Gadahi JA, Saleha S, Ali Q. Histological and haematological disturbance caused by arsenic toxicity in mice model. Pak J Biol Sci 2008; 11(11):1405-1413.
42. Rael LT, Ayala-Fierro F, Bar-Or R, Carter DE, Barber DS. Interaction of arsine with hemoglobin in arsine-induced hemolysis. Toxicol Sci 2006;90 (1):142-148.
43. Winski SL, Carter DE. Interactions of rat red blood cell sulfhydryls with arsenate and arsenite. J Toxicol Environ Health 1995;46(3):379-397.
44. Govindaswamy S, Schupp DA, Rock SA. Batch and continuous removal of arsenic using hyacinth roots. Int J Phytoremediation 2011;13(6):513-527.