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Research Article

Phytoremedial effect of *Asparagus racemosus* on sodium arsenite-induced toxicity in Charles Foster rats

Sandhya Kumari
Department of Biotechnology, Magadh University, Bodh Gaya (Bihar), India
Santwana Rani
Department of Botany, College of Commerce, Patliputra University, Patna, Bihar (India)
Arun Kumar*
Mahavir Cancer Sansthan and Research Centre, Patna, Bihar (India)

*Corresponding author. E-mail: drarunk31@gmail.com

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Abstract
Arsenic poisoning has recently resulted in significant health problems in the exposed population. In India, the Indo-Gangetic plains are the region where the arsenic threat has increased. In Bihar, it is estimated that 10 million people are exposed to arsenic poisoning. The present study aims to develop a novel drug as an antidote against arsenic-induced toxicity in rats. In the present study, arsenic in sodium arsenite at a dose of 8 mg/kg body weight per day was induced (Group-II) in Charles Foster rats at 2 or 6 months to observe chronic exposure. In the arsenic pretreated group (Group-III), *Asparagus racemosus* was administered at a dose of 400 mg/kg body weight per day for 8 weeks. Normal control group (Group I) was taken without any treatment. After completion of the entire experiment, the animals were sacrificed, and their blood samples were obtained for hematological and biochemical evaluation. At the same time, vital tissues, such as the liver and kidney, were fixed in preservatives for the histopathological study. The study showed that the hematological levels, such as RBC, WBC, platelet counts and hemoglobin percentage, were significantly restored by administering *A. racemosus* (Group-III), against the arsenic-exposed group. Furthermore, biochemical parameters such as SGPT, SGOT, ALP, bilirubin, urea, uric acid and creatinine were significantly recovered ($p<0.05$) against arsenic-induced toxicity. The histopathological study also showed remarkable restoration in hepatocytes and nephrocytes by *A. racemosus* against arsenic-induced toxicity. Therefore, it can be concluded from the entire study that *A. racemosus* has a significant antidote effect against arsenic-induced toxicity.

Keywords: Arsenic treatment, *A.racemosus,* Charles Foster rats, Drug development

INTRODUCTION

Arsenic poisoning in groundwater has caused serious health risks for the exposed population around the world. An estimated 300 million people around the world are exposed to arsenic, while approximately 70 million people are affected in India. In Bihar, approximately 18 districts out of 38 are affected by ground water arsenic poisoning, while an estimated 10 million people are exposed to arsenic poisoning in the state (Hassan, 2018; Kumar *et al*., 2019[a]; 2019[b]; 2015; 2016; Singh and Geetanjali., 2014). After the intake of contaminated water, the arsenic reaches the bloodstream through the gastrointestinal tract in the toxic trivalent form, which is converted into pentavalent form and finally degraded to the low toxic compound DMA, which is still a carcinogen (Zheng *et al*., 2017). According to the WHO and EPA, the maximum limit of arsenic contamination in water is 10 µg/L. However, in the exposed population of Bihar, severe health risks were observed in the population as a result of chronic exposure (US NRC (National Research Council)), 2001; Kenneth and Gilbert, 2002). It has led to dysfunction of vital organs, such as the liver, kidney (Smith *et al*., 1998; Kannan *et al*., 2001), cardiovascular system (Vahidnia *et al*., 2008), nervous system, endocrine system, etc., with inhibition of DNA repair capability (Hartwig *et al*., 2002; Andrew *et al*., 2006; Patlolla and Tchounwou 2005), leading to carcinogenesis in vital tissues such as the skin, lungs, urinary bladder, colon, gallbladder, liver and kidney (Martinez *et al*., 2011; Minatel *et al*.,2018; Kumar *et al*., 2021[a]).

Chronic exposure of the population to arsenic has resulted in serious health issues, such as skin manifesta-

tions, diarrhea, constipation, loss of appetite, shortness of breath, diabetes, and cardiovascular disease. Various studies have been carried out to determine the health status of the exposed population of Bihar (Rahman *et al*., 2019; Kumar *et al*., 2020[a]; 2021[a]; 2021[b]; 2021[c]; 2021[d]).

Therefore, to combat the current problem, developing an antidote as a dietary supplement against arsenic toxicity is necessary. Various studies have been conducted that demonstrated the antidote and antioxidant properties of medicinal plants against arsenic-induced toxicity in preclinical studies. These plants have proven a promising role in controlling arsenic-induced toxicity in rat models (Kumar *et al*., 2022; 2020[b]; Bhattacharya 2017).

Asparagus racemosus was used in the present study as an antidote for arsenic-induced toxicity in rats. It is also known as Shatavari in India, with a wide range of phytochemicals – asparagmine A, steroid saponins, oligospirostanoside, alkaloid aspargamine, isoflavones, flavonoids and many other compounds. Various studies speculate that it possesses hepatoprotective as well as renal-protective activities (Palanisamy and Manian, 2012; Acharya *et al*., 2012; Goyal *et al*., 2003).

Therefore, this study addresses assessing the phytocurative impact of *A. racemosus* root extract against hepatorenal toxicity induced by sodium arsenite in rats.

MATERIALS AND METHODS

Animals and diets

A total of n=24, 8-week-old male Charles Foster rats weighing approximately 15-180 g were procured from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India (CPCSEA Reg. No. 1129/PO/ReBi/S/07/CPCSEA. Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) with IAEC No. 2015-16/12/2015. Agenda No. 3 (c). The rats were acclimatized for 15 days prior to the beginning of the experiment with the laboratory conditions maintained under 12 hours of light and dark cycles and room temperatures maintained at 22 ± 2°C. The experimental animals were provided food and water *ad libitum.*

Chemicals

The arsenic-based chemical used in the experiment was sodium arsenite (98.5%) manufactured by Sigma–Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736 V, PCode 1001683292. The chemical was purchased from the Scientific store of Patna, Bihar, India and was used for the entire experiment.

Preparation of *A. racemosus* root ethanolic extract

In the present study, *A.s racemosus* plant roots were procured from the local market of Patna, Bihar and were identified by Dr. Santwana Rani (Botanist), Department of Botany, Patliputra University, Patna, Bihar, India. The plant roots were cleaned in running water and dried at 37°C in an incubator. The dried roots were subsequently crushed to form a fine powder. In addition, the dried roots were soaked in absolute ethanol for 48 hours and extracted with absolute ethanol using Buchi's Rota Vapour device. The ethanolic extract dose was calculated after LD_{50} estimation, which was 4800 mg/kg body weight. The final dose calculation was 400 mg/kg body weight for the administration to the sodium arsenite-treated rats for 8 weeks.

Experimental design

Rats were randomly divided into four groups containing six rats and categorized as follows. Group I: Normal control group without any treatment. Group II: Treated with arsenic- Rats were treated orally with sodium arsenite at a dose of 8 mg/kg body weight/day for 6 months. Group III: *A. racemosus* administration- Rats were pretreated with sodium arsenite 8 mg/kg body weight/day for 6 months followed by administration of *A. racemosus* ethanolic extract of root - 400 mg/kg body weight/day for 8 weeks. After the end of the entire experiment, the rats were anaesthetized and sacrificed. Blood was removed through the orbital puncture of every group of rats. The serum was later segregated for various biochemical estimations.

Histopathological study

Vital organs, such as liver and kidney tissues, were removed from all rat Groups (Group I to III) for the histopathological study. The dissected tissues were washed in normal physiological serum, grossed into small pieces, and then fixed in the fixative 10% formalin for 24 hours. The tissues were thereafter processed through a series of graded ethanol solutions and finally embedded into paraffin blocks. Sections of 5 µm thickness were cut through a microtome and stained with hematoxylin and eosin (H&E). For observation, the stained slides were viewed under a light microscope (Cardiff *et al*., 2014).

Biochemical analysis

Biochemical analysis was performed by the standard kit process (coral crest) using a spectrophotometer (UV - Vis) (UV-10, Thermo Fisher, USA). The Liver Function Test comprised the analysis of Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT), which were estimated according to the method (Reitman & Frankel, 1957), Alkaline Phosphate (ALP) by the method (Kind & King, 1954), total bilirubin activity by the method (Jendrassik and Grof, 1983) and albumin level measured by the BCG method. The kidney function test (KFT) was performed with urea by (Fawcett, 1960 and Berthelot,

1859), creatinine by (Toro and Ackermann 1975), and uric acid by (Bones and Tausky, 1945).

Lipid peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS) are markers of LPO. This was evaluated through the double heating method (Draper and Hadley, 1990) based on the principle of spectrophotometric measurement of color reproduced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this study, 2.5 ml of 100 g/L solution of trichloroacetic acid (TCA) was mixed with 0.5 ml serum in a centrifuge tube and heated in a water bath at 90°C for 15 minutes. After cooling at room temperature, the mixture was further allowed to centrifuge at 3000 rpm for 10 minutes, and 2 ml of the supernatant was mixed with 1 ml of 6.7 gm/L TBA solution in a test tube, which was further heated in a water bath at 90°C for 15 minutes and left for cooling at room temperature. Subsequently, an additional absorbance was measured using a UV-visible spectrophotometer (Thermo Scientific UV-10 USA) at 532 nm.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) for six individual rat groups, and the total variation represented in a set of data was analysed through one-way analysis of variance (ANOVA). Differences among the means and variances were analysed by applying Dunnett's 't' test at a 99.9% ($p < 0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

RESULTS

Haematological Study

The hematological parameters showed a significant decrease ($p<0.001$) in leukocyte count (WBC), hemoglobin percentage, red blood cell count (RBC), hematocrit percentage, MCV, MCH and MCHC in rats exposed to arsenic for six months. However, after administration of *A. racemosus* root extract, significant normalization in hematological parameters was observed (Table 1).

Biochemical assay

After 6 months of arsenic treatment (Group-II), in rats, there was a significant ($p<0.0001$) increase in the biochemical parameters observed, especially in SGPT, SGOT, ALP, creatinine and ($p<0.05$) urea levels, in comparison to the control group (Group-I) of rats. However, after 8 weeks of arsenic treatment, the concentrations of SGPT, SGOT, ALP, urea and creatinine increased significantly ($p<0.0001$) in comparison to the control rats. In contrast, after the administration of *A. racemosus* root extract to rats intoxicated with arsenic, there was a significant ($p<0.0001$) decrease in the serum levels of SGPT, SGOT, ALP, urea, uric acid, and creatinine compared to those in rats treated (Group-II) with arsenic for 6 months. In the control group of rats, the lipid peroxidation level was 2.86 ± 0.76 nmol/ml, and after 6 months of arsenic treatment, it was 105.1 ± 17.23 nmol/ml, while it was 17.68 ± 3.30 nmol/ml after 8 weeks of *A.racemosus* administration (Group-III) (Table 2).

Histopathological study

In the present histopathological study, the liver sections showed normal architecture of hepatocytes, central vein, and sinusoids, denoting normal cellular function in the liver (Fig. 1A). The arsenic treatment (Group -II) group showed a very high degree of degeneration in hepatocytes with haemorrhage in the central vein. Acute sinusoid vacuolization was also visualized (Fig. 1B). However, after the administration (Group-III) of *A. racemosus*, significant normalization was observed in the hepatocytes, central vein and sinusoids. However, moderate haemorrhages were still persistent (Fig. 1C). Kidney sections had a normal architecture of nephrocytes, Bowman capsule and glomeruli, convoluted tubules and distal tubules (Fig. 2A). The arsenic-treated

Table 1. Changes in the haematological parameters of Charles foster rats exposed to sodium arsenite at a dose of 8 mg/kg body weight per day for 6 months and its phytoremediation by *Asparagus racemosus* at a dose of 400 mg/kg body weight for 8 weeks.

Blood parameters	Control (Group-I)	Arsenic treated for 6 months (Group-II)	*A.racemosus* treated for 8 weeks (Group-III)
WBC (Cu.mm)	8200 ± 14.84	3500 ± 23.56**	10221 ± 7.62**
RBC Counts (x10^6/mm^3)	8.42 ± 5.32	3.01 ± 1.48***	5.56± 2.43***
Hb (g/dL)	14.4 ± 2.08	7.12 ± 2.15**	11.49 ± 1.84**
Haematocrit percentage (Hct) (%)	43 ± 3.08	21.4 ± 2.87**	34.5 ± 3.14**
MCV (fL)	51.1 ± 18.6	71.1 ± 8.96**	62.1 ± 6.43***
MCH (pg)	17.1 ± 1.40	23.7 ± 1.79*	20.7 ± 2.01*
MCHC (g/dL)	33.5 ± 0.036	33.3 ± 0.01**	33.3 ± 0.34*

Data are given as the mean ± SD (n=6 each group); p value ***($p<0.0001$), **($p<0.001$), *($p<0.05$) vs control.

Table 2. Changes in liver and kidney function biochemical parameter activity and LPO activity after different treatments in rats. Data are given as the mean ± SD (n=6 each group); p value $^{***}(p<0.0001)$, $^{**}(p<0.001)$, $^{*}(p<0.05)$ vs control.

Parameters	Control (Group-I)	Arsenic treated for 6 months (Group-II)	*racemosus* treated for 8 weeks (Group-III)
SGPT (U/mL)	28.12 ± 4.86	193.55 ± 7.34**	56.42 ± 4.23***
SGOT (U/mL)	35.10 ± 5.26	180.41 ± 3.51***	48.81 ± 2.56***
ALP (KA units)	4.31 ± 0.28	21.56 ± 2.88***	12.46 ± 2.92***
Urea (mg/dL)	25.19 ± 3.54	57.18 ± 4.22***	32.72 ± 3.62***
Uric acid (mg/dL)	2.82 ± 0.94	9.10 ± 1.58*	5.94 ± 0.99**
Creatinine (mg/dL)	0.85 ± 0.42	3.14 ± 0.54**	0.97 ± 0.64**
Lipid Peroxidation (nmol/ml)	2.86 ± 0.76	105.1 ± 17.23*	17.68 ± 3.30*

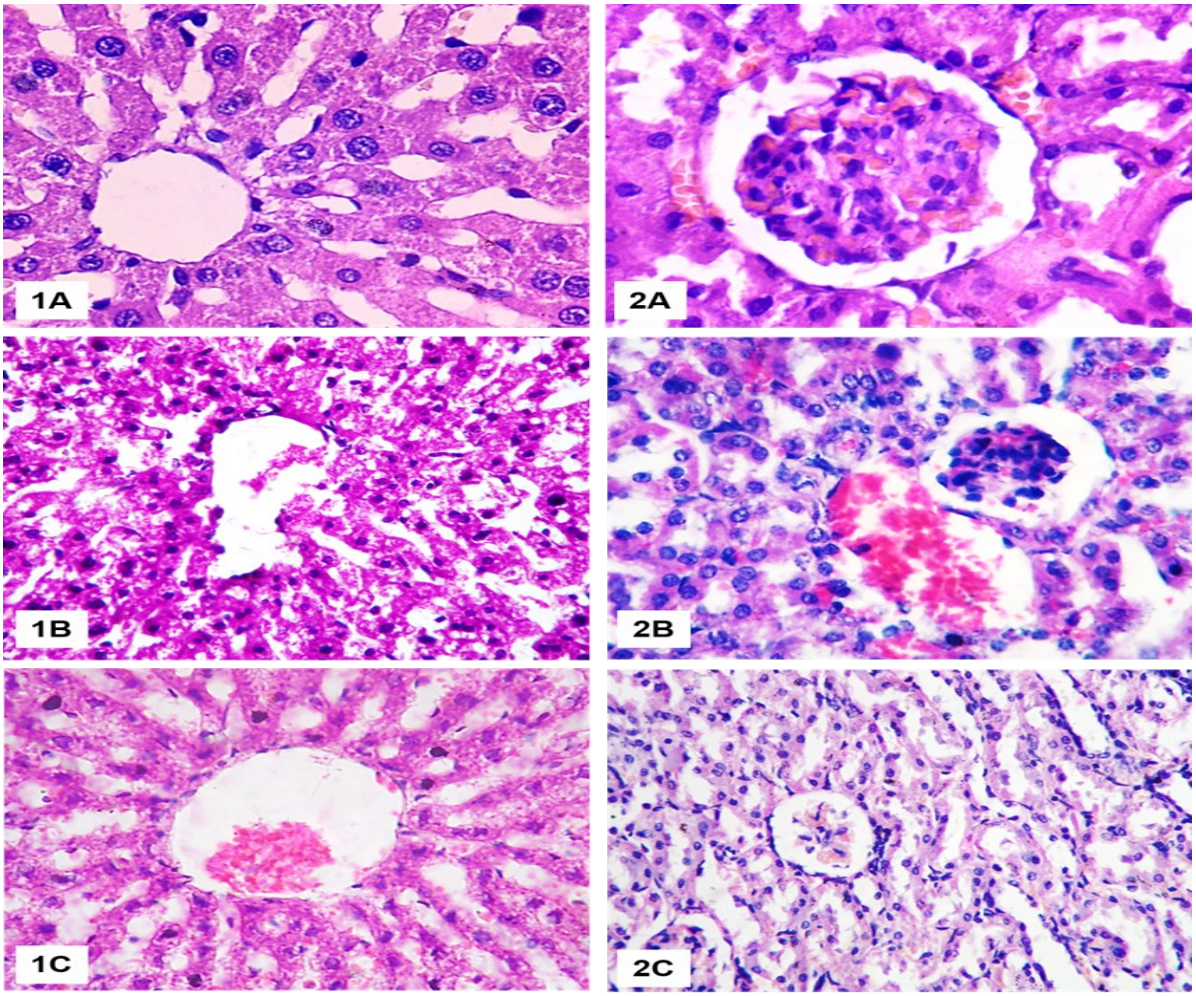

Fig. 1-2. ***1A.*** *Microphotographic section of liver tissue, hematoxylin and eosin-stained control rats showing polygonal hepatocytes with central nucleus with intact dense cytoplasm. Sinusoids are clearly seen, which are opening in the Central Vein X500.* ***1B.*** *Rats treated with arsenic for 6 months showed degenerated hepatocytes with degenerated nuclei and degenerated cytoplasm. Central venous endothelial cell degeneration was observed. X500.* ***1C.*** *In A. racemosus, which was administered to arsenic-treated rats for 6 months for 8 weeks, the hepatocytes normalized as degeneration was minimal. Granulated nuclei were observed in liver cells with little vacuolated space. The sinusoids and the central vein were also significantly restored with nuclear material. X500. 2A. Microphotographic section of kidney tissue of hematoxylin and eosin-stained control rat showing normal nephrocyte architecture, Bowman capsule, glomeruli. X800* ***2B.*** *Rats treated with arsenic for 6 months had severe nephrocyte degeneration with haemorrhage in the Bowman capsule and convoluted and distal tubules X500.* ***2C.*** *In A. racemosus, administered to arsenic-treated rats for 6 months for 8 weeks, nephrocytes showed significant restoration. However, mild haemorrhages continued to persist X500*

(Group-II) group showed degeneration in the nephrocytes and hemorrhage in the glomeruli. The intermingling of two Bowman's capsules with severe haemorrhage was also visualized (Fig. 2B). However, after the administration (Group-III) of *A. racemosus,* there was significant normalization in the nephrocytes, with the Bowman capsule and glomeruli, convoluted tubules and distal tubules (Fig. 2C).

DISCUSSION

Groundwater arsenic poisoning has led to serious health hazards among the exposed population. The trivalent form of arsenic is more toxic than the pentavalent form and causes severe damage to vital tissues. In the body system, arsenic reaches the body's vital organs through drinking water. It is methylated in vital organs, such as the liver, causing disrupted metabolic functions and disease progression (Yamanaka, 2004).

There was a remarkable change in the studied parameters in the present study. In the haematological parameters, there was a significant decrease in the WBC counts, RBC counts, platelet count, haemoglobin, HCT, MCV, MCH MCHC and percentage (Table 1) after the exposure of arsenic in the rats (Group-II). Similarly, there was significant damage to the biochemical parameters – liver function tests such as SGPT, SGOT, ALP and bilirubin levels while in kidney function tests as urea, uric acid and creatinine levels (Table 2). The significantly increased lipid peroxidation levels denote the suppression of the defense mechanism system in the cells. The histopathological study also showed a very high degree of degeneration in the hepatocytes due to arsenic toxicity in rats.

Various studies have reported that arsenic-induced toxicity leads to severe damage to the vital organs of the system (Yamamoto *et al*., 1995; Wanibuchi, *et al*., 1996; Cohen, *et al*., 2006). Arsenic exposure usually damages the glycolysis pathway, which causes disrupted ATP synthesis (Helleday *et al*., 2000). Moreover, long-duration exposure leads to metabolic disorders, which may further cause mutations in the cells (Mallikarjuna *et al*., 2003; Yamanaka *et al*., 2004); Soni *et al*., 1993. In the present study, there was a significant increase in the glucose-mediated functions of liver function tests such as SGPT and SGOT. This finding indicates that arsenic exposure causes severe damage to liver and kidney functions. Arsenic is a genotoxicant and can mutate DNA, leading to the formation of incorrect proteins and disrupting metabolic functions. Chromosomal aberrations further influence gene functions in cells (Miller *et al*., 2002; Huang *et al*., 2004; Duker *et al*.,2005; Faita *et al*., 2013; Roy *et al*., 2018).

Arsenic toxicity causes severe damage to liver and kidney tissues. In the liver, it usually disrupts the glucose signaling pathway, leading to the production of less ATP synthesis. This ATP deficiency causes irregularity in the function of the organ system. Moreover, macrophagic activities, such as Kupffer cell numbers, also increase many folds due to continuous inflammation, which in long-term impact also causes malignancy (Tao *et al*., 2020; Liu and Waalkes 2008). Similarly, the kidney, a metabolic organ, is also severely damaged due to arsenic-induced toxicity. Nephrocytes, the glomerular filtration rate, and increased urine albumin excretion are hampered. Dimethyl arsenic acid (DMA^V) is a more toxic compound form of arsenic that causes subcellular damage with disrupted renal functions (Chen *et al*., 2011; Hsueh *et al*., 2009; Zheng *et al*., 2013 & 2014; Jha *et al*., 2013) (Styblo *et al*., 2002; Carter *et al*. 2003). There was also significant damage to the biochemical parameters and cellular damage in hepatocytes and nephrocytes in the present study.

In this study, *A. racemosus* was used as an antidote against the arsenic-induced toxicity model in rats. The arsenic-treated animals (Group-II) showed a significant increase in biochemical parameters such as SGPT, SGOT, ALP, bilirubin, urea, uric acid creatinine, and lipid peroxidation levels. However, after the administration of *A. racemosus* (Group-III)*,* there was a significant decrease in the biochemical levels and cellular restoration. The root extract of asparagus contains the polycyclic alkaloid asparagine A and a few saponins. Asparagine A has the capability to eliminate arsenic-induced toxicity by increasing detoxication activity followed by increasing permeability in absorption through nephrons. Moreover, the antioxidant activity of asparagine A plays a vital role in controlling arsenic-induced cellular degeneration by increasing free radical scavenging activity. Asparagamine A plays a vital role in modulating normal cellular function by restoring cellular architecture (Wiboonpun *et al*., 2004). The histopathological damage caused by arsenic leads to severe damage to hepatocytes, central veins, portal veins, nephrocytes-glomeruli and Bowman's capsule. However, there was significant restoration at the cellular level in the asparagus extract group. Asparagine A likely restored cellular activity. Its powerful active antioxidant properties have free radical scavenging properties. It also catalyzes the synthesis of asparagine and glutamate through the reaction between aspartate and glutamine in an ATP-dependent reaction regulating normal cellular functioning. Hence, the root extract constituent asparagine A plays a vital role in combating arsenic-induced toxicity compared to the other constituents. It was also proven from the present study that compared to the other plant constituents, *A. racemosus* has a significantly promising role against arsenic induced toxicity (Acharya *et al*., 2012; Palanisamy and Manian 2012; Singh 2016; Bopana and Saxena 2007; Lomelino *et al*., 2017; Maurya and Srivastava 2011).

Conclusion

The present study concluded that arsenic causes a high degree of degeneration at the haematological, biochemical and histopathological levels in rats, disrupting the body's normal functioning. However, after *A. racemosus* administration, there was significant restoration in haematological parameters. Liver and kidney enzyme functions have been normalized, while marked cellular normalization indicated the normalization in physiological activity at the tissue level. Thus, *A. racemosus* has a hepato-nephroprotective effect against arsenic-induced toxicity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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