Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45** Issue **5 Year 2024** Page 45-**56***

DOI: 10.53555/jaz.v45i5.4686

Antidote effect of *Bacopa moneri* against arsenic induced toxicity in rats

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Abstract

Arsenic catastrophe has caused serious health hazards to the exposed population. An estimated 10 million people are exposed to arsenic poisoning through groundwater. The arsenic contamination through ground water has caused health diseases such as skin manifestations, loss of appetite, nausea, bowel disorders, neurological disorders etc. Therefore, there is need for search for proper therapeutic drug against arsenic induced toxicity in rats.

In the present study, arsenic induced toxic model was developed by treating Charles Foster rats (n=18) with sodium arsenite orally at the dose of 8 mg/kg body weight daily for 60 days followed by administration of *Bacopa moneri* (Brahmi) leaf extract at the dose of 30mg/kg body weight daily by gavage method for 60 days. After the completion of the experiment, all the animals were sacrificed and their blood samples were collected along with their vital tissues. Following parameters were assayed such as haematological assay, biochemical assay and histopathological evaluation in the present study. The haematological study comprised of RBC counts, WBC counts, platelets counts and heamoglobin percentage. For biochemical evaluation, the liver function test and the kidney function test parameters along with free radical assay were carried out. The histopathological study also showed very high degree of degeneration in the hepatocytes and the nephrocytes in the vital organ's liver and kidney. But, after the administration of ethanolic leaf extract of *Bacopa moneri*, there was marked restoration in the studied parameters. The haematological parameters, the biochemical parameters as well as the histopathological study showed very significant outcomes. Therefore, the present studied medicinal plant *Bacopa moneri* possesses antidote effect against arsenic induced toxicity in Charles Foster rats which can be used as therapeutic drug in the future after clinical approval.

Keywords: Arsenic toxicity, biochemical assay, liver and kidney toxicity, Bacopa moneri leaf extract, antidote effects, Charles Foster rats.

Introduction

The levels of pollution have been steadily rising over the last several decades, with groundwater contamination emerging as an especially serious concern in recent years. One of the biggest threats to the health of humans across the world is arsenic poisoning in groundwater. Arsenic poisoning affects an estimated 300 million people worldwide, including 70 million in India and 10 million in the Bihar Gangetic plains. Health issues affecting these exposed group vary from skin manifestations to neurological illnesses,

hormone abnormalities, gastrointestinal disorders, cardiovascular ailments, and more. It has also become an enormous problem since the incidence of cancer has grown exponentially in this exposed region. The water is the only area that has been addressed in terms of problem mitigation. However, the notion of arsenic-free water has reduced the level of damages. The crops are being irrigated with water that is polluted with arsenic. Because of this, arsenic has been able to bio-magnify and enter human systems (Shaji et al., 2021; Hassan, 2018; Kumar et al., 2022[a]; Richards 2022,2021, 2020).

People in the state of Bihar are particularly vulnerable to the harmful effects of arsenic poisoning. Serious health risks have been presented to the arsenic-exposed population as a result of their long-term use of water contaminated with arsenic. Skin manifestations, gastrointestinal issues, lung diseases, cardiovascular issues, hormone imbalance, decreased appetite, lowered immunity, bowel abnormalities, neurobehavioral alterations, and cancer poisoning are among the usual symptoms suffered by the exposed population. (Chakraborti et al., 2003 & 2016; Kumar 2022[a], Kumar et al., 2022[b,c]; 2020[a,b]; 2021[a,b,c,d]; 2020; 2016; 2015).

Therefore, in order to deal with this expansive issue, a bio-remedial strategy is required. Ayurveda, the ancient Indian medical system, makes use of the herb *Bacopa moneri* as a medication to treat a wide range of illnesses. Memory enhancement, sleeplessness, epilepsy, and anxiolytic are the primary uses of Bacopa in Ayurvedic medicine. Multiple clinical trials have shown that Bacopa may help with anxiety reduction, delayed word recall, memory acquisition, and verbal learning. The Ayurvedic book "Caraka Samhita" describes its use as a remedy for a number of mental diseases (Russo & Borrelli, 2005). The Ayurvedic term for Bacopa is a "Medhya rasayana," which refers to a group of plants said to have rejuvenating and life-extending properties as well as positive effects on the mind, memory, and intelligence (Shinomol et al., 2011; Chaudhari et al., 2011). In Ayurvedic medicine, Brahmi is primarily used to treat memory loss, sleeplessness, epilepsy, and anxiety. Bacopa has been shown in several clinical trials to alleviate anxiety, increase verbal learning, delay word recall, and memory acquisition. One way to put it is that it helps with concentration and calmness (Kumar et al., 2016). Therefore, the present study aims to find out the antidote effect of the leaves of *Bacopa moneri* against the arsenic induced toxicity in Charles Foster rats.

Materials and Methods

Ethics approval: This study was conducted after the approval of the research work from the Institutional Animal Ethics Committee of Mahavir Cancer Sansthan and Research Centre, Phulwarisharif, Patna, Bihar, India.

Animals: The study used male Charles Foster rats that were healthy condition and weighed between 150 and 180 grams on average. All of the animals were given the right to eat and drinkon an as-needed basis and kept in appropriate group cages.

Chemical: For this experiment, the chemical utilized was Sodium Arsenite, a form of arsenic procured from Sigma Aldrich (Merck). After dose titration, the treatment dose for the animals was adjusted to 8 mg/Kg body weight.

Antidote Plant: *Bacopa moneri* leaves were collected from a garden of Patna, Bihar, India, and was verified by a botanist from A.N. College in Patna, Bihar, for being used as an antidote for rats treated with arsenic. Before drying in the oven for 48 hours, the leaves were thoroughly rinsed with distilled water after being properly cleaned with water. The next step was to soak the leaves in alcohol for 48 hours after they were ground to a fine powder. For the final ethanolic extract, the powdered leaves were put through to a vacuum evaporation run. The reference dosage was determined to be 30 mg/Kg body weight after the titration.

Experimental design: Animals were divided into 03 groups - Group-I – Control group (n=6), Group-II– Arsenic treated group (n=12), Group-III – *Bacopa moneri* leaf extract treated group(n=6). The control group received only plain water and food to eat. The arsenic treated group were given sodium arsenite at the dose of 8 mg/Kg body weight per day for 90 days. The *Bacopa moneri* leaf extract was administered at the dose of 30 mg/Kg body weight per day for 60 days to the arsenic pretreated rats (arsenic treated for 90 days). After the completion of the entire experiment, all the rats were sacrificed, their blood samples were collected for haematological and biochemical study, while vital tissues such as liver and kidney were dissected out for histopathological study.

Haematological study: Standard methods were used to analyze the obtained blood samples for haematological parameters, including RBC counts, WBC counts, platelet counts, and hemoglobin percentage.

Biochemical assays: From the collected blood samples, serum was isolated by centrifuging it for 5 minutes at 3000rpm. The obtained serum was then utilized for the biochemical assays of liver function tests and kidney function tests. The liver function test consisted of Serum Glutamate Pyruvate Transaminase (SGPT)

assay, Serum Glutamate Oxalate Transaminase (SGOT) assay, alkaline phosphatase assay and bilirubin assay. The kidney function test consisted of urea, uric acid and creatinine assay. All the protocols for the assay were kit based and for this Coral Crest test kits were utilized. All the assays were carried out with standard protocol and proper calibration for the authenticated results.

Histopathological study: For the histopathological study, the dissected tissues were fixed in 10% neutral formalin for minimum 24 hours. Then after, the tissues were embedded in paraffin wax blocks after process through various grades of alcohol. The paraffin blocks were then cut at 5µm for fine sections and were then stained with Delafield's haematoxylin and eosin Y and through various grades of alcohol microscopic slides were obtained. The stained slides were then viewed under microscope for histopathological evaluation.

Statistical analysis: Utilizing the statistical software GraphPad 5.0, one-way ANOVA was used for the statistical analysis. All other variables were tested using Dunnett's test, except forthe p-value.

Results

Haematological study: In the study on blood cells, it was found that rats treated with arsenic had significantly lower red blood cell (RBC) counts, white blood cell (WBC) counts, platelets count, and hemoglobin percentage were compared to the control group. On the other hand, rats treated with *Bacopa moneri* leaf extract had significantly normalized levels of RBC, WBC, platelets counts and hemoglobin percentage.

Table 1.: Haematological parameters in different treatment group of rats. All data are expressed in Mean ± SE *(One way ANOVA Test in various group of rats (n=6))*

Group	Control	90 Days arsenic treated	60 Days *B. moneri* treated
RBC ($\times10^6$/mm^3)	5.82 ± 1.76	2.34 ± 3.21	5.14 ± 2.15
HGB (g/dL)	14.14± 2.86	6.34 ± 3.29	13.49 ± 2.42
HCT (%)	42.34 ± 3.29	19.59 ± 4.17	40.5 ± 3.74
MCV (fL)	72.7 ± 6.24	83.7 ± 3.78	78.8 ± 3.72
MCH (pg)	24.3 ± 4.23	27.1 ± 4.58	26.2 ± 4.83
MCHC (g/dL)	33.4 ± 2.38	32.24 ± 2.51	33.3 ± 2.36
WBC ($\times10^3$/mm^3)	8.44 ± 3.81	14.22 ± 8.95	9. 7.67± 4.22
Platelets ($\times10^3$/mm^3)	420 ± 16.2	77 ± 34.2	363 ± 12.77

Biochemical Study:

1) SGPT Assay: There was a substantial rise ($p<0.005$) in SGPT levels in the arsenic treated group of rats as compared to the control group. The SGPT levels in the arsenic-treated rat group were significantly ($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment. (Figure 1).

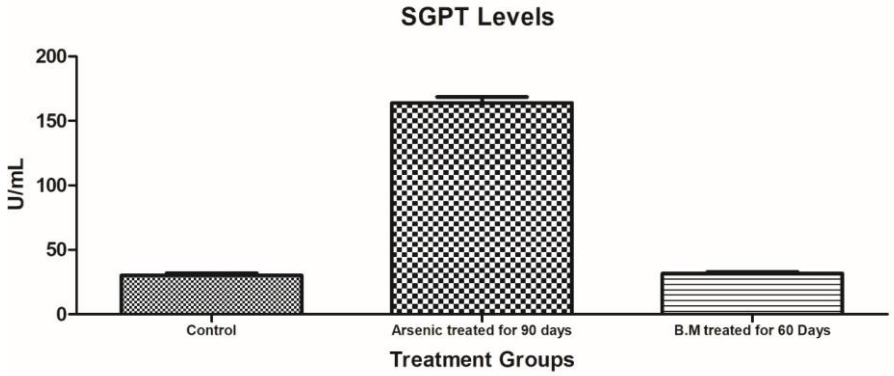

Figure 1. SGPT Levels of the treated groups (One way ANOVA Test in various group of rats(n=6), values displayed as Mean ± SE)

2) SGOT Assay: There was a substantial rise ($p<0.005$) in SGOT levels in the arsenic treated group of rats as compared to the control group. The SGOT levels in the arsenic- treated rat group were significantly($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 2).

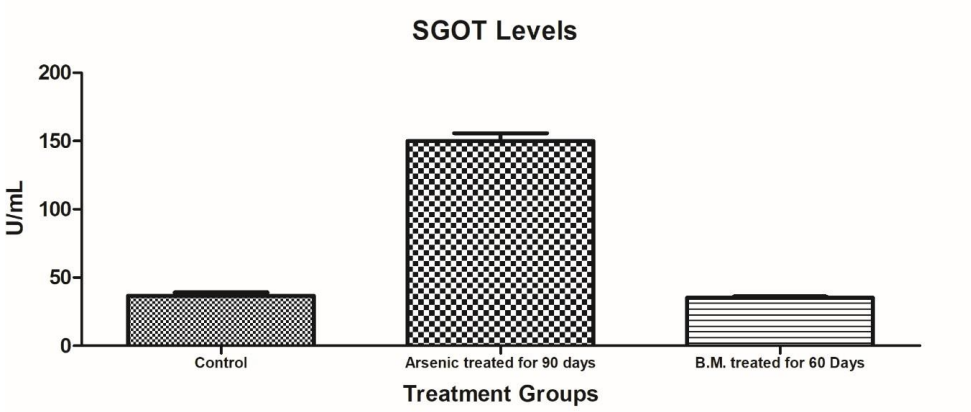

Figure 2. SGOT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

3) Alkaline Phosphatase (ALP) Assay: There was a substantial rise ($p<0.005$) in ALP levels in the arsenic treated group of rats as compared to the control group. The ALP levels in the arsenic-treated rat group were significantly($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 3).

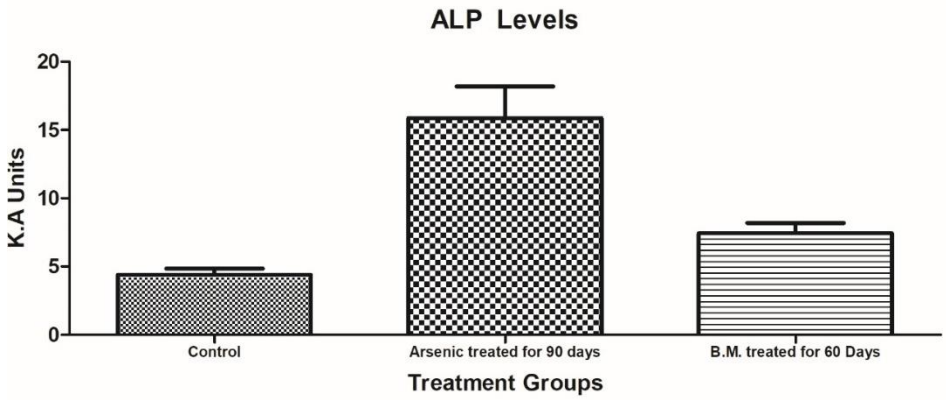

Figure 3. Alkaline phosphatase Levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

4) Bilirubin Assay: There was a substantial rise ($p<0.005$) in bilirubin levels in the arsenic treated group of rats as compared to the control group. The bilirubin levels in the arsenic-treated rat group were significantly($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 4).

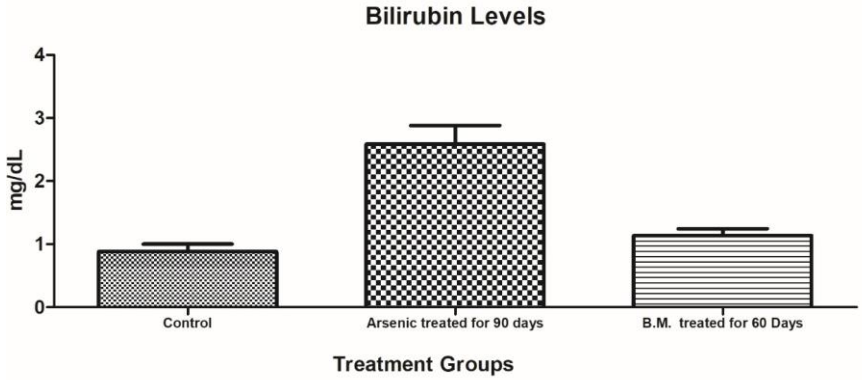

Figure 4. Bilirubin Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

5) Urea Assay: There was a substantial rise ($p<0.005$) in urea levels in the arsenic treated group of rats as compared to the control group. The urea levels in the arsenic-treated rat group were significantly($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 5).

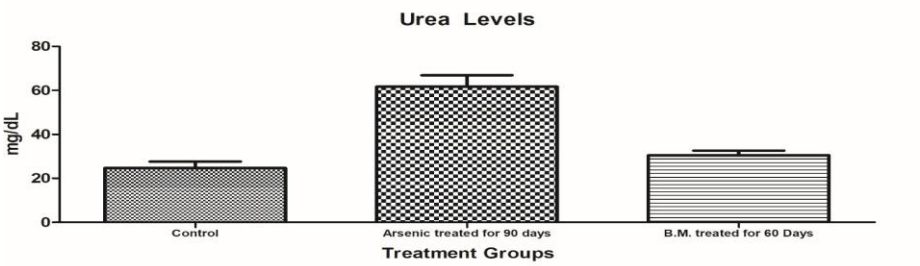

Figure 5. Urea Levels of the treated groups (One way ANOVA Test in various group of rats(n=6), values displayed as Mean ± SE)

6) Uric Acid Assay: There was a substantial rise ($p<0.005$) in Uric acid levels in the arsenic treated group of rats as compared to the control group. The Uric acid levels in the arsenic-treated rat group were significantly ($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 6).

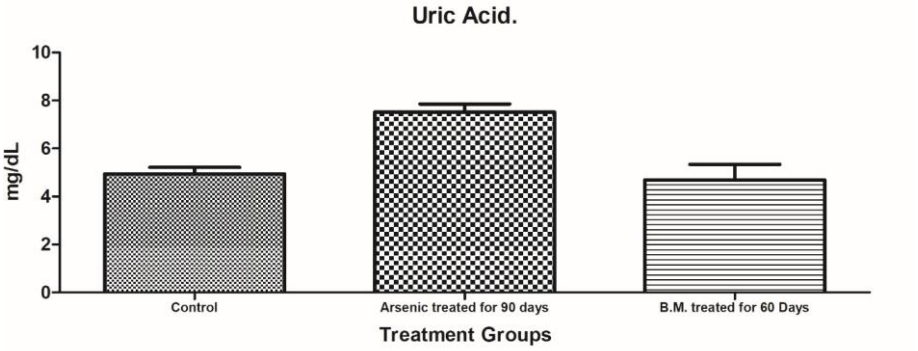

Figure 6. Uric acid Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

7) Creatinine Assay: There was a substantial rise ($p<0.005$) in creatinine levels in the arsenic treated group of rats as compared to the control group. The creatinine levels in the arsenic-treated rat group were significantly($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 7).

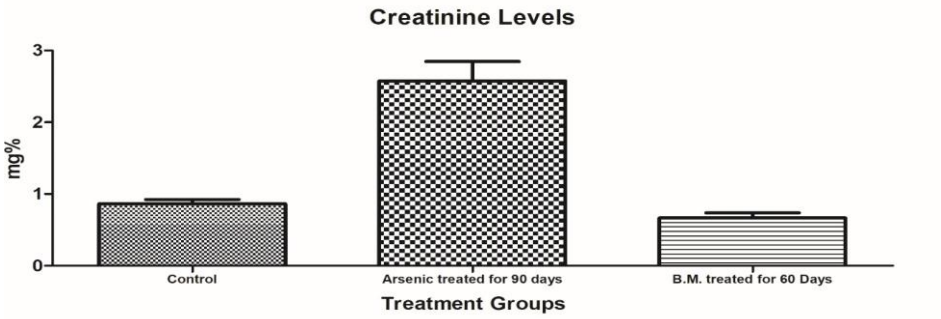

Figure 7. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

8) Lipid Peroxidation (LPO) Assay: There was a substantial rise ($p<0.005$) in LPO levels in the arsenic treated group of rats as compared to the control group. The LPO levels in the arsenic-treated rat group were significantly ($p<0.005$) normalized following *Bacopa moneri* leaf extract treatment (Figure 8).

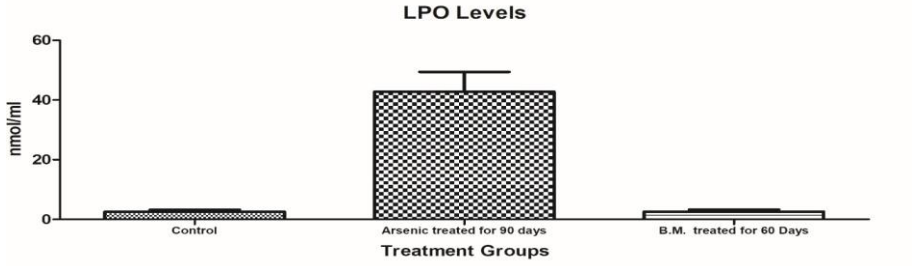

Figure 8. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

Histological investigation:

Liver histopathology sections show normal architecture of hepatocytes with central vein, indicating significant modifications in the studied groups. Figure 9A shows that hepatocytes are observed in the sinusoids, which means that the liver cells are functioning efficiently. Hepatocytes with pyknotic nuclei significantly degrade in the arsenic-treated rat liver section.Macrophage activity has increased observed by an upsurge in the number of Kupffer cells. Thecentral vein membrane's endothelial cells have also ruptured and caused the sinusoidal spaces to rupture. Vacuolations in the sinusoidal spaces are also seen in the section (Figure 9B). Hepatocytes, central veins, and sinusoids showed significant improvement after 60 days of therapy with *Bacopa moneri* leaf extract. A regular arrangement of hepatocytes in the sinusoids indicates that the liver is functioning effectively. In addition, as seen in Figure 9C, the liver is functioning appropriately as there are no Kupffer cells. Figure 9D shows that the kidney histological sections show typical glomerulus, Bowman's capsule, convoluted tubules, and distal tubules. A damaged glomerulus and Bowman's capsule are seen in the kidney section that was exposed to arsenic. In addition, severe hemorrhaging inside the kidneys may be observed, which could be a sign of an abnormal kidney filtration mechanism caused on by arsenic poisoning (Figure 9E). But after treating the nephrocytes with *Bacopa moneri* leaf extract, they showed significant improvement, especially in the glomerulus, Bowman's capsule, and convoluted tubules, which means that their activity is restored to normal (Figure9F).

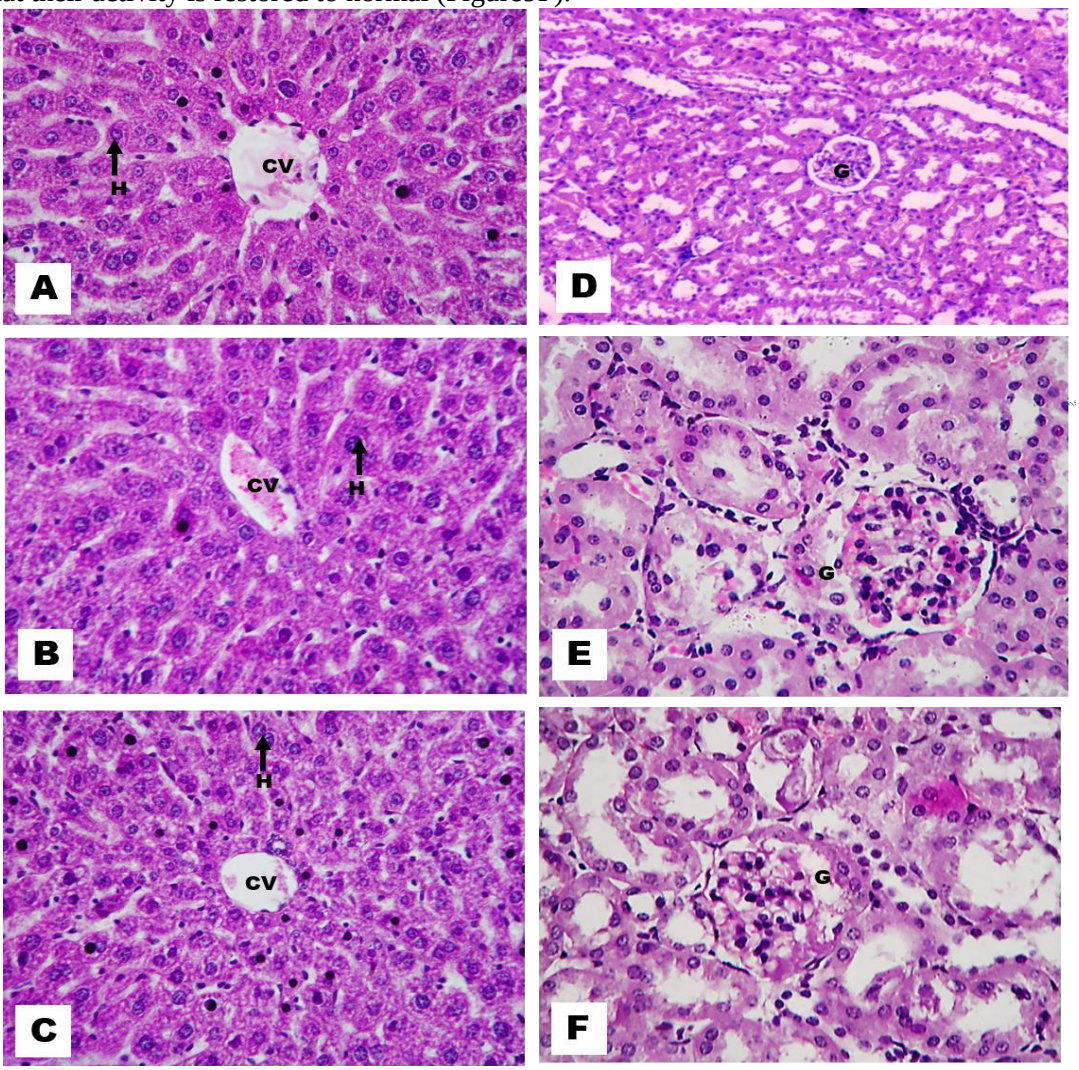

Figure 9: Microphotograph of Haematoxylin and eosin (H&E 500x) stained sections of rat **[A]** Liver of a control rat showing typical architecture of hepatocytes (H), central veins (CV), and sinusoids. Hyper-condensed hepatocytes are seen in sinusoids. **[B]** Sections of arsenic-treated rat liver showing hepatocytes (H) that have degraded and a central vein (CV). Additionally, hepatocyte pyknotic nuclei may be seen. An indicator of tissue inflammation is an increase in the number of pin-shaped Kupffer cells. There is also bleeding in the areas between the sinuses. **[C]** After treating the rats with *Bacopa moneri* leaf extract, there is a significant normalizationof the hepatocytes (H) with central vein (CV) in the liver sections. **[D]** Rat control kidney sections stained with H&E (haematoxylin and eosin) 500x reveal normative glomerulus architecture

(G) with Bowman's capsules. In a normal architecture, the endothelial cells of convoluted tubules are also present. **[E]** Sections of the kidneys of rats exposed to arsenic reveal widespread hemorrhage, extensive damage to Bowman's capsule and the glomerulus (G), and other abnormalities. Significant damage has also occurred to the convoluted tubules (CT). **[F]** Sections of kidney treated with *Bacopa moneri* leaf extract shows significant improvement in the nephrocytes, namely in the glomerulus (G), Bowman's capsule, and convoluted tubules (CT), suggesting normal kidney tissue function.

Quantification histopathological study of liver tissue

Comparing the liver sections of the 90-days arsenic-treated group to the control sections, the research reveals a significant deterioration. However, the liver tissue significantly restored to normal after the *Bacopa moneri* treatment. (Table 2).

Table 2: Quantified scoring of histopathological damage in the liver tissue

Group	Control	90 Days arsenic treated	60 Days *B. moneri* treated
Degenerated hepatocytes	0.84 ± 0.54	68.34 ± 3.89	17.93 ± 3.56
Vacuolization	0.64 ± 0.45	17.31 ± 4.38	3.37 ± 2.82
Haemorrhage	0.45 ± 0.67	13.09 ± 5.23	2.40 ± 2.73
Central vein degeneration	0.98 ± 0.90	16.95 ± 4.28	1.69 ± 0.78
Portal vein degeneration	0.56 ± 0.23	14.37 ± 3.76	3.78 ± 1.83

Quantified histological damage scoring in liver tissue (n=6, values reported as Mean S.D.). Each rat's hepatocyte degeneration was measured by counting the degeneration among 100 hepatic cells; the remainder of the histopathological abnormalities were investigated randomly in 20 microscopic fields (X40; H&E).

Quantification histopathological study of kidney tissue

Comparing the kidney sections of the 90-days arsenic-treated group to the control sections, the research reveals a considerable deterioration. However, there was a substantial recovery to normalcy in the kidney tissue after the administration of *Bacopa moneri*. (Table 3).

Table 3: Quantified scoring of histopathological damage in kidney tissue.

Group	Control	90 Days arsenic treated	60 Days *B. moneri* treated
Tubular degeneration	0.58 ± 0.38	44.32 ± 5.36	20.18 ± 3.87
Glomerulus degeneration	0.98 ± 1.93	16.34 ± 4.27	7.89 ± 3.63
Haemorrhage	0.72 ± 0.98	10.42 ± 3.68	1.36 ± 1.00
BC membrane degeneration	0.55 ± 0.59	19.23 ± 3.94	2.84 ± 1.86
Vacuolization	0.21 ± 0.38	18.20 ± 5.42	7.79 ± 1.11

Quantified histological damage scoring in kidney tissue of control and treated rats (n=6, values are expressed as Mean ± S.D). Tubular degeneration was counted among 100 tubules in each rat, and the remainder of the histopathological alterations were investigated randomly in 20 microscopic fields (X40; H&E).

Discussion

Arsenic has an effect on the animal's organ systems and general body because, after ingestion,it travels via the gastrointestinal tract to the blood and other vital organs. The current study found that compared to the control group, rats treated with arsenic had significantly lower red blood cell (RBC) counts, white blood cell (WBC) counts, and hemoglobin percentage. All of the blood tests came to the same conclusion, which suggests that the arsenic poisoning has damaged the rats' hematopoietic stem cells, which in turn has produced noticeable alterations in the animals' haematological parameters. After pre-treatment with arsenic, rats administered with *Bacopa moneri* leaf extract showed significant improvement at the haematological level,with red blood cell (RBC) counts, white blood cell (WBC) counts, and hemoglobin percentagereturning to baseline. A second level of indicators is the biochemical parameters, which indicate the biochemical amount of deterioration at the tissue level. Enzyme indicators which provides the most relevant information about toxicity in the body are those that measure the performance of the liver and the kidneys. The results of this research showed that SGPT, SGOT, alkaline phosphatase, bilirubin, urea, uric acid, and creatinine levels

significantly increased ($p<0.005$) indicate that these vital organs are severely damaged by arsenic poisoning. However, when rats that had been pre-treated with arsenic were given *Bacopa moneri* leaf extract, there was a significant improvement in the levels of these liver and kidney function tests. The third stage of toxicity assessment includes observational data provided by the histology of exams. The histological analysis of the kidney and liver tissues in this study showed substantial damage to both organs. Significant damage was discovered in the hepatocytes, central vein, portal vein, and sinusoidal spaces in the arsenic-treated liver tissue sections. The presence of an increased number of Kupffer cells in the liver is a marker of acute inflammation caused by the adverse effects of arsenic. In above that, the sinusoidal gaps showed hemorrhage. With this toxin in the kidney tissue, damage was made to the glomerulus, Bowman's capsules, convoluted tubules, and ductal tubules. As a result of arsenic's toxicity, kidney tissue deteriorated and hemorrhaged, making glomerular filtration more challenging. The liver and kidney tissues of the arsenic-pretreated rats showed a significant improvement after receiving an administration of *Bacopa moneri* leaf extract. There was significant improvement in the restoration of hepatocytes, central veins, and sinusoidal gaps when compared with the arsenic-treated rat liver. Also, the kidney tissue, namely the glomerulus, Bowman's capsule, convoluted tubules, and distal tubules, showed significant signs of repair in the nephrocytes. According to these findings, *Bacopa moneri* leaf extract may have antidote properties that may reduce the harmful effects of arsenic poisoning. *Bacopa moneri* leaf extract's rejuvenating properties and antioxidant properties are responsible for the complete healing seen at the organ level. The adverse effect made by the arsenic-induced toxicity has been mitigated by these qualities. (Rathour 2024; Sumathi et al., 2009; Shinomol et al., 2013;Evan Prince et al., 2016; Shahid et al., 2016; Janani et al., 2009 & 2010; Sabina et al., 2019; Kamesh and Sumathi 2014; Kumar et al., 2022[d], Kumar et al., 2020[b]).

Protective mechanisms are sustained at the cellular level via the oxidant-antioxidant system. The lipid peroxidation levels were much greater in the arsenic-treated rats compared to the control group, suggesting that the defense mechanism of the arsenic-treated rats was not effective. The two groups of rats were compared with one another, the lipid peroxidation levels were significantly restored after treatment with *Bacopa moneri* leaf extract. Thus, *Bacopa moneri* has antioxidant properties (Dinh et al., 2023; Oyeleke e al., 2022; Kapoor et al., 2009;Bafna and Balaraman 2005).

The leaf extract of *Bacopa moneri* contains active components such as flavonoids and saponins, which played a crucial role in the normalization of cellular processes in the arsenic-induced toxicity. These active chemicals assisted with restoring the cellular activities. Through the antioxidant mechanism, they restore the damage caused by arsenic, therefore restoring the activities of the organism on the haematological, biochemical, and histopathological levels.

Conclusion

In this study, the antioxidant properties of *Bacopa moneri* leaf extract were shown to potentially protect rats against arsenic-induced toxicity. An excellent antidote for arsenic poisoning is the *Bacopa moneri* leaf extract, which has antioxidant and anti-protective qualities.

Acknowledgements

The authors are thankful to Department of Zoology, Patliputra University, Patna (Bihar) India for infrastructural facilities and Mahavir Cancer Sansthan and Research Centre, Patna (Bihar) India for experimental rats, ethical approval and laboratory facilities.

Declarations

Competing interests

The authors declare that they have no conflicts of interest.

Consent for publish

All the authors provide their consent to publish this article.

Author contributions

The entire experimental work was conceptualized by Z.H., R.K. and A.K. The manuscript's principal author

Z.H contributed the majority of writing activities, but support was also provided by R.K, and A.K., Literature search was done by Z.H. Figures were developed by
Z.H. and A.K. The experimentation and data analysis were carried out by Z.H. The figures were designed by Z.H. and A.K. The statistics and data interpretation were done by Z.H. The final manuscript writing was done by Z.H. R.K. and A.K. All authors read and approved the final manuscript.

Funding

No funding was received for this work.

Availability of data and materials

None of the data has been fabricated or manipulated (including image) to support thisinvestigational study. Data supports the findings.

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