JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Therapeutic Effect Of *Vinca rosea* Against Arsenic Induced Toxicity In Charles Foster Rats

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Abstract

In India, an estimated 70 million population are exposed to arsenic due to drinking of contaminated groundwater. In state of Bihar about 10 million people are exposed to arsenic poisoning. Out of 38 districts of the state, 18 districts are severely affected. The exposed population exhibit various symptoms such as skin manifestations - hyperkeratosis, melanosis, loss of appetite, neuro-behavioral disorders etc. Therefore, the present study aims to develop novel drug discovery against arsenic induced toxicity in Charles Foster rat models.

After the ethical approval from the Institutional Animal Ethics Committee, the animals were grouped into 03, the first group was the control group, the second group was arsenic treated and the third group was *Vinca rosea* leaf extract treated upon arsenic pre-treated group. In the arsenic treated group, the rats received sodium arsenite orally at the dose of 8 mg/kg body weight daily for 90 days followed by administration of *Vinca rosea* leaf extract at the dose of 300 mg/kg body weight daily by gavage method for 60 days. Their biochemical levels like liver and kidney function tests were assayed and were found with elevated levels. Furthermore, their free radical assessment such as lipid peroxidation levels were assayed which was also found to be many folds higher. Furthermore, the arsenic concentration in the tissue of liver and kidney was significantly very high. But, after the administration of ethanolic leaf extract of *Vinca rosea*, there was significant restoration in the biochemical and lipid peroxidation levels. Moreover, there was also reduction in the arsenic content in the liver and kidney tissues of rats. The entire study reveals that, *Vinca rosea* possesses therapeutic effect against arsenic induced toxicity.

Keywords: Charles Foster rats, arsenic induced model, biochemical assays, liver and kidney toxicity, *Vinca rosea* leaf extract, therapeutic effects.

Introduction

Recent decades have seen a dramatic rise in pollution levels worldwide, with groundwater contamination emerging as a particularly pressing issue. Groundwater contaminated with arsenic is a significant cause of health risks for people across the world. There are an estimated 300 million individuals worldwide who are at risk of arsenic poisoning; this includes an estimated 70 million in India and 10 million in the Gangetic plains of Bihar. These people suffered from a wide range of illnesses as a result of their exposure, including skin manifestations, neurological disorders, hormone abnormalities, gastrointestinal disorders, cardiovascular diseases, etc. Cancer rates have also risen in this exposed region, becoming a serious cause for alarm. Only water-related measures are being taken to address the issue. However, the notion of arsenic-free water has mitigated the toxicity to a significant degree. However, the crops continue to be grown using the same water that contains arsenic. As a result, arsenic has been biomagnified and transformed from the environment into the human body system (Shaji et al., 2021; Hassan, 2018; 2019; 2020; Kumar et al., 2022[a]; Richards et al., 2021 & 2020).

Arsenic poisoning poses major health risks to the inhabitants of the Indian state of Bihar. People who have been drinking arsenic-tainted water for a long period are at high risk for major health problems. Arsenicosis symptoms, including skin manifestations, gastrointestinal illness, lung disease, cardiovascular disease, hormone imbalance, loss of appetite, poor immunity, bowel alterations, neurobehavioral abnormalities, and cancer poisoning, have been seen in the exposed population (Chakraborti et al., 2003 & 2016; Kumar 2022[a], Kumar et al., 2022[b,c]; Kumar et al., 2020[a,b]; Kumar et al., 2021[a,b,c,d]; Kumar et al., 2020; Kumar et al., 2016; Kumar et al., 2015; Rahman 2019[a,b]; Kumar and Ghosh 2021 & 2019[b]).

Therefore, a bio-remedial strategy is required to address this intricacy. Ayurvedic medicine makes use of the plant's leaves, flowers, and roots. The plant extract is used to treat a variety of illnesses in traditional Chinese medicine, including diabetes, malaria, leukemia, and Hodgkin's disease. Traditional medicine has found uses for the juice of the leaves in the treatment of wasp stings, as a gargle for sore throats, and as an eye wash for newborns made from flower extracts. The alkaloids responsible for the plant's anti-tumor and anti-cancer effects are found in the plant's leaves and stems. The leaves are used to manage blood sugar levels and hypertension. The alkaloids may calm and relax the body and mind. Because of its ability to detoxify and neutralize poison, it is used to alleviate the pain and sadness caused by wasp stings. Nosebleeds, gum bleeding, mouth sores, and sore throats may all be stopped with the help of this herb. When used internally, it helps with a variety of conditions, including gastritis, cystitis, enteritis, diarrhea, diabetes, and so on. The *Vinca rosea* plant is beneficial to mental well-being. The active chemicals in this product boost the amount of oxygen that the brain can absorb from the blood. In addition to increasing serotonin, it also stops the blood from clotting abnormally. Vincamine, an alkaloid, improves memory and keeps blood pressure down. Therefore, it is helpful in warding off dementia, and in particular vascular dementia. They have found use in the treatment of diabetes, hypertension, and as a disinfectant. The vinca alkaloids also have a significant role in combating against cancer. In medicine, four primary vinca alkaloids stand out: Vinodil, Vincristine, Vinorelbine, and Vindesine; abbreviations for their chemical names.

Therefore, the purpose of this research is to determine whether or not arsenic-induced toxicity in Charles Foster rats may be mitigated by administering a leaf extract of *Vinca rosea.*

Materials and Methods

Ethics approval: The Institutional Animal Ethics Committee of Mahavir Cancer Sansthan and Research Centre in Phulwarisharif, Patna, Bihar, India granted its approval for this study.

Animals: Male Charles Foster rats weighing an average of 180g were utilized in this investigation. The animals had adequate housing, including group cages with food and drink available at all times.

Chemical: In the study, arsenic utilized was in the form of sodium arsenite (purchased from Merck – Sigma Aldrich). After dosage titration, a dose of 8 mg/Kg body weight was determined for administration to the animals.

Medicinal Plant Used: In the study, arsenic utilized was *Vinca rosea* leaves were obtained from a garden in Patna, Bihar, India, and later recognized by a Botanist at A.N. College, Patna, Bihar, India, as an antidote for arsenic-induced poisoning in rats. After gathering the leaves, they were cleaned well, given a final rinsing in distilled water, and then dried in the oven for 48 hours. After being soaked in alcohol for 48 hours, the ground-up leaves were finally ready to use. To obtain the ethanolic extract, the powdered leaves were run through a Vacuum evaporator. The titration resulted in a reference dosage of 300 mg/Kg.

Experimental design: Animals were divided into 03 groups - Group-I – Control group (n=6), Group-II– Arsenic treated group (n=12), Group-III – *Vinca rosea* 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 arsenic-pretreated rats (treated with arsenic for 90 days) received *Vinca rosea* leaf extract at a dosage of 300 mg/Kg body weight each day for 60 days. At the completion of the experiment, all of the rats were euthanized, blood was drawn for haematological and biochemical analysis, and important organs including the liver and kidney were removed for histological analysis.

Haematological study: Standard procedures were used to analyze the obtained blood samples for haematological parameters such as red blood cell count, white blood cell count, platelet count, and hemoglobin percentage.

Biochemical assays: From the collected blood samples, serum was isolated by centrifuging it for 15 minutes at 3000rpm. The obtained serum were then utilized for the biochemical assays of liver function tests and kidney function tests. The biochemical study was carried out through the standard kit process (Coral crest) using the Spectrophotometer (UV - Vis) (UV-10, Thermo Fisher, USA). The biochemical parameters used in the present study were - Liver Function Tests- Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) estimated through the method of (Reitman & Frankel, 1957), Alkaline Phosphate (ALP) assay by the method of (Kind & King, 1954), total bilirubin activity by method of (Jendrassik & Grofs, 1938). The Kidney Function Tests (KFT) were assayed as urea by the method of

(Fawcett 1960, Berthelot 1859), creatinine assay by the method of (Toro and Ackermann 1975), and uric acid assay by the method of (Bones and Tausky, 1945). The lipid peroxidation study was carried out through the method of (Draper and Hadley, 1992).

Group	Control	90 Days arsenic treated	60 Days *V.rosea* treated
RBC ($\times 10^6/mm^3$)	6.23 ± 1.65	1.96 ± 2.55	3.90 ± 1.96
HGB (g/dL)	13.98 ± 3.79	5.69 ± 1.45	12.94 ± 2.90
HCT (%)	42.28 ± 3.88	16.8 ± 1.92	39.32 ± 2.64
MCV (fL)	67.9 ± 5.82	85.7 ± 8.45	100.8 ± 5.20
MCH (pg)	22.4 ± 3.28	29.0 ± 3.04	33.2 ± 3.19
MCHC (g/dL)	33.1 ± 1.09	33.9 ± 3.78	32.9 ± 2.93
WBC ($\times 10^3/mm^3$)	7.24 ± 4.44	17.94 ± 6.43	8.90 ± 1.21
Platelets ($\times 10^3/mm^3$)	330 ± 16.2	84 ± 34.2	190 ± 10.19

Histopathological study: Histopathological analysis included fixing the tissue samples for at least 24 hours in 10% neutral formalin. The tissues were then processed via a series of ethanol concentrations before being embedded in paraffin wax blocks. Fine sections (5 mm) were cut from paraffin blocks, which were then stained with Delafield's haematoxylin and Esosin Y and subjected to different concentrations of alcohol to make microscopic slides. Histopathological analysis was performed by examining at the stained slides under a microscope (Cardiff et al., 2014).

Statistical analysis: GraphPad 5.0 was used to perform one-way ANOVA tests for the statistical analysis. All other variables were tested using Dunnett's method for significance.

Results

1) **Haematological study:** The haematological study revealed that the red blood cell count, white blood cell count, platelet count, and haemoglobin percentage were all significantly lower in the arsenic-treated rats compared to the control group rats, while all of these parameters were significantly normalized in the Vinca rosea leaf extract-treated rats ($p<0.05$) (Table 1).

Table 1.: Rats of several treatment types were tested for various haematological markers. Statistics are presented as Mean ± SE. *(One way ANOVA Test in various group of rats (n=6))*

Biochemical Study:

2) **SGPT Assay:** When comparing the levels of SGPT in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal SGPT levels in arsenic-treated rats. (Figure 1).

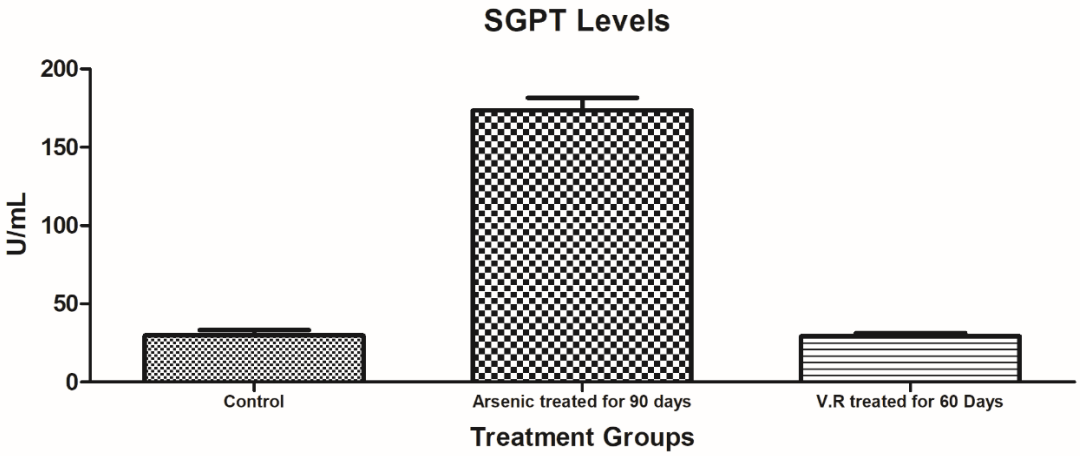

Figure 1. SGPT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

3) **SGOT Assay:** When comparing the levels of SGOT in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal SGOT levels in arsenic-treated rats. (Figure 2).

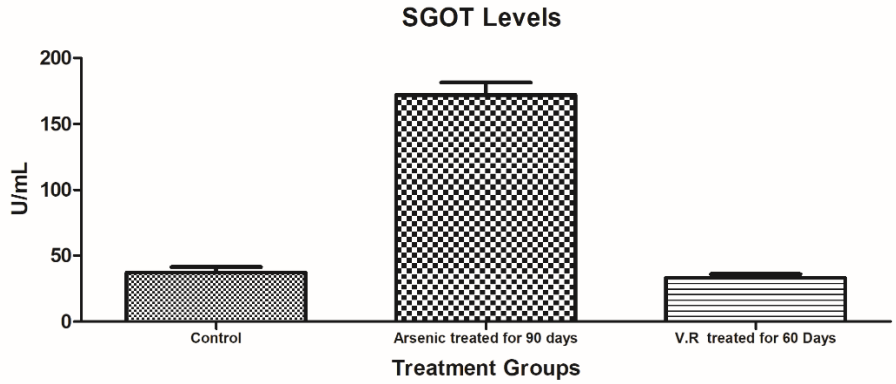

Figure 2. SGOT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

4) **Alkaline Phosphatase (ALP) Assay:** When comparing the levels of ALP in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal ALP levels in arsenic-treated rats (Figure 3).

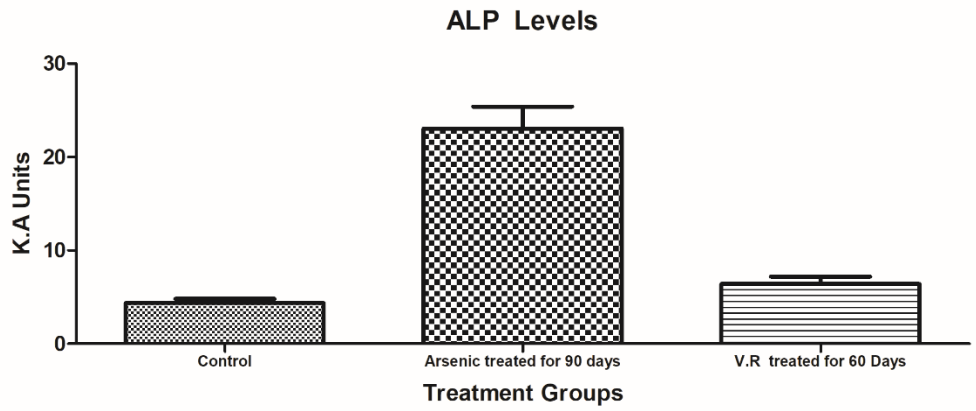

Figure 3. Alkaline phosphatase Levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

5) **Bilirubin Assay:** When comparing the levels of bilirubin in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal bilirubin levels in arsenic-treated rats. (Figure 4).

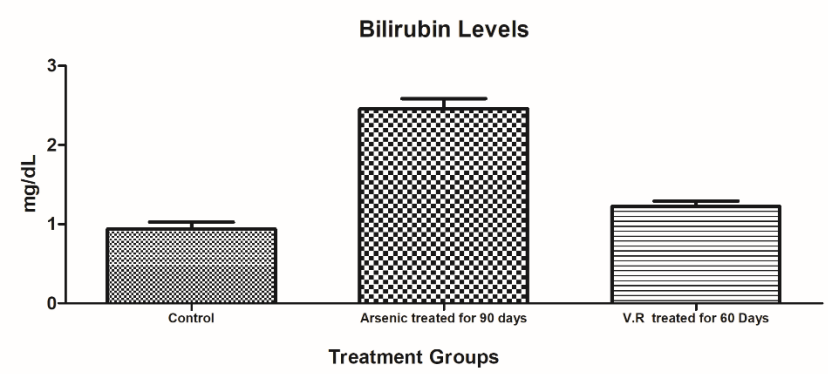

Figure 4. Bilirubin Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

6) **Urea Assay:** When comparing the levels of Urea in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal Urea levels in arsenic-treated rats. (Figure 5).

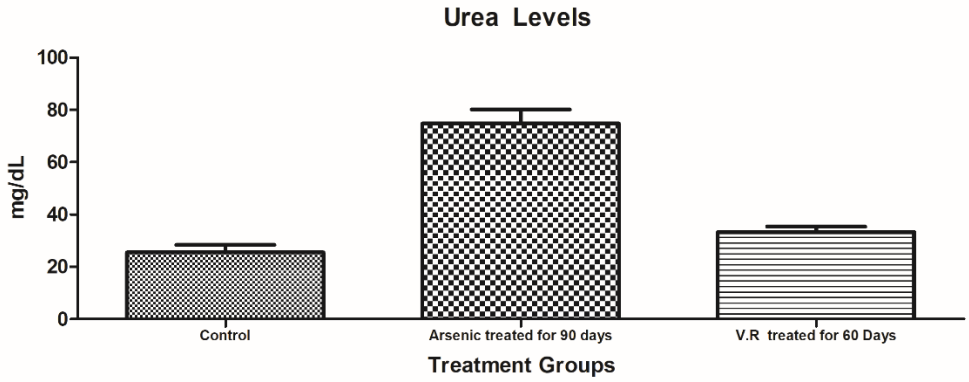

Figure 5. Urea Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

7) **Uric Acid Assay:** When comparing the levels of Uric acid in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal Uric acid levels in arsenic-treated rats. (Figure 6).

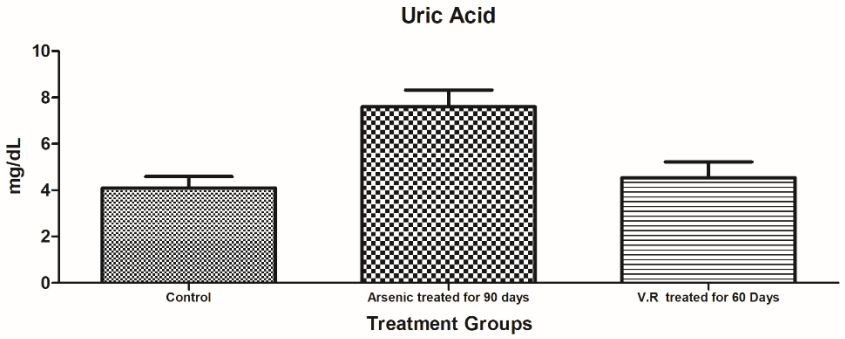

Figure 6. Uric acid Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

8) **Creatinine Assay:** When comparing the levels of creatinine in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal creatinine levels in arsenic-treated rats. (Figure 7).

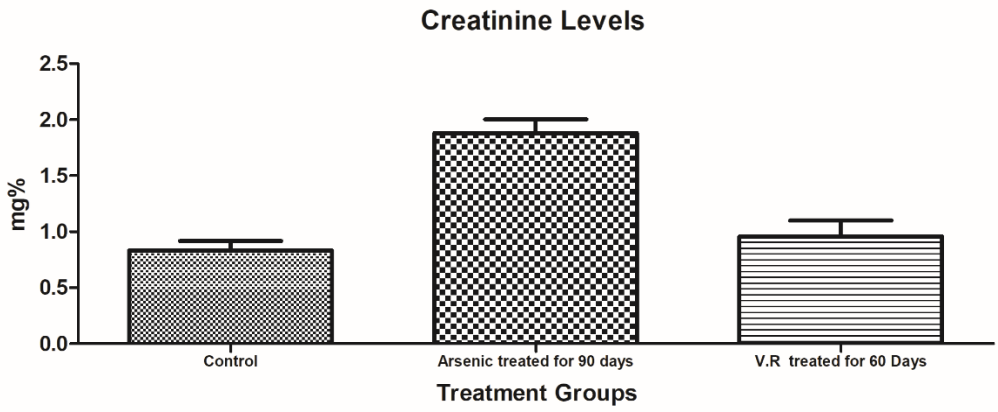

Figure 7. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

9) **Lipid Peroxidation (LPO) Assay:** When comparing the levels of LPO in the arsenic-treated rats to those in the control rats, there was a significant increase ($p<0.05$). Treatment with *Vinca rosea* leaf extract substantially ($p<0.05$) restored normal LPO levels in arsenic-treated rats. (Figure 8).

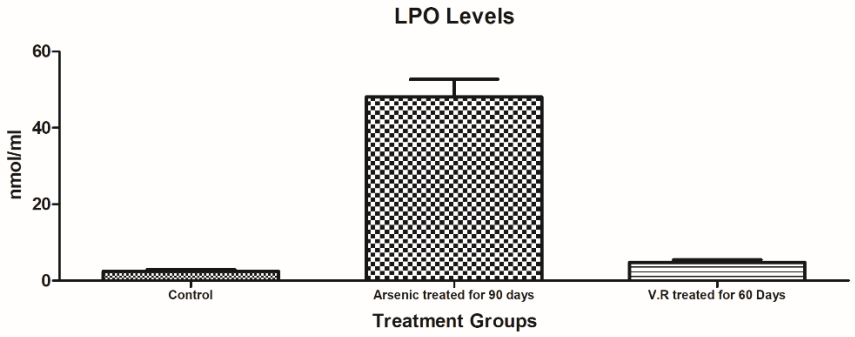

Figure 8. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

Histopathological Study:

In the present study, the microphotograph section of liver shows normal architecture of liver with hepatocytes well arranged in the sinusoids, denotes the normal functioning of the liver cells (Figure 9A). The arsenic treated liver section shows high degree of generation in the liver cells as the central vein endothelial cells appears to be ruptured at many places. Increase in the number of Kupffer cells indicate the increased macrophagic activity in the liver cells. Moreover, haemorrhages in the sinusoids are also clearly visible in the liver tissue. This denotes the malfunctioning of the liver cells (Figure 9B). However, following 60 days of *Vinca rosea* leaf extract treatment, there was significant restoration in the hepatocytes, central vein, and sinusoids can be seen. Hepatocytes are appropriately arranged in the sinusoids and functions appears in normal condition. Furthermore, the absence of Kupffer cells indicates that the liver is working normally (Figure 9C). The glomerulus, Bowman's capsule, convoluted tubules, and distal tubules are all normal in the kidney histological sections (Figure 9D). The arsenic-treated kidney section exhibits a deformed glomerulus and Bowman's capsule with haemorrhage in them. Furthermore, extensive bleeding in the renal tissue may be seen, indicating an aberrant filtration mechanism in the kidney caused by arsenic poisoning (Figure 9E). However, following *Vinca rosea* leaf extract treatment, there was considerable improvement in the nephrocytes, particularly in the glomerulus, Bowman's capsule, and convoluted tubules, indicating normal nephrocytic activity (Figure 9F).

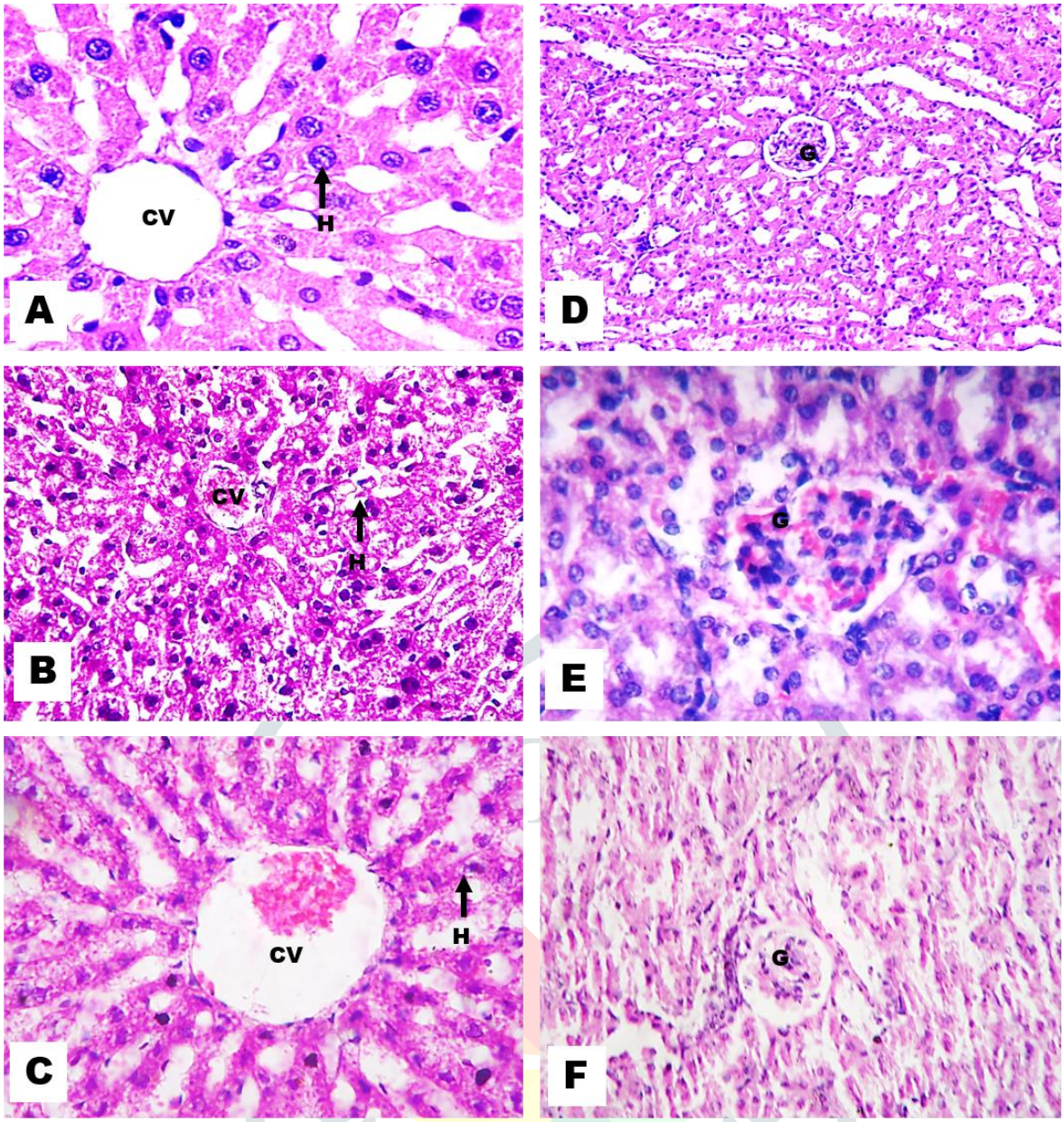

Figure 9: Microphotographs of rat liver sections stained with haematoxylin and eosin (H&E 500). **[A]** Hepatocyte (H), central vein (CV), and sinusoidal structure are all visible and functioning properly in a healthy control rat liver. Hepatocytes are well organized in sinusoids. **[B]** The hepatocytes (H) and central vein (CV) of arsenic-treated rat liver sections are shown in Pyknotic nuclei in hepatocytes may also be detected under a microscope. The extent of tissue inflammation may be evaluated by the abundance of Kupffer cells (pin-shaped cells). The sinusoidal spaces are also hemorrhaging. **[C]** Sections of rat liver treated with leaf extract of *Vinca rosea* show significant normalization of hepatocytes (H) with central vein (CV). Sections of rat kidney stained with haematoxylin and eosin (H&E 500) show **[D]** Sections of normal rat kidney displaying Bowman's capsules and a normal glomerulus (G). The endothelial cells lining the tubules of a normal kidney are also an integral part of the structure. **[E]** Kidney tissue from arsenic-treated rats has extensive hemorrhaging and degeneration in the glomerulus (G) and Bowman's capsule. The CT, or convoluted tubules, have been severely damaged as well. **[F]** The nephrocytes in the leaf extract of *Vinca rosea* extract-treated rat kidney segment shows significant recovery, with the glomerulus (G), Bowman's capsule (B), and convoluted tubules (CT) all recovering to normal.

Quantified histological damage scoring in liver tissue of control and treated rats (n=6, values are expressed as Mean ± S.D). Hepatocytes degeneration was assessed in each rat by counting the degeneration among 100 hepatic cells; rest histopathological changes were examined randomly in selected 20 microscopic fields (X40; H&E) (Table 2.).

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

Group	Control	90 Days arsenic treated	60 Days *V.rosea* treated
Degenerated hepatocytes	0.42 ± 0.54	53.88 ± 2.67	16.15 ± 2.99
Vacuolization	0.51 ± 0.32	22.84 ± 3.93	6.05 ± 3.83
Haemorrhage	0.12 ± 0.23	18.18 ± 2.19	4.04 ± 1.92
Central vein degeneration	0.76 ± 0.11	33.96 ± 5.88	2.39 ± 0.54
Portal vein degeneration	0.24 ± 0.03	20.33 ± 4.86	4.05 ± 2.87

Quantified histological damage scoring in kidney tissue of control and treated rats (n=6, values are expressed as Mean ± S.D). Tubular degeneration was assessed in each rat by counting the degeneration among 100 tubules; rest histopathological changes were examined randomly in selected 20 microscopic fields (X40; H&E) (Table 3.).

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

Group	Control	90 Days arsenic treated	60 Days *V.rosea* treated
Tubular degeneration	0.23 ± 0.38	78.34± 8.85	14.3 ± 4.98
Glomerulus degeneration	0.83 ± 2.67	23.93 ± 3.64	3.93 ± 1.03
Haemorrhage	0.34 ± 0.73	19.92 ± 1.05	2.99 ± 0.83
BC membrane degeneration	0.29 ± 0.19	29.46 ± 2.67	3.04 ± 4.22
Vacuolization	0.18 ± 0.21	29.17 ± 4.05	4.93 ± 2.83

Discussion:

Groundwater arsenic pollution poses significant health risks to those who consume it. Arsenic binding to the sulfhydryl group and producing methylation of it increases its probability of arsenic causing metabolic dysfunction since the toxicity induced by As^{+3} is greater than that caused by As^{+5}. The exposed populace becomes ill as a result (Yamanaka, 2004).

Significant changes in the parameters examined were seen in the arsenic-exposed rat model. Significant reductions were seen in white blood cell count, platelet count, and RBC indices factors as haemoglobin %, Hct percentage, and MCHC in the haematological investigation. This indicates that the defensive system collapsed, in the form of myelosuppression, owing to dangerous levels of arsenic. The levels of SGPT, SGOT, ALP, bilirubin, urea, uric acid, and creatinine all increased up noticeably in the biochemical parameters. Changes in oxidative activity in the cells are also indicated by an increase in lipid peroxidation levels. After being exposed

to arsenic for a long time, the animal's body begins to break down, eventually leading to death. Similar deterioration was seen in the hepatocytes, central vein, and sinusoids of arsenic-exposed animals compared to the control group, correlating with the findings of the histological criteria. The failure of the liver's defensive system is shown by an increase in Kupffer cells. Nephrocytes, particularly those in the glomerulus, Bowman's capsule, convoluted, and ductal tubules, were significantly damaged in kidney tissue when compared to the control.

Arsenic typically enters the body via the digestive tract, makes its way to the circulatory system, and stays there for the shortest possible time owing to its very short half-life (Lu et al., 2007). Arsenic is mostly eliminated in the bile and urine. Cellular energy is lost due to arsenic's As+3 form inhibiting pyruvate dehydrogenase (Helleday et al., 2000). Apoptosis inducing factors (AIF) are secreted by cells when the body's defensive mechanisms fail, leading to cell damage and death. Carcinogenesis in important organs including the liver, kidney, bladder, etc. occurs when the apoptotic mechanism fails to activate at the cellular level (Mallikarjuna et al., 2003; Yamanaka et al., 2004). Methylated arsenic, like DMA+5, has harmful effects and promotes tumor growth (Yamamoto et. al., 1995; Wanibuchi et. al., 1996; Cohen et al., 2006 & Cohen 2014; Kumar et al., 2023[a,b]). Arsenic accumulates in the body over time, especially in the liver and the kidneys (Soni, et al., 1993). Arsenic inhibits glucocorticoid receptor (GR) mediated transcription by direct interaction with GR. Disruption of the spindle apparatus (Miller et al., 2002) leads to chromosomal damage and mutation (Huang et al., 2004; Duker et al., 2005), including aneuploidy, micronucleus formation, DNA-protein cross linking, and sister chromatid exchange. Arsenic has been linked to severe chronic kidney disease in recent years, with research suggesting that it decreased glomerular filtration rate and increased urine albumin secretion (Chen et al., 2011; Hsueh et al., 2009; Zheng et al., 2013 & 2014; Jha et al., 2013). The nephrocytes are damaged by the DMAV that is exported from the liver to the kidney (Styblo et al., 2002; Carter et al., 2003; Yousefsani et al., 2018; Zhang et al., 2014). Significant biochemical and cellular alterations in liver and kidney tissues were detected in the current investigation, supporting the aforementioned hypotheses. Arsenic was also shown to be significantly flushed out of the body through the kidneys and liver, as was the case in the current investigation.

In addition, the antitoxic and antidote action of leaf extract of *Vinca rosea* was significantly ameliorated. The result is consistent with the findings of related research on various hazardous models. The leaf extract of *Vinca rosea* was used as an antidote against arsenic toxicity in the current study, and it was found to significantly restore all of the parameters examined, including haematological, biochemical, and lipid peroxidation levels. The cellular level was also significantly restored, with both hepatocyte and nephrocytic functions returning to normal. The four primary vinca alkaloids - Vinodil, Vincristine, Vinorelbine, and Vindesine of *Vinca rosea* play the vital role in controlling the toxicity caused due to arsenic poisoning (Moudi et al., 2013; Kumar et al., 2022[e]; Li et al., 2022; El-Beltagi et al., 2023; Chen et al., 2021: Kumar et al., 2022[d], Kumar et al., 2020[b]; Kumar 2015[a,b]).

Conclusion

Arsenic induces significant deterioration in rats across several levels of analysis, including the haematological,

biochemical, and histological. However, considerable cellular repair occurred when leaf extract of Vinca rosea was administered. Enzyme functioning in the liver and kidneys returned to normal, and there was a noticeable return to normalcy at the cellular level, indicating typical physiological activity in the tissues. This suggests that leaf extract of *Vinca rosea* has a protective effect on the liver and kidneys against the toxicity caused by arsenic. Therefore, it may be used as a potential new and non-hazardous anti-arsenic medication.

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.

Author contributions

M.K., R.K., and A.K. conceived of the whole experimental effort. M.K., the manuscript's primary author, did the bulk of the writing, with assistance from R.K. and A.K. M.K. conducted the literature review. M.K. and A.K. designed the graphical sections. M.K. conducted the experiments and analyzed the results. M.K. and A.K. designed the figures. M.K. analyzed the data and drew the necessary conclusions. M.K., A.K. and R.K. wrote the final draft of the text. Each contributor has reviewed the final version of the paper.

Funding

No funding was received for this work.

Conflict of interest

The authors declare that they have no conflicts of interest.

Consent for publish

All the authors provide their consent to publish this article.

Availability of data and materials

All the data on request is available.

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