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Phytoremedial Effect Of *Ocimum sanctum* Against Arsenic Induced Toxicity In Charles Foster Rats

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Abstract

Globally, 300 million people worldwide are exposed to groundwater arsenic poisoning in the recent time, while an estimated 10 million population are exposed to arsenic poisoning in Bihar (India). The arsenic contamination in ground water has caused serious health hazards in the exposed population. The typical symptoms of arsenicosis are observed in the exposed population mostly skin manifestations such as hyperkeratosis, melanosis, loss of appetite, neuro-behavioral disorders etc. Hence, the present study aims to develop novel drug discovery against arsenic induced toxicity in rat models.

In the present study treatment groups received sodium arsenite orally at the dose of 8 mg/kg body weight daily for 90 days followed by administration of *Ocimum sanctum (*Tulsi) seed extract at the dose of 500 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 seed extract of *Ocimum sanctum,* 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 phytoremedial effect of this novel plant (*Ocimum sanctum)* denotes that it possesses antidote effect against arsenic induced toxicity.

Keywords: Arsenic toxicity, biochemical assay, liver and kidney toxicity, Ocimum sanctum seed extract, phytoremedial effects, Charles Foster rats.

Introduction

In the recent times, the pollution levels have gradually increased many folds globally and the groundwater pollution has become a major problem in the recent times. Arsenic poisoning in groundwater has become a major problem in causing the health hazards in the exposed population globally. It is estimated that about 300 million population are exposed to arsenic poisoning and about 70 million people in India, while 10 million people in the Gangetic plains of Bihar. These, exposed population have now various types of health-related

problems especially the skin manifestations, neural disorders, hormonal disorders, gastrointestinal disorders, cardiovascular disorders etc. Moreover, the cancer incidences have also increased many folds in this exposed area, where it has become major matter of concern. The mitigation related to the problem is only restricted to the water. The concept of arsenic free water has although lowered down the magnitude of the toxicity. But still the crops are cultivated from the same arsenic contaminated water. This has posed biomagnification and transformation of arsenic from environment to the human body system (Shaji et al., 2021; Hassan, 2018; Kumar et al., 2022[a]; Richards 2022, 2021, 2020).

In state of Bihar, arsenic poisoning has caused serious health hazards in the exposed population. The arsenic exposed population due to drinking of arsenic contaminated water for very long time has posed serious health hazards to them. The exposed population are exhibiting typical symptoms of arsenicosis such as skin manifestations, gastrointestinal disease, lung disease, cardiovascular disease, hormonal imbalance, loss of appetite, low immunity, bowel changes, neuro-behavioral changes and disease of cancer poisoning (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).

Hence, there is need of bio-remedial approach to cater this complex problem. *Ocimum sanctum* is the Indian holy basil, which has been used as drug to cure various diseases in Indian Medicine System called as *Ayurveda*. The holy basil has been used for the treatment of diseases such as lung, low immunity, hepatoprotective, anti-inflammatory, anti-microbial and anti-cancer. Hundreds of scientific investigations, including in vitro, animal, and human trials, have been conducted on tulsi in order to investigate its potential therapeutic uses. According to the findings of these studies, tulsi has a remarkable array of health benefits, including the following: antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and anthelmintic), mosquito repellent, anti-diarrheal, anti-oxidant, anti-cataract, anti-inflammatory, chemopreventive, radioprotective, hepato-protective, neuro-protective, cardio- cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic, analgesic, anti-pyretic, anti-allergic, immunomodulatory, central nervous system depressant, memory enhancement, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritic, adaptogenic, anti-stress, anti-cataract, anti-leukodermal and anti-coagulant activities (Kumar & Patel., 2023; Baliga et al., 2016; Cohen 2014; Singh et al., 2007; Uma Devi 2001).

Therefore, the present study aims to find out the phytoremedial activity of the seeds of *Ocimum sanctum* 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: Healthy male Charles Foster rats were used for this study with an average body weight of 150-180g. The animals were properly caged in groups and were provided proper food and water *ad libidum.*

Chemical: Arsenic in the form of Sodium Arsenite was used for the study. The chemical was procured from the Merck – Sigma Aldrich. After the titration of the dose, the dose was prepared to 8 mg/Kg body weight for the treatment to the animals.

Antidote Plant: For the administration of antidote against arsenic induced toxicity in rats, *Ocimum sanctum* seeds were collected from the local garden of Patna, Bihar, India and was identified by a Botanist, A.N. College, Patna, Bihar. The collected seeds were properly washed with water and finally rinsed with distilled water and then dried in the oven for 48 hours. After that seeds were grinded to fine powder and were soaked in alcohol for 48 hours. The powdered seeds were run on the Vacuum evaporator to get the final ethanolic extract. After the titration, the reference dose was calculated to 500 mg/Kg body weight.

Experimental design: Animals were divided into 03 groups - Group-I – Control group (n=6), Group-II– Arsenic treated group (n=12), Group-III – *Ocimum sanctum* seed 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 *Ocimum sanctum* seed extract was administered at the dose of 500 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: The collected blood samples were assayed for haematological parameters – RBC counts, WBC counts, Platelets counts and haemoglobin percentage using the standard protocols.

Biochemical assays: From the collected blood samples, serum was isolated by centrifuging it for 15 minutes at 3000rpm. The obtained serum was 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 & Grof, 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 Taskuy, 1945). The lipid peroxidation study was carried out through the method of (Draper and Hadley, 1992).

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 Esosin Y and through various grades of alcohol microscopic slides were obtained. The stained slides were then viewed under microscope for histopathological evaluation (Cardiff et al., 2014).

Statistical analysis: For the statistical analysis, one way ANOVA were used using the statistical software GraphPad 5.0. Apart from this for p- value all the variables were assayed for Dunnett's test.

Results

Haematological study: The haematological study, showed significant decrease ($p<0.005$) in the RBC counts, WBC counts, platelets counts and haemoglobin percentage in arsenic treated rats in comparison to the control group rats, while there was significant normalization ($p<0.005$) in the levels of RBC counts, WBC counts, platelets counts and haemoglobin percentage in *Ocimum sanctum* seed extract treated rats.

Group	Control	90 Days arsenic treated	60 Days *O.sanctum* treated
RBC ($\times10^6$/mm^3)	5.82 ± 1.76	2.34 ± 3.21	4.22 ± 3.42
HGB (g/dL)	14.14± 2.86	6.34 ± 3.29	11.37 ± 1.68
HCT (%)	42.34 ± 3.29	19.59 ± 4.17	34.31 ± 2.73
MCV (fL)	72.7 ± 6.24	83.7 ± 3.78	81.3 ± 7.45
MCH (pg)	24.3 ± 4.23	27.1 ± 4.58	26.9 ± 6.21
MCHC (g/dL)	33.4 ± 2.38	32.24 ± 2.51	33.1 ± 3.74
WBC ($\times10^3$/mm^3)	8.44 ± 3.81	14.22 ± 8.95	9.94 ± 3.32
Platelets ($\times10^3$/mm^3)	420 ± 16.2	77 ± 34.2	176 ± 10.19

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))*

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 *Ocimum sanctum* seed extract treatment. (Figure 1).

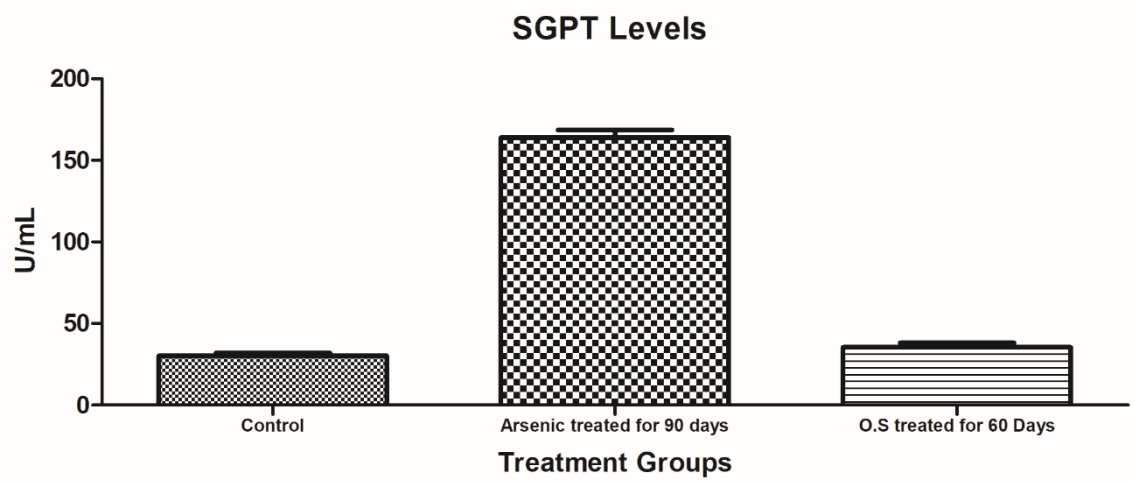

Figure 1. SGPT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 2).

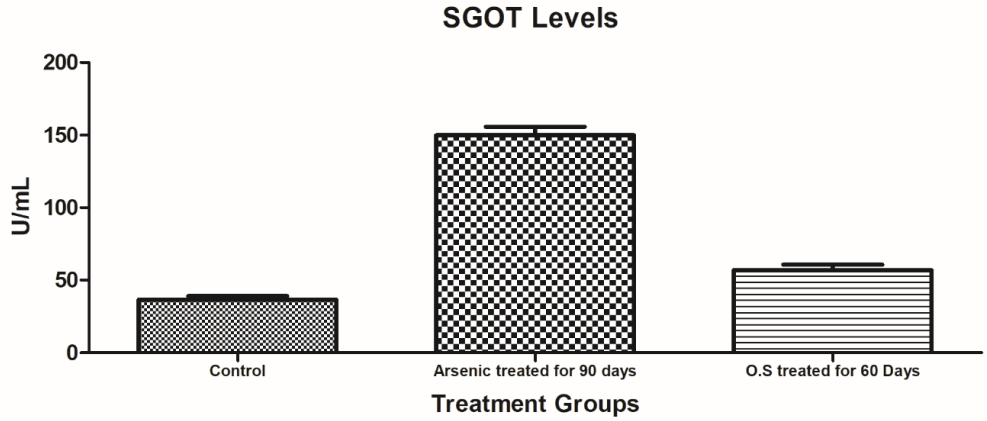

Figure 2. SGOT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 3).

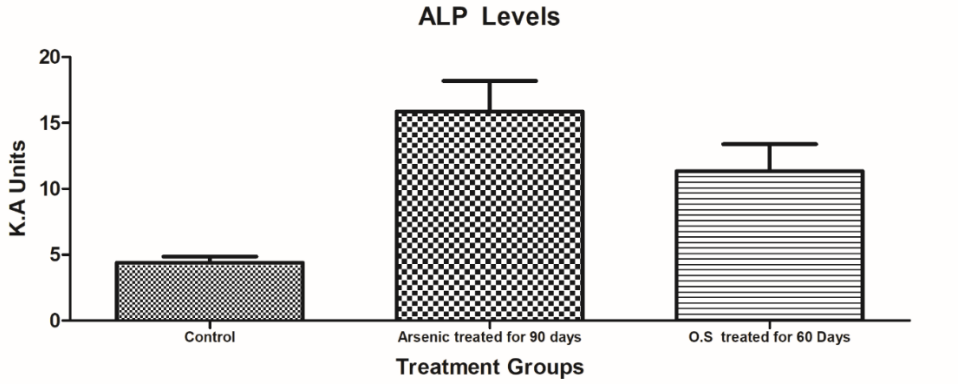

Figure 3. Alkaline phosphatase Levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 4).

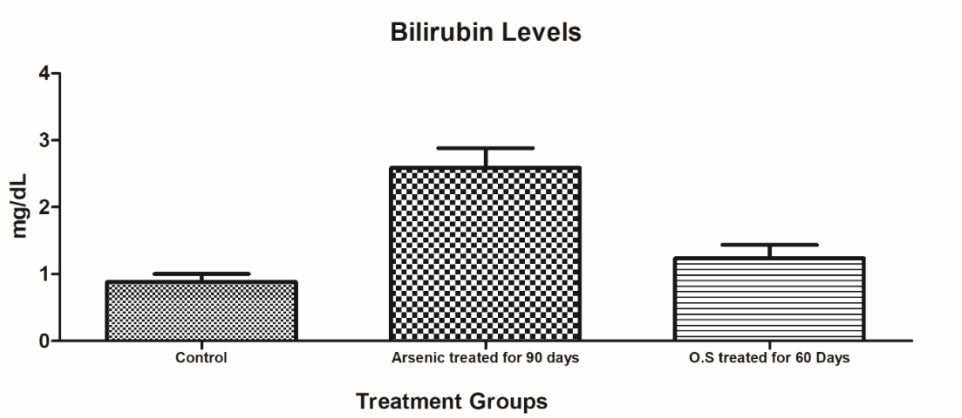

Figure 4. Bilirubin Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 5).

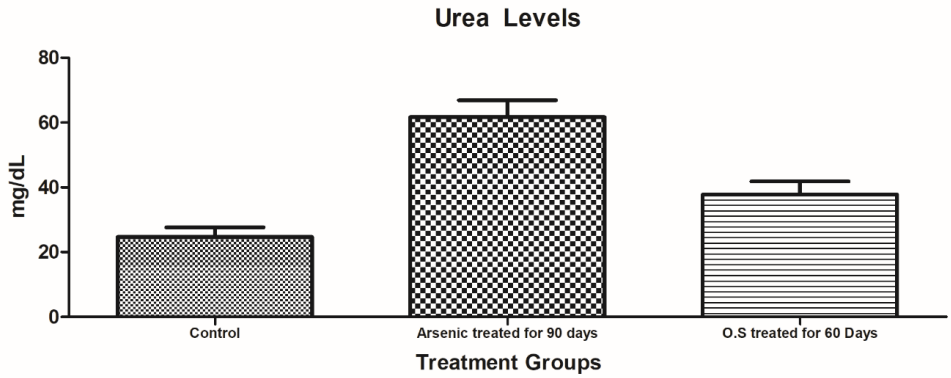

Figure 5. Urea Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 6).

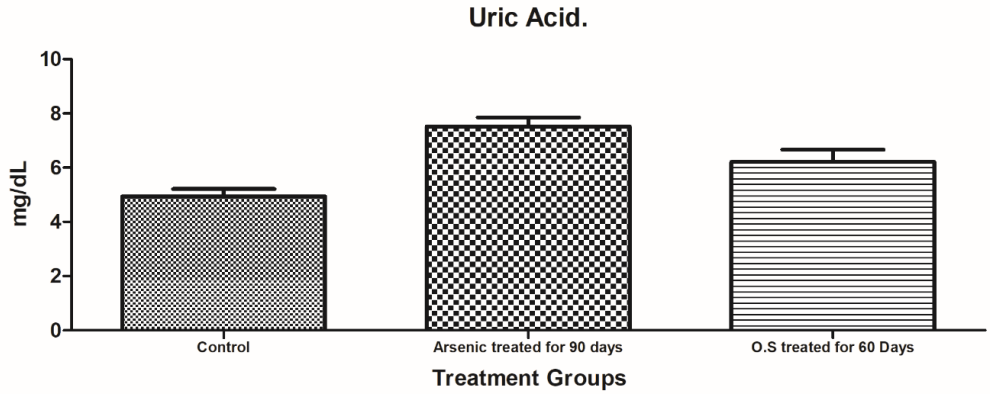

Figure 6. Uric acid Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 7).

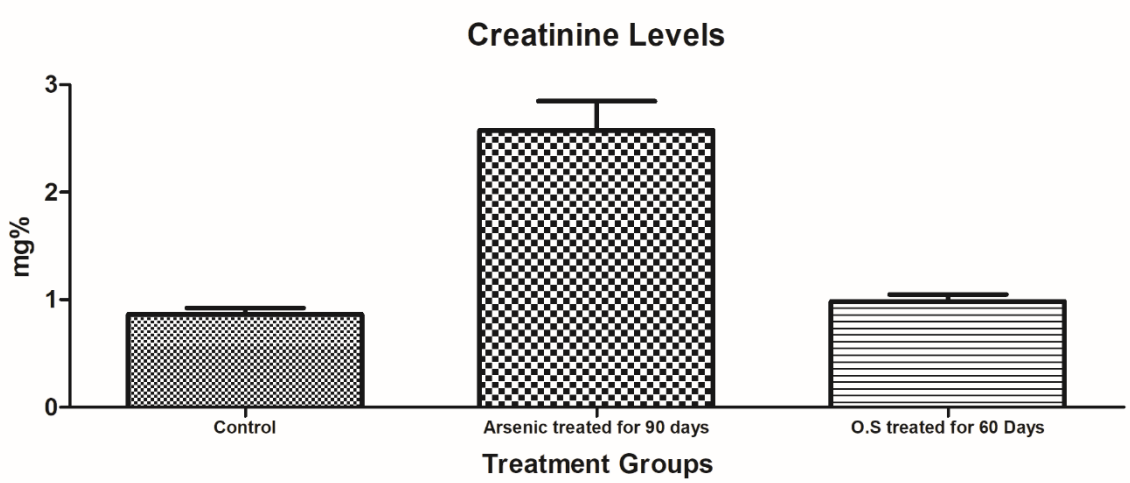

Figure 7. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

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 *Ocimum sanctum seed* extract treatment (Figure 8).

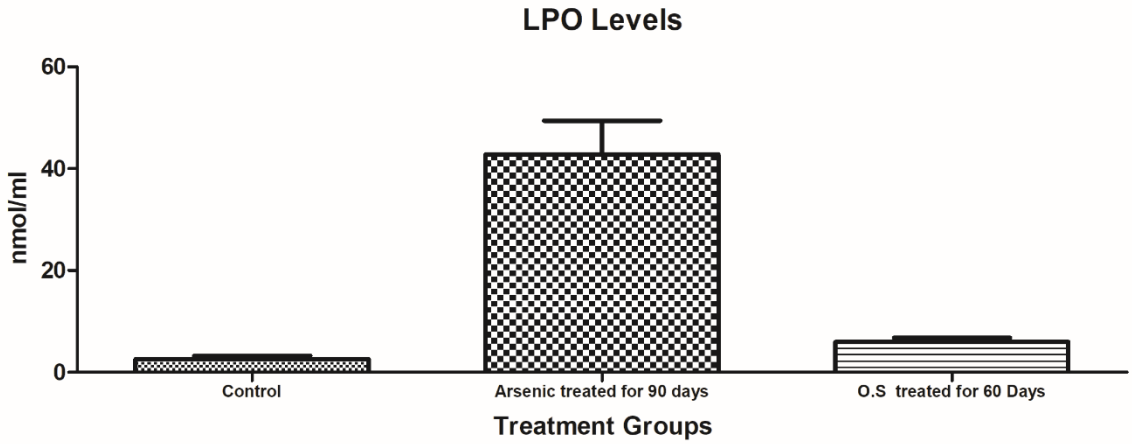

Figure 8. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

Histological investigation:

Significant alterations in the analyzed groups were identified in the histological investigation - the liver histopathological sections indicate normal architecture of hepatocytes with central vein. The presence of hepatocytes in the sinusoids indicates that the liver cells are functioning normally (Figure 9A). The hepatocytes with pyknotic nuclei in the arsenic-treated rat liver segment exhibit a significant degree of deterioration. The increased number of Kupffer cells indicates increased macrophagic activity. Furthermore, the endothelial cells of the central vein membrane have ruptured extensively, resulting in haemorrhaging in the sinusoidal spaces. The section also shows vacuolations in the sinusoidal spaces (Figure 9B). However, following 60 days of *Ocimum sanctum* seed extract treatment, there was considerable repair seen in the hepatocytes, central vein, and sinusoids. Hepatocytes are appropriately arranged in the sinusoids and functions normally. 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.

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 *Ocimum sanctum* seed 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).

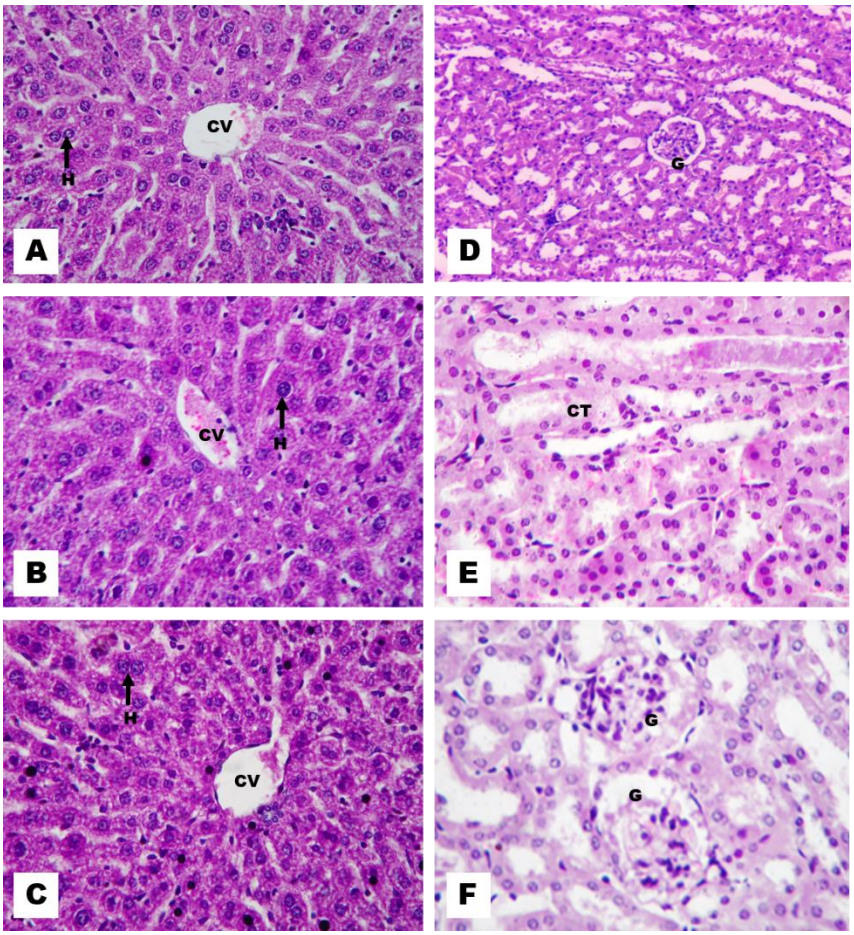

Figure 9: Microphotographs of rat liver sections stained with haematoxylin and eosin (H&E 500). **[A]** Control rat liver with normal hepatocyte (H), central vein (CV), and sinusoids architecture. In sinusoids, hepatocytes are highly structured **[B]** Arsenic-treated rat liver sections with deteriorated hepatocytes (H) and a central vein (CV) are exhibited. Hepatocyte pyknotic nuclei are also clearly seen. The increased number of Kupffer cells (pin-shaped) in the tissue indicates the degree of inflammation. There is also hemorrhage in the sinusoidal spaces. **[C]** The rat liver sections demonstrate considerable normalization in the hepatocytes (H) with central vein (CV) after *Ocimum sanctum* seed extract treatment. Sections of rat kidney stained with haematoxylin and eosin (H&E 500) show **[D]** Control rat kidney sections with normal glomerulus architecture (G) with Bowman's capsules. Normal architecture also includes the endothelial cells of convoluted tubules. **[E]** Arsenic-treated rat kidney sections show severe deterioration in the glomerulus (G) and Bowman's capsule, as well as hemorrhaging throughout the kidney tissue. The convoluted tubules (CT) have also suffered significant damage. **[F]** The *Ocimum sanctum* seed extract treated rat kidney segment demonstrates remarkable improvement in the nephrocytes, particularly the glomerulus (G), Bowman's capsule, and convoluted tubules (CT), indicating normal kidney tissue function.

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

Group	Control	90 Days arsenic treated	60 Days *O.sanctum* treated
Degenerated hepatocytes	0.84 ± 0.54	68.34 ± 3.89	25.14 ± 2.56
Vacuolization	0.64 ± 0.45	17.31 ± 4.38	5.88 ± 2.76
Haemorrhage	0.45 ± 0.67	13.09 ± 5.23	6.38± 3.46
Central vein degeneration	0.98 ± 0.90	16.95 ± 4.28	9.39 ± 2.35
Portal vein degeneration	0.56 ± 0.23	14.37 ± 3.76	7.67 ± 1.45

Quantified histological damage scoring inliver 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).

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

Group	Control	90 Days arsenic treated	60 Days *O.sanctum* treated
Tubular degeneration	0.58 ± 0.38	44.32 ± 5.36	29.19 ± 4.94
Glomerulus degeneration	0.98 ± 1.93	16.34 ± 4.27	12.37 ± 3.56
Haemorrhage	0.72 ± 0.98	10.42 ± 3.68	3.40 ± 3.89
BC membrane degeneration	0.55 ± 0.59	19.23 ± 3.94	6.99 ±1.04
Vacuolization	0.21 ± 0.38	18.20 ± 5.42	10.38 ± 4.23

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

When arsenic is ingested, it passes via the gastrointestinal tract to the blood and other key organs of the body, which, in turn, indicates the influence that arsenic has on the organ system and the overall body of the animal. In the existing investigation, there was a significant decrease in the haematological parameters such as RBC counts, WBC counts, and haemoglobin percentage in the arsenic-treated rats as compared to the control group of rats. This was the case in all of the haematological measurements. This indicates that the toxicity of the arsenic has caused injury to the hematopoietic stem cells of the rats, which has led to significant changes in the haematological parameters of the rats being detected. However, when *Ocimum sanctum* seed extract was administered to the rats that had previously been pre-treated with arsenic, there was a considerable restoration to their baseline levels in the RBC counts, the WBC counts, and the haemoglobin percentage, which indicates an improvement at the haematological level. The biochemical parameters are the second level of indicators, and they are responsible for reflecting the damage that has occurred at the biochemical level at the tissue level. Tests of liver and kidney function are the most important enzyme indicators in the body because they give the most relevant information on toxicity in the body. The fact that there was a substantial rise in the levels of SGPT, SGOT, alkaline phosphatase, bilirubin, urea, uric acid, and creatinine in this research ($p<0.005$) indicates that arsenic intoxication causes severe harm to these essential organs. However, there was a considerable restoration in these liver function tests and kidney function tests levels after administration of *Ocimum sanctum* seed extract to rats that had previously been pre-treated with arsenic. The histology of examinations provides the observational component of the third level of toxicity evaluation. In the current research, the histological examination of liver and kidney tissues revealed extensive damage in both organs' tissues. In the liver tissue sections that had been treated with arsenic, considerable damage was found in the hepatocytes, as well as in the central vein, the portal vein, and the sinusoidal spaces. The presence of a greater number of Kupffer cells in the liver is indicative of the presence of significant inflammation brought on by the toxic effects of arsenic. In addition to this, there had been haemorrhaging in the sinusoidal spaces. The glomerulus, Bowman's capsules, convoluted tubules, and ductal tubules were all damaged while this poison was present in the tissue of the kidney. The deterioration that was brought on by the toxicity produced by arsenic led to haemorrhaging in the kidney tissue, which in turn made the process of glomerular filtration more difficult. However, there was a very large restoration detected in the liver and renal tissues of the rats that had previously been arsenic pre-treated following the injection of *Ocimum sanctum* seed extract to the rats. In compared to the rat liver that had been treated with arsenic, the hepatocytes, the central vein, and the sinusoidal gaps have all been greatly repaired. In a similar fashion, there was a large amount of restoration in the nephrocytes seen in kidney tissue, particularly in the glomerulus, Bowman's capsule, convoluted tubules, and distal tubules. This suggests that *Ocimum sanctum* seed extract has antidote qualities, which allow it to mitigate the damage produced by the arsenic-induced toxicity. The whole restoration in the organ level that was detected is due to the rejuvenating capabilities and antioxidant characteristics that the *Ocimum sanctum* seed extract has. These attributes have helped counteract the damage that had been caused by the arsenic-induced toxicity (Yadav et al., 2020; Kumar & Patel 2023; Hasan et al., 2023; Jayapal et al., 2021; Parajuli-Baral 2023; Prakash & Gupta 2005; Yousefsani et al., 2018; Duan et al., 2016; Kumar et al., 2022[d], Kumar et al., 2020[b]).

At the level of the cell, the oxidant-antioxidant system is responsible for the maintenance of the defensive mechanism. The fact that the arsenic-treated group of rats in this research had much higher levels of lipid peroxidation than the control group of rats indicates that this particular group's defense mechanism was unable

to function properly. The comparison was made between the two groups of rats. However, following treatment with *Ocimum sanctum* seed extract, there was a considerable restoration in the levels of lipid peroxidation. This indicates that *Ocimum sanctum* has antioxidant qualities (Chaudhary et al., 2020; Muralikrishnan et al., 2012; Pasangulapati et al., 2020; Ramesh & Satakopan 2010; Kath & Gupta 2006).
The seed extract of *Ocimum sanctum* contains active components such as polyphenols and eugenol, 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. (Utispan et al., 2023 & 2020; Khatoon et al., 2022; Pattanayak et al., 2010; Bhattacharyya et al., 2013; Baliga et al., 2013; Kim et al., 2010; Ighodaro & Ebuehi 2009).

Conclusion

The results of this research show that *Ocimum sanctum* seed extract may protect rats from arsenic-induced toxicity attributed to its antioxidant characteristics. *Ocimum sanctum* seed extract antioxidant and anti-protective properties make it an effective antidote for the poisoning caused by arsenic.

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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.

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Availability of data and materials
None of the data has been fabricated or manipulated (including image) to support this investigational study. Data supports the findings.

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