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Bio-Remedial Impact of *Elaeocarpus sphaericus* Seed Extract (ESSE) Against Sodium Arsenite (As)-Induced Nephrotoxicity in Charles Foster Rats

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Abstract:

The presence of arsenic (As) metalloid in groundwater poses serious threat to human and animal's health. Approx. 300 million people of about 105 countries in the world are affected due to arsenite poisoning. Except mitigation there is no such mode by which the population can be prevented from being exposed to arsenic. *Elaeocarpus sphaericus* (*E. sphaericus*) is widely used in the folk medicine system for the treatment of various diseases. Hence, present study aimed to investigate the Bio-remedial Impact of ESSE against As-Induced Nephrotoxicity in Charles foster rats. Male twenty-four rats (weighing 160 ± 20 g) were randomly assigned into two groups, where Group-I (n=6) rats were used as control. Group-II (n=18) rats were treated with sodium arsenite at 8 mg/Kg body weight for 90 days daily and then further divided into three sub-groups. Sub-Group I (n=6); rats were sacrificed and data were collected, Sub-Group II (n=6); rats were left for 60 days for auto recovery (as As-pre-treated group), and Sub-Group II (n=6); rats were administrated with *E. sphaericus* at 20mg/kg body weight for 60 days. After the completion of entire experimental dose all the control and treatment group were sacrificed to evaluate the various parameters. As-Induced rats had Significant ($p<0.0001$) alteration in haematological parameters. As-Induced serum levels of urea, uric acid, creatinine and albumin had significant ($p<0.0001$) alteration. Level of MDA and BUN were significantly ($p<0001$) increased. However, ESSE administration significantly reduced the adverse effect related to test of nephrological functions, MDA level significantly ($p<0.0001$) reduced. Dose dependent ESSE administration combat As-Induced toxicity and significantly ($P<0.0001$) normalise the level of haematological parameters. Hence, the study concluded that *E. sphaericus* seed might be used as a nutritional supplement to combat the arsenic led toxicity among the exposed population.

***Keywords**: Arsenic, E. sphaericus, Nephrotoxicity, and Bio-remediation.*

Introduction

Arsenic metalloid known as environmental toxins that has two most common forms As^{III} (arsenite) and As^{V} (arsenate); globally affect humans and animals **(Richards, et al., 2020; Mawia, et al., 2021)**. These metalloid naturally present in the earth crust and widely distributed in water, soil, food, and air. It combined with oxygen, chlorine, and sulphur to form inorganic arsenic compounds. The presence of arsenic in animals and plants combines with carbon and hydrogen to form organic arsenic compounds. Inorganic form of arsenic compounds are mainly used as pesticides, wood preservatives, and paint pigment **(Borah, et al., 2020)**. Moreover, its organic forms; used in antibiotics for the treatment of spirochetal and protozoal disease. The Arsenobetine and Arsenocholine are the organic form of arsenic known as "fish arsenic" which is relatively nontoxic to humans. The toxins of arsenic metalloid enters the body via consumption of contaminated drinking water. Prolonged consumption of arsenic contaminated drinking water results in the manifestations of toxicity in practically all systems of the body **(Cao, et al., 2019; Hull, et al., 2021; Kumar, et al., 2021)**.

Scientifically, the kidney is a known target organ for arsenic and is critical for both arsenic bio-transformation and elimination. Epidemiologic research and animal experiments have proven that acute and chronic arsenic exposure can cause injury to the kidney and raise the risk of renal cancer **(Palma-Lara, et al., 2020)**. Epithelial cells located on the surface of proximal convoluted tubules have been found to be more sensitive to arsenic-induced toxicity due to their high re-absorptive activity and anatomical positions as the first renal tubular epithelial cells to be exposed to filtered toxicants **(Rana, et al., 2018)**. Various research combined ultra-structural/biochemical analysis in the kidney of rats exposed to arsenate revealed swelling of mitochondria accompanied by decreased respiratory function. Moreover, it has been observed that arsenic increases the number of lysosomes **(Santra, et al., 2022)**.

Exposure of chronic arsenic leads to irreparable damage in several vital organs. It may also result in persistent methyl exhaustion leading to DNA hypo-methylation which may after the gene expression making the cells susceptible to carcinogenesis. Arsenic metalloid has high binding affinity with Sulfhydryl (SH) group of proteins, which leads to inhibition of cellular respiration, degeneration of glycolysis and oxidative process leading to cell death **(Kumar, et al., 2020; Shankar, et al., 2023)**. Many researchers have reported that, during arsenic biotransformation, cell generates many reactive oxygen species (ROS) and nitrogen species which are then subsequently converts into more damaging species as hydroxyl radicals (OH), thereby leads to oxidative damage of cellular biomolecules, lipid peroxidation, DNA damage and the activation of cascades of signalling pathways associated with tumorigenesis. Altogether, arsenic toxicity and its impact causes severe damages to the metabolic activities of the body system. Over the past few years, use of naturally occurring antioxidants dietary substance for prevention of various toxicants and environmental agents including metals/metalloids is gaining interest **(Tam, et al., 2020; Ghosh, et al., 2023)**.

E. sphaericus (***Syn**: E. ganitrus*) belongs to the family of Elaeocarpaceae owes an important status in Ayurveda as well as Hindu mythology for its cultivate and spiritual benefits **(Joshi, et al., 2020)**. The physical wearing of rudraksha has been cited for its pharmacological actions against vast range of medical ailments including anxiety, lack of concentration, insomnia, depression, hypertension, palpitation, infertility, cardio disorders, hepato-renal disorders, asthma, and rheumatism **(Aryal, et al., 2021)**. It has variety of antioxidants as alkaloids, glycoside, Phytosterols, carbohydrate, tannin, flavonoid, amino acid, saponins, and terpenoids **(Prasannan, et al., 2020)**. It's proven that *E. sphaericus* (Rudraksha) works on electromagnetism and controls bioelectric energy of human body, which in turn is responsible for mind-body coordination and its induced health benefits. The Elaeocarpus alkaloids have an affinity with the delta-opioid receptors (DOR). Many extract of Elaeocarpus are rich in phenolic and flavonoid compounds that are potent antioxidants and inhibitors of several health related enzymes **(Rai, et al., 2019)**. Therefore, present study was aimed to investigate the bio-remedial impact of ESSE against As-Induced nephrotoxicity in Charles foster rats.

Materials and methods

Chemical

Arsenic element was used as Sodium (meta) arsenite (90%) manufactured by Sigma Aldrich, USA (CAS number: 7784-46-5: S7400 100G), Lot number # SLBH5736V, P Code – 1001683292 was purchased from approved scientific store of Patna, Bihar, India.

***Elaeocarpus sphaericus* seed identification and ethanolic extract preparation**
The seed of the medicinal plant purchased from Patna Herbal store, Patna (Bihar), India. These medicinal plant were identified and certified by the Department of Botany, Jai Prakash University, Chapra Bihar (India). The identified *E. sphaericus* seed were washed, cleaned and dried in incubator at 37ºC temperature. Then after the seed was grinded to thin powder. The powder of the seed was macerated and soaked in absolute ethanol for 48 h. The ethanolic extract was separated using rota vapour apparatus.

Animals
Healthy male rats (n=24) weighing 160 ± 20g of 2 to 2.5 months old were obtained from MCSRC (Mahavir Cancer Sansthan and Research Centre), Phulwari Sharif, Patna (Bihar), India. The obtained rats were comfortably accommodated in clean polypropylene case which has the stainless-steel grill top facelifts. In animal house laboratory; temperature was regulated at 24 ± 2°C for 12 h light and dark cycles. Fed-nourishment (nurtured by laboratory itself) and water were provided through ad libitum procedure.

Grouping of animals
Healthy male rats were randomly distributed into control and treated groups.
Group I: Control (n=6).
Group II: As-Treated; (n=18). These group of rats were orally induced with sodium arsenite at the dose of 8mg/Kg body weight/day for 90 days. The dose of sodium arsenite were calculated at LD_{50}. After completion of 90 days As-treatment rats were further divided into three sub-group (n=6; each).
Sub-group I: (As-Treated); rats were sacrificed to evaluate status of arsenic toxicity.
Sub-group II: (As-Pre-Treated); rats were left without any treatment up to next 60 days for auto-recovery.
Sub-group III: (*E. sphaericus* administrated group against sodium arsenite); As-Treated rats were orally administrated with ESSE at the dose of 20mg/Kg body weight/day for further next 60 days. The administrated dose of *E. sphaericus* was also calculated at LD_{50} estimation.

Sample Collection and Prevention
After the completion of entire treatment all groups and sub-groups rats were anaesthetized through xylazine or Ketamine and blood sample were collected and positioned on ice. For the determination of biochemical assay in the serum the blood sample were centrifuged at 4° C under 3000 g for 15 minutes and serum was obtained which was stored at -20° C for further analysis. The tissue of the kidney were also dissected out for the histopathological studies.

Body weight Analysis
The analytical balance was used for weight analysis of each rat's group. Initial and final weight was recorded to recognise body weight variations. Examined rat's body weight was determined at mean ± SD in g/kg.

Haematological assay
Whole blood samples were collected in EDTA coated vacutainers and were analysed for haematological parameters. By using fully automated haematological analyser RBCs (red blood cells), WBCs, (White blood cells), PLT (platelets), HGB (haemoglobin), HCT (haematocrit), MCH (mean corpuscular haemoglobin), and MCV (mean corpuscular volume) were performed.

Biochemical assay
To the detection of nephro markers blood serum were used. The level of urea was measured by the **Fawcett and Scott (1960)** technique, uric acid levels was measured by the **Fossati and Prencipe (1980)** technique, levels of serum creatinine was measured by **Bones and Tausky (1945)** technique, and the level of albumin were performed through **Doumas and Watson (1971)** technique. To the detection of Blood Urea Nitrogen (BUN); **Hosten (1990)** technique were used.

Malondialdehyde (MDA) assay
To evaluate the level of lipid peroxidation **Draper and Hadley (1990)** technique were used. Ensuing this technique TBARS marker (Thiobarbituric acid reactive substance) and double heating process were performed. These technique based on the principle of spectrophotometric measurement of colour reproduced in reaction with thiobarbituric acid (TBA) and malondialdehyde (MDA). For this experiment 2.5ml of 100 g/L TCA (trichloroacetic acid) were mixed with 0.5ml serum in a centrifuge tube and heated in the water bath at 90ºC

for 15min. After cooling at RT (room temperature) the mixture was further allowed to centrifuge at 3000 rpm for 10min. and supernatant was separated out. Two mille litre of separated supernatant was mixed with 1ml of 6.7g/L TBA solution in test tube which were further heated in water bath at 90ºC for 15min. and left for cooling at RT. In last, absorbance was measured by UV visible spectrophotometer (UV 10, Thermo scientific USA) at 532 nm and the final values were drawn in nmol/ml after calculation.

Histopathological assay

The collected renal tissue were sectioned in two pieces and fixed into 10% of formalin for 24 h. Thereafter, tissues were dehydrated through graded ethanol concentration and embedded into paraffin. Tissue section were grosses at 4 -5µm thickness by using digital rotary microtome (Microtome HM 340E, Thermo Scientific, USA) and stained with haematoxylin and eosin (H&E). All microscopic slides were examined for the assessment of histopathological changes.

Statical analysis

The result were presented through mean ± standard deviation (SD); where data were analysed through one-way analysis of variance (ANOVA) and Turkey's multiple range test with multiple compressions. The value of $P < 0.05$ were considered as statically significant. All calculations were performed with the Graph Pad Prism program (Graph Pad software, Inc., San Diego, U.S.A.).

Results

Effects on Body weight

Compare to control the body weight of As-treated rat's group significantly had ($p<0.0001$) reduction. Mild weight gain were observed in As-pre-treated group in comparison with As-treated group. ESSE administration upon As-treated group shown its good effect where significant ($P<0.0001$) weight gain was observed in comparison with As-Pre-Treated group **[Table-1].**

Parameter	Control	As-Treated	As-Pre-Treated	As-Treated + ESSE administration
Body weight examination	330.0 ± 5.164	188.0 ± 7.367	243.5 ± 4.724	344.2 ± 3.607

Table-1: Body weight variations in each group of rats (n=6, values are expressed as mean ± SD).

Haematological Analysis

Parameters	Control	As-Treated	As-Pre-Treated	As-Treated + ESSE administration
RBCs ($X10^6/mm^3$)	7.65 ± 0.92	3.01 ± 0.77	5.70 ± 1.13	7.28 ± 0.43
WBCs ($X10^3/mm^3$)	7.25 ± 1.16	16.18 ± 1.32	14.42 ± 1.50	8.32 ± 0.92
HGB (g/dL)	13.15 ± 1.36	4.14 ± 0.77	4.58 ± 1.37	12.14 ± 0.63
PLT ($X10^3/mm^3$)	658.4 ± 122.6	405.2 ± 103.3	611.3 ± 153.4	648.4 ± 122.3
HCT (%)	38.10 ± 3.42	20.34 ± 4.12	24.22 ± 4.02	41.38 ± 2.35
MCV (fL)	52. 03 ± 4.23	74.23 ± 18.33	61.12 ± 12.09	47.55 ± 4.52
MCH (pg)	17.74 ± 2.14	28.41 ± 5.34	24.38. ± 2.52	16.48 ± 1.54

Table-2: Haematological parameters in various group of rats (n=6, values are expressed as mean ± SD).

Compare to control haematological findings in arsenic treated rats showed significant ($P<0.0001$) changes in HGB, RBCs, WBCs, HCT, MCH ($p<0.05$) and non-significant changes were observed in PLT and MCV. As-Pre-Treated group had non-significant changes in HGB, RBCs, WBCs, PLT, HCT, MCV, and MCH in comparison with As-Treated group. However, ESSE administration upon As-treated group shown greatly significant ($p<0.0001$) restoration in HGB, RBCs, WBCs, HCT and non-significant changes in MCV, MCH and PLT as compared to the As-Pre-Treated auto recovered group **[Table-2]**.

Biochemical KFT (Kidney Functional Test) Analysis

Compare to control group the As-Treated rats showed significant ($p<0.0001$) changes in serum level of urea, uric acid, creatinine, and albumin. However, compare to As-Treated group vs. As-Pre-Treated group which were left for auto recovery had significant ($p<0.0001$) restoration in the level of serum creatinine and uric acid.

Non-significant changes were observed in the level of urea and albumin. Compare to As-Pre-Treated group vs. ESSE administration upon As-Treated group had significant ($p<0.0001$) restoration in the serum level of urea, creatinine, and albumin, while non-significant changes were observed in uric acid **[Figure- 1 (a), (b), (c), and (d)]**.

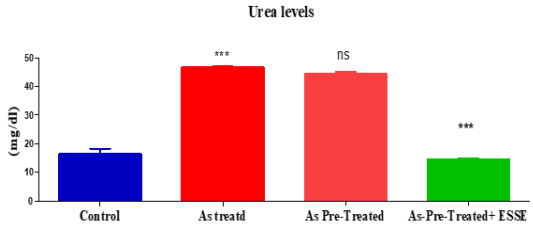

Figure-1 (a): Comparative level of Urea in various group of rats (n=6); value expressed as mean ± SD.

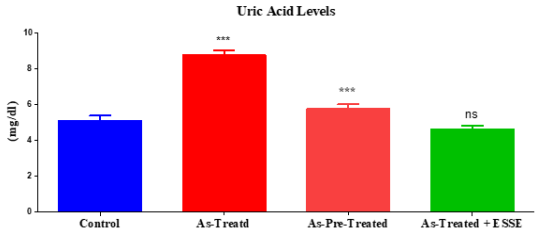

Figure-1 (b): Comparative level of Uric acid in various group of rats (n=6); value expressed as mean ± SD.

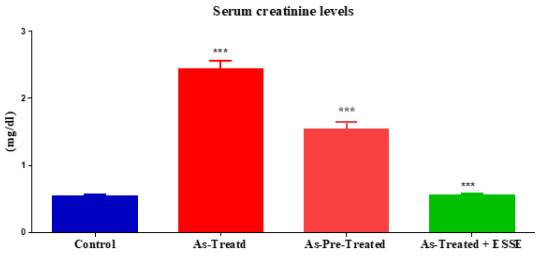

Figure-1 (c): Comparative level of Creatinine in various group of rats (n=6); value expressed as mean ± SD.

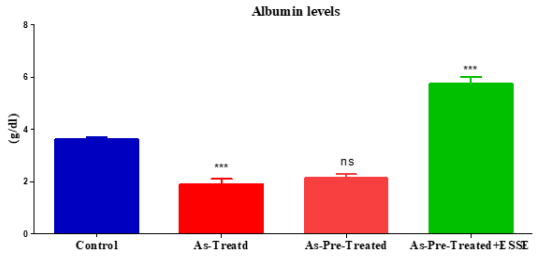

Figure-1 (d): Comparative level of Albumin in various group of rats (n=6); value expressed as mean ± SD.

Effect on Blood Urea Nitrogen (BUN) and Malondialdehyde (MDA) level

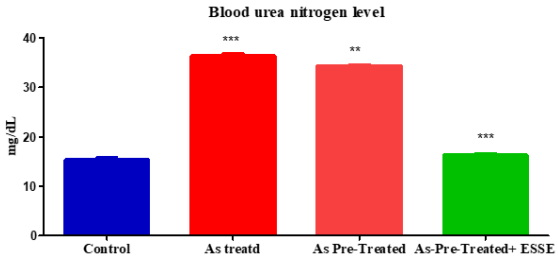

Figure-2 (a): Comparative level of BUN in various group of rats (n=6); value expressed as mean ± SD.

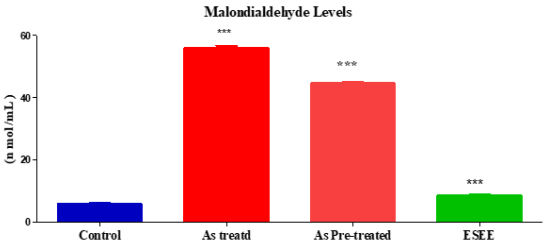

Figure-2 (b): Comparative level of MDA in various group of rats (n=6); value expressed as mean ± SD.

The serum tested Malondialdehyde (MDA) level and Blood urea nitrogen (BUN) and had significant ($p<0.0001$) elevation in the arsenic treated rats in comparison to the control group. In the As-Pre-Treated group which were left for auto recovery significantly had mild ($p<0.0001$) reduction as compared to As-Treated group. However, compare to as-Pre-Treated group vs. ESSE administration upon As-Treated group had significant ($p<0.0001$) reduction in both Malondialdehyde (MDA) level and Blood urea nitrogen (BUN) level **[Figure-2 (a), and (b)]**.

Histopathological Analysis

The renal histopathological section reveals that the control group had normal architecture of Bowman's capsule, glomerulus along with the distal convoluted tubules and proximal convoluted tubules, which denotes normal function of kidney **[Figure-3 (A)]**. The As-Treated group showed enormous degeneration in tubules with glomerulus. Huge amount of damages were prominently observed in the brush border of Bowman's capsule along with the vacuolization as well as degeneration in the distal and proximal convoluted tubules **[Figure-3 (B)]**. The As-Pre-treated group also showed significant degeneration in glomerulus and Bowman's capsule along with the vacuolization in renal convoluted tubules denotes mild recovery in nephrocytes **[Figure-3 (C)]**. However, the *E. sphaericus* (ESSE) administration upon As-Treated group had significant restoration

in the nephrocytes as the architecture of glomerulus with Bowman's capsule appears to be normal along with the distal convoluted tubules and proximal convoluted tubules **[Figure-3 (D)]**.

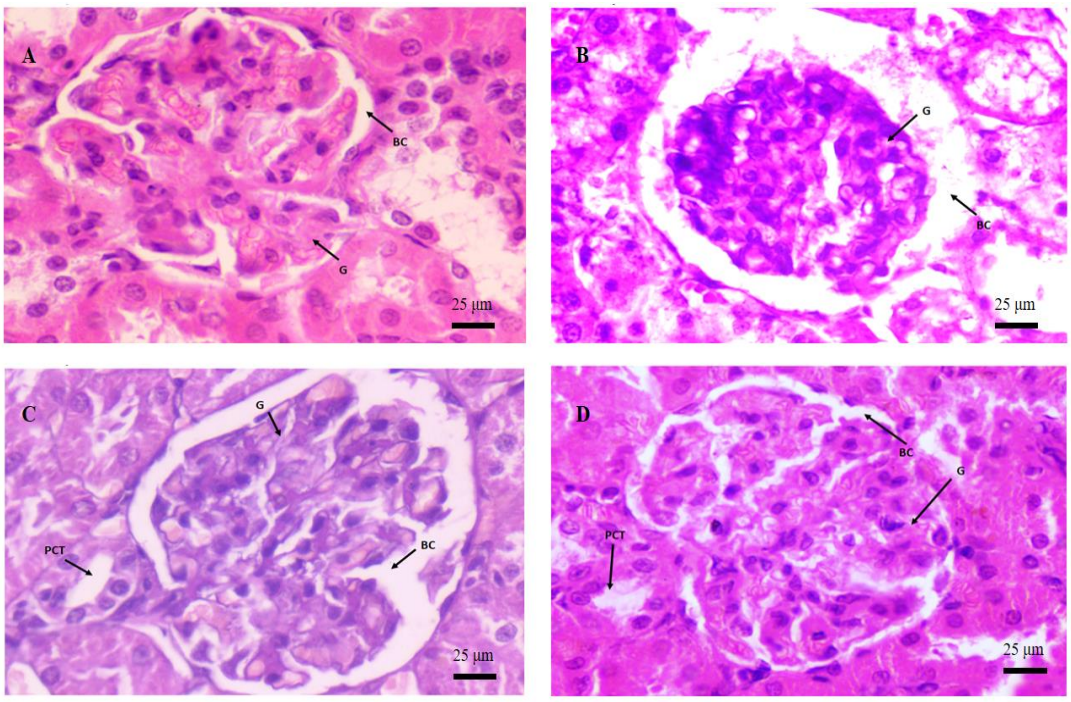

Figure-3 - Microphotograph of rat kidney stained with haematoxylin and eosin (H & E). (A) Section of control rat kidney showing normal architecture of nephrocytes with glomerulus in Bowman's capsule (H&E × 800). B) In arsenic treated rat kidney shows high magnitude of degeneration in the Bowman's capsule along with the glomerulus (H&E × 800). (C) In the arsenic pre-treated control rats shows persistence of degeneration in the glomerulus in in the Bowman's capsule as there is mild haemorrhage in the nephrocytes (H&E × 800). (D) In *E. sphaericus* administered group there was significant restoration in the nephrocytes as the architecture of glomerulus with Bowman's capsule appears to be in normal.

Discussion

The arsenic poisoning is a well environmental toxin associated with severe health hazards including risk of cancer in humans **(Obasi, et al., 2020)**. It has been demonstrated that arsenic compounds have toxin effect in almost all targeted organs including kidney. Chronic arsenic exposure are attributed to its ability to induce formation of ROS **(Juan, et al., 2021)**. Various natural substances including chelating agents have been investigated for their protective potential in arsenic-induced toxicity **(Najafi, et al., 2023)**. However, most of them have not yet been proved safe for clinical application. To address this multifaceted issue, bio-remedial strategy is required. *E.sphaericus* (Rudraksha) seed has a long history of medicinal use in Ayurvedic medicine and are also known to be potential heavy metal chelators including antioxidant agent **(Primiani, et al., 2022)**. Accordance with the finding of present research, arsenic concentration led to renal dysfunction, as there was significant ($p<0.0001$) elevation in serum level of urea, uric acid, creatinine, and albumin in As-treated group. As-Pre-Treated group had mild restoration in comparison with As-Treated group, which is in consistent with previous findings **(Kumar, et al., 2020)**. These altered KFT parameters is an indicator of kidney damage. Urea, uric acid and creatinine are the primary waste products of protein metabolism that are eliminated through kidney. Acute inflammation and the development of tubular necrosis was suggested by high serum creatinine level, which was also associated with significant changes in cellular proliferation events and a decrease in glomerular filtration rate (GFR). The reduced level of albumin in As-Treated group could be the outcome of severe nephrotoxic effect of arsenic resulting in the excretion of protein through urine. The study also observed the elevated level of BUN in As-Treated group. BUN analysis is primarily used to evaluate kidney function in a wide range of circumstances, to monitor people with acute or chronic renal dysfunction. The elevated level of BUN directly interlinked with the degeneration of renal tissue, which is observed in this study. These series of test reports support our research. In previous research it was declared that, methylated arsenic reach kidney which is filtered in the glomerulus and later reabsorbed in the proximal tubules caused oxidative stress and

necrosis in proximal tubular epithelial cell. These severe changes result in expansion and back leakage of the filtration leading to decreased excretion and increased retention of urea **(Dutta, et al., 2018)**.

The histopathological examination revealed that there were significant changes in renal architecture in arsenic intoxicated rats, including degeneration in the distal and proximal convoluted tubules followed by glomerular degeneration and thickening of space in bowman's capsules. Our findings support the previous studies reported by several researchers **(Ahmed, et al., 2019)**. In the study, histological alteration in renal tissue may be caused by excessive deposition of arsenic metalloids and its metabolites in the renal tissues, which is sensitive to nephrological damage **(Garla, et al., 2021)**. The present study assessed the nephroprotective activity of *E.sphaericus* on arsenic induced toxicity. The administration of *E.sphaericus* seed extract (ESSE) upon arsenic intoxicated rats significant ($p<0.0001$) amelioration were observed in the level of urea, uric acid, creatinine, albumin, and blood urea nitrogen. The membrane stabilizing properties of *E.sphaericus* was helpful significantly to restore the histopathological alterations caused by arsenic induced nephrotoxicity in rats. The outputs of this research are in line with previous findings. *E.sphaericus* shows strong antioxidant cascade mechanism that is particularly due to the occurrence of indolizidine type of alkaloids in addition with variety of minerals, flavonoids, vitamins, tannins, steroids, and glycosides, which play an important role by providing protection against nephrotoxicity **(Dixit, et al., 2018)**.

The output of haematological assay showed significant changes in RBCs, WBCs, HGB, HCT, MCH, and non-significant changes in PLT, and MCV in As-Treated rats vs. control group of rats. The inorganic form of arsenic considered to be a protoplasmic compound, which has strong binding affinity with hemoglobin containing sulfhydal (SH) group on protein. By this means, it getting accumulation in blood and leading haematological changes such as anaemia, and leucopenia **(Kumar, et al., 2020; Tuteja, et al., 2021)**.. Thereafter, ESSE treatment caused significant ($p<0.0001$) restoration in the above mentioned haematological alterations, thus reducing the haemato toxicity in arsenic intoxicated rats. ESSE administration possibly acts by stimulating liver and spleen to remove defective and damaged RBCs from peripheral blood circulation and also stimulating haemopoiesis in the bone marrow for the production of PLT, PRBs, and WBCs **(Kumawat, et al., 2022)**. Present study also epitomise that, As-Treated group had significant elevation in the serum level of MDA, which is the most important sign of oxidative (lipid peroxidation) damage occurring in the cell membrane. The As-Pre-Treated group which were left for 60 days auto recovery had mild reduction in comparison to As-Treated group. Study reveals that under moderate or high lipid peroxidation rates (toxic conditions) the extent of oxidative damage increases the repair capacity, and cells induce apoptosis or necrosis programmed cell death **(Ahangarpour, et al., 2018; Shankar, et al., 2023)**. The ESSE administrated group ameliorate the oxidative stress as it significantly reduce the serum level of MDA in arsenic exposed rats. The reduction of MDA levels indicates that the antioxidant ingredients of *E.sphaericus* seed extract can improve the reactive oxygen species (ROS) activity additionally maintain the cellular redox, which usually gets imbalanced by arsenic toxicity **(Deepika, et al., 2018; Brindha, et al., 2022)**.

Conclusion

Therefore, the present research suggest that supplementing with seed extract reduces oxidative stress and restore serum biomarkers levels, thereby protecting kidney against arsenic-induced nephrotoxicity.

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