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Therapeutic Protection of Arsenic-Induced Oxidative Stress and Hepato-Nephro Toxicity by *Syzygium cumini* (Seed) Ethanolic Extract (SCEE) in Charles Foster Rats

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Abstract

Arsenic (As) is an environmental toxin distributed in groundwater which is presently a global concern. The present study aimed to investigate the efficacy of SCEE in the mitigation of arsenic-induced hepato-renal dysfunction and oxidative stress in rats. Thirty male Charles foster rats (140-160 g) were randomly assigned into three groups. Group I (n = 6) - Control, Group II (n = 6) - SCEE control was administrated with 600 mg kg^{-1} body weight daily for 60 days and the last Group III (n = 24) was As-treated at 8 mg kg^{-1} body weight daily for 90 days. Subsequently, Group III was further divided into three subgroups. The subgroup I (n = 6) was immediately sacrificed to observe the arsenic-induced toxicity. Subgroup II (n = 6) was kept on a normal diet for 60 days for auto recovery. Subgroup III (n = 6) was administrated orally by SCEE at 600 mg kg^{-1} body weight daily for 60 days to decipher the therapeutic potential against arsenic-induced toxicity. The experimental exposure reveals various changes in biochemical parameters of the liver function test and kidney function test in addition to histopathological studies. Chronic exposure to arsenic significantly ($p < 0.0001$) increased the levels of lipid peroxidation along with significantly reducing the activity of glutathione, superoxide dismutase and catalase. Significant ($p < 0.0001$) arsenic accumulation was observed in the hepatic-nephron tissues. The dose-dependent SCEE administration against As-toxicity had progressive survival benefits on antioxidant levels, enzymatic activities and histopathological changes. Thus, the study concludes that *S.cumini* seed has a protective effect against As-induced oxidative stress and hepato-renal intoxication.

Keywords: Hepato-Nephro Protective Activity, Oxidative Stress, *S. cumini*, Sodium Arsenite

List of abbreviations: **As**: Arsenic, **LD**: Lethal Dose, **mg**: Mille Gram, **g**: Gram, **Kg-1**: per Kilogram, **B.W.**: Body Weight, S.cumini: *Syzygium cumini*, **SCEE**: *Syzygium cumini* Ethanolic Extract, **LFT**: Liver Functional Test, **KFT**: Kidney Functional Test, **SGPT**: Serum Glutamate Pyruvate Transaminase, **SGOT**: Serum Glutamate Oxaloacetate Transaminase, **ALP**: Alkaline Phosphate, **ROS**: Reactive Oxygen Species, **LPO**: Lipid Peroxidation, **SOD**: Superoxide Dismutase, **CAT**: Catalase, **GSH**: Glutathione, **GF-AAS**: Graphite Furnish Atomic Absorption Spectrophotometer, **NIOSH**: National Institute for Occupational Safety and Health.

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1. Introduction

Arsenic (As) is a ubiquitous element that exists naturally in the earth's crust affecting humans and animals globally[1]. According to the International Agency for Research on Cancer, it is the 20th most prevalent element and a Category-I carcinogen[2,3]. It exists in both organic and inorganic forms but its inorganic form is highly toxic than organic[4,5]. These metalloids get into the ecosystem by way of natural activities such as the mineralization of the earth's crust and weathering[6]. Anthropogenic activity is another source of arsenic poisoning which harms the ecosystem by the consumption of arsenic-based pesticides, insecticides, and fertiliser[2,7,8].

An estimated 300 million people globally are exposed to groundwater arsenic poisoning[1,9]. Residents of the southern Asian nations of Afghanistan, Bangladesh, India, Nepal, Pakistan, and Sri Lanka suffer from the most severe types of arsenic poisoning[10]. In Asia, the severity of arsenic poisoning mostly affects the areas near the Brahmaputra and the upper, middle, and lower Gangetic plains[1,10,11]. States in India along the Gangetic basin like Bihar, Jharkhand, West Bengal, Uttar Pradesh and Uttarakhand are severely affected by groundwater arsenic poisoning[8,10,12].

Groundwater arsenic poisoning in Bihar is severely affected in 22 districts[8,11,13]. Naturally, both drinking water and groundwater contain significant amounts of inorganic arsenic[10,12,13]. Most of the arsenic-prone Asian countries including the World Health Organisation (WHO) have set the acceptable limit of arsenic in drinking water as 10 µg/L and recommended max of 50 µg/L; if there is unavailability of nearby source[14–16].

The major route of exposure of this metalloid to humans and animals is via ingestion of drinking water and food with a relatively minor route being inhalation and skin absorption[17,18] Long-term exposure to arsenic poisoning caused multiple damages to the body's metabolic processes associated with a plethora of disorders as melanosis, keratosis, raindrop pigmentation, generalised weakness, biochemical-hormonal incontinence, neuro-behavioural activity, low intellect, reproductive issues and cancer[8,11,19–21]. The toxicity of these toxins has harmful effects on vital organs such as the liver, kidney, lungs, bladder, prostate and skin[14,19,22]. The symptoms and severity of arsenic concentration also reflect the risk of nutritional superiority and hereditary influences[15,23].

The mechanism of arsenic toxicity has been reported to generate Reactive Oxygen Species (ROS) initiating oxidative stress and by their differing ability to cause DNA methylation (both hypo-methylation and hyper-methylation via interaction with DNA methylating enzymes or by depleting S-Adenosyl Methionine (SAM)[24]. Its strong binding affinity with the thiol (-SH) group of proteins and enzymes resulting the disruption of their functions[25]. Science revelations state that arsenic toxicity contributes to the cellular production of Reactive Oxygen Species (ROS), biotransformation and nitrogen species that are converted into more vicious species in the form of Hydroxyl Radicals (OH), which leads to oxidative damage of cellular biomolecules, lipid peroxidation, DNA damage and the activation of signalling pathway associated with tumorigenesis[26,27]. Given this, therapeutic herbicides can be used to target arsenic-mediated toxicity. The search for a specific, reliable and safe treatment for arsenic toxicity is still continuous. Various studies on mediational plants have been reported to be very much effective against metal toxicity such as arsenic, lead and cadmium-induced toxicity in animal models[26,28–30].

Syzygium cumini (**Family**: Myrtaceae) also entitled by the name of Malabar plum, Java plum, Jambul, black plum and Indian blackberry is a worldwide medicinal plant traditionally has a long history of traditional medicinal uses[31]. It is aboriginal to Indian Subcontinent, also found nearby Florida and Hawaii in the United States contiguous to Southeast Asian nations[32]. Globally, this plant is utilized for medicinal purposes by having various compounds containing anthocyanin, glucoside, ellagic acid, isoquercitrin, kaempferol and myricetin[31,33]. *Syzygium cumini* plant has an important role with its different parts as barks, leaves, seeds and fruits[34,35].

Pharmacologically, the potential of the *S. cumini* plant is reported to have anti-diabetic, antihyperlipidaemic, antioxidant, antiulcer, hepato-protective, antiallergic, antiarthritic, antimicrobial, anti-inflammatory, antifertility, antipyretic, antiplaque, radioprotective, neuro-psychopharmacological nephroprotective and antidiarrheal activities[34–37]. The health-beneficial effect of *S. cumini* is mainly attributed to various phytoconstituents such as flavonoids, steroids, alkaloids, glycosides, tannins, anthocyanin, terpenoids, fatty acids, ascorbic acid phenols, minerals, carbohydrates and vitamins[36,38,39].

Its remedial components also help to prevent skin cancer and gastrointestinal cancer[39]. By having a wide range of innumerable bioactive compounds and antioxidant activity the plant can be used to control arsenic-induced oxidative stress and hepato-nephro toxicity[40,41]. Therefore, this study was conducted to evaluate the therapeutic protection of As-induced oxidative stress and hepato-nephro toxicity by *Syzygium cumini* (Seed) Ethanolic Extract (SCEE) in Charles Foster Rats.

2. Materials And Methods

2.1 Animals

To perform this experiment; thirty Charles Foster rats, weighing 140 g to 160 g at 8 weeks old, were obtained from MCSRC (Mahavir Cancer Sansthan and Research Centre) Patna, India (under the supervision of an animal committee) under the controlled condition of 24 ± 2 °C (maintained temperature), 50 ± 15 % relative humidity and normal photoperiod (12 hour of light and dark cycle). The water and food were given to the rat *ad libitum* (food was blended and prepared in the lab itself)[42]. The experimental rats were randomly divided into control and treatment groups and kept in small standard polypropylene cages (two in each). Locally purchased sterile paddy husk was used as bedding in the case which was changed every day.

2.2 Ethics Approval

Investigation of this current study was carried out with prior permission from the Institutional Animal Ethics Committee (IAEC); (IAEC No. 2020/1D-27/08/20) by the standards of the Committee for Control and Supervision of Experiments on Animal (CPCSEA), New Delhi. The experimental facilities (Rats, polypropylene case including competence of animal house) and the regular management procedures including 12-hour light and dark cycle were obtained from Mahavir Cancer Sansthan and Research Centre (MCSRC), Patna, India.

2.3 Chemicals

Sodium (*Meta*) *arsenite* ≥ *90* %; CAS Number: 7784-46-5. S7400-100G), Lot# SLBH5736V, P Code 1001683292 manufactured by Sigma-Aldrich, USA; were purchased from a certified and accredited scientific store in Patna (Bihar), India.

2.4 Plant Material

Syzygium cumini (Jamun) dry seeds were purchased from the local market in the Patna district of Bihar, India. The faculty of Jai Prakash University's, Department of Botany, Chapra Bihar (India) identified and certified the Seeds of *S.cumini* which were authenticated and matched with its *Taxonomy ID: 260142, NCBI: txid260142 and IPIN Life Sciences Identifier (LSID) ID: 601603-1.*

2.5 Preparation of Seed Extract[43]

The *S. cumini* seeds were washed, dried at 37 °C in an incubator for four days, and were then crushed into fine powder. Rotatory evaporation was applied in combination with an ethanolic solvent to prepare the seed extract. After the entire process, the liquid extract was cooled and concentrated by vacuum distillation to eliminate its liquid content. Later on, the dried seed powder and 10.8% of the extract were collected and until its next usage, the seed extract was dried and kept at a suitable temperature for storage.

2.6 Phytochemical Screening[44]

The SCEE was subjected to a phytoremedial test using Trease and Evans methods for alkaloids, flavonoids, tannins, steroids, phenols, anthraquinones and minerals.

2.7 Dose Selection

- Sodium arsenite: Oral median Lethal Dose (LD_{50}) of sodium arsenite has been estimated at 8mg kg^{-1} body weight[45]. The dosage was prepared by dissolving 8 mg of Sodium arsenite in 10 ml of distilled water.
- *Syzygium cumini*: The SCEE oral median Lethal Dose (LD_{50}) was calculated and ultimately established at 600 mg kg^{-1} body weight[46]. The dosage was prepared by dissolving 600 mg of SCEE in 10 ml of 5% hydroxyethanol water.

2.8 Experimental Strategy

Rats (n = 30) were casually assigned and distributed into control, *S.cumini* control and treated groups. **The gavage method** was used to feed the dose of arsenic and SCEE (Figure 1).

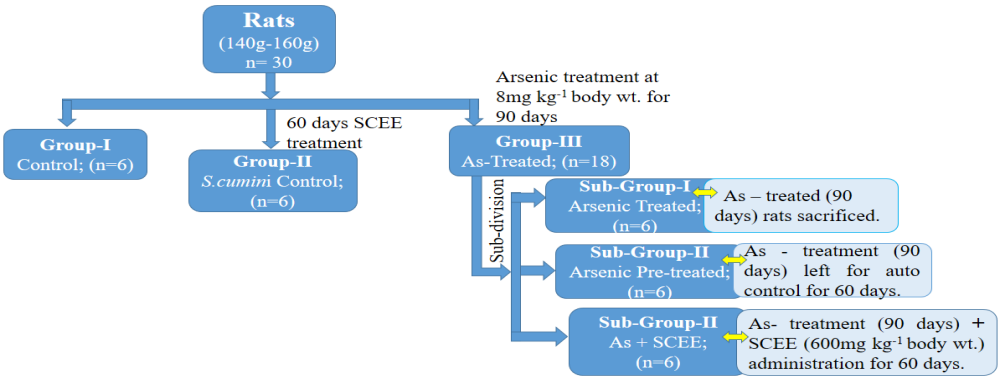


Figure 1: Experimental design.

Group I: Rats (n = 6) were kept as control and received no treatment.

Group II: Rats (n = 6), a model designed as *S.cumini* control; where 600mg kg^{-1} body weight SCEE administration was given on fresh rats for 60 days.

Group III: (n = 18) As-treated rats received treatment of sodium arsenite at 8mg kg^{-1} body weight daily for 90 days.

Then after, Group III (90 days As-treated) rats were subsequently separated into three subgroups (n = 6 each).

Subgroup I: (n = 6; As-treated) – These sub-group rats were sacrificed to evaluate the effect of arsenic toxicity.

Subgroup II: (n = 6; As- pre-treated control) – These sub-group rats were left for 60 days without any treatment to identify the status of their auto recovery.

Subgroup III: (*S.cumini* administered sub-group) – This sub-group (n = 6) rats were orally administrated with *S.cumini* ethanolic seed extract (SCEE) at 600mg kg^{-1} body weight per day for further next 60 days on arsenic pre-treated rats.

After completion of the entire experimentation, rats were lastly sacrificed after proper anaesthetization. Heparinised tubes were used to collect blood samples by cardiac puncture. Moreover, the vital organs such as liver and kidney tissues were dissected for free radical study, and arsenic contamination study through Atomic Absorption Spectrophotometer and were also fixed in 10% formalin for histopathological study.

2.9 Tissue Preparation

Each liver and kidney tissue was homogenised separately in 2ml of phosphate buffer saline 1:2 (w/v; 1g tissue with 2ml PBS, pH7.4) solution. Both homogenates were centrifuged at 10,000 g for 15 minutes at 4 ^{0}C. The supernatant was collected to evaluate the concentration of Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) in both liver and kidney tissues.

2.10 Serum Separation

To separate the serum; blood was centrifuged at 3000g for 15 minutes. The serum supernatant was collected in an eppendorf tube. The collected serum was used to evaluate the concentration of biochemical assay as Liver Functional Test (LFT), Kidney Functional Test (KFT) and the level of Lipid Peroxidation (LPO).

2.11 Biochemical Assay

Functions of both serum level Liver and kidney (LFT and KFT) tests were examined by using the Coral Crest standard kit technique. Lipid Per-Oxidation (LPO) tests were performed by using Draper and Hadley method[47]. All absorbances were taken using a UV spectrophotometer (UV - 10, Thermo Scientific; USA).

2.12 Determination of Liver Function Test (LFT)

Reitman and Frankel's methods were followed to the determination of Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT)[48]. Kind and King technique was used to analyse Alkaline Phosphate (ALP)[49]. The measurement of total bilirubin activity was analysed through Gambino *et al.*, (modified Jendrassik and Grofs method[50]).

2.13 Determination of Kidney Function Test (KFT)

To determine the kidney function test method was followed for Urea - Fawcett and Scott method[51], Uric acid - Fossati and Prencipe method[52], and Creatinine – Bones and Tausky method[53], While Doumas and Watson method[54], were used to determine the albumin levels.

2.14 Determination of Lipid Per Oxidation (LPO)[47]

Draper and Hadley; the "double heating method" was utilized to determine the level of lipid peroxidation[47]. The reactive substance TBARS (thiobarbituric acid reactive compounds) has been used to examine the level of Lipoprotein which is the product of lipid peroxidation. Based on the principle of spectrophotometric colour measurement which is reacted in reaction with Thiobarbituric Acid (TBA) and Malondialdehyde (MDA). The coupled solution of Trichloroacetic Acid (TCA) 2.5ml of 100g/L and 0.5ml serum was heated in a water bath at 90 0^0C for 15 minutes. The mixture was allowed to centrifuge at 3000g for 10 minutes after cooling at Room Temperature (RT) and then the supernatant was separated. The separated 2ml of supernatant and 1ml of 6.7g/L TBA solution was boiled together once again for 15 minutes at 90 °C in a water bath. Before letting the absorbance samples were stored to cool at RT. The measurement of absorbance was taken through UV-Vis spectrophotometer (Thermo Scientific UV-10; USA) at 532nm. Findings were calculated in nmol/ml.

2.15 Determination of Cellular Antioxidant Status

2.15.1 Glutathione (GSH) Levels[55]

The Moron *et al.*,[55] technique was used to analyse the level of GSH. Homogenated tissue samples supernatants were mixed with 2ml of sodium phosphate EDTA and 0.6 mol/L DTNB 5,5 ′ dithiobis – 2 (nitro benzoic acid) were added. The optical density of the yellow substance generated by GSH and DTNB (Ellman's reagent) was evaluated by using UV-Vis spectrophotometer (Thermo scientific UV-10; USA) at 405 nm. The concentration was expressed in µg/g tissue.

2.15.2 Superoxide Dismutase (SOD) Activity[56]

Mishra *et al.*,[56] epinephrine technique was used to quantify SOD activity in the supernatant. This technique relies on measuring the level of epinephrine auto-oxidation inhalation by SOD that exist in the examined sample in 50 mM sodium carbonate buffer pH 10.2, within the linear range of the auto-oxidation curve and U/mg proteins unit, which were used to assess the expression of SOD activity.

2.15.3 Catalase (CAT) activity[57]

To determine the Catalase (CAT) activity Goth *et al.*,[57] technique was used. A technique using a supernatant sample which was incubated in substrate containing 65 µmol/ml H_2O_2 (Hydrogen Peroxide) in 60mmol/L $KNaO_4P$ (sodium potassium phosphate) buffer and pH 7.4 were maintained for the 60s at 37 °C. Under these reactive circumstances, one unit of CAT was decomposed into 1µmol of hydrogen peroxide per minute. Instantaneously 32.4mM $(NH_4)_2MoO_2$ (Ammonium Molybdate) were used to stop the enzymatic reaction. The appearance of yellow complex molybdate and hydrogen peroxide was detected at 405nm in comparison to a blank that contained all of the components except enzymes.

2.15.4 Determination of Arsenic (As) Concentration in Tissues[58]

To estimate the concentration of arsenic 500mg tissue from each sample was placed in a conical flask and added concentrated HNO_3 (Nitric acid) and placed in the conical flask on a Hot plate [Thermotech PID 420 (SSI LASTh)] for digestion as directed by NIOSH protocol[58]. The observance was taken by using GF-AAS (Graphite Furnish Atomic Absorption Spectrophotometer); Model number- Pinnacle 900T (Perkin Elmer, Singapore) at Mahavir Cancer Sansthan and Research Centre (MCSRC), Patna (Bihar), India.

2.16 Histopathological Studies

To investigate the histopathological alterations in both organs (kidney and liver), tissue specimens were obtained from the all-experimental groups of rats that were fixed in the 10% solution of concentrated formaldehyde. Before paraffin sectioning, the sections were cut usually less than 4-5 µm thickness and stained in by the little dip with haematoxylin-eosin stain. Then, DPX fixatives were used

to cover the specimen slide with a cover slip for further studies.

2.17 Statistical Analysis

To examine the outcomes of this study ANOVA (One-Way Analysis of Variance) and TMET (Tukey's Multiple Range Test) were used. Statical calculations were used to perform through Graph Pad Prism (GraphPad Software, Inc., San Diego, U.S.A.). A value of $P < 0.05$ was deemed to be significant where the finding was presented through Mean ± Standard Deviation (SD).

3. Results

3.1 Effect of *S. cumini* on Liver Function Test (LFT)

Arsenic exposure significantly increased ($p < 0.0001$) the LFT parameters (SGPT, SGOT, ALP and total bilirubin) in comparison between the control and treatment groups. Elevated levels of LFT tests indicate and specify that the liver functions were in the normal range. In comparison to the As-treated group vs. As-pre-treated group that were left for auto recovery for 60 days had significant ($p < 0.0001$) auto recovery with mild restoration in serum levels of SGPT, SGOT, total bilirubin and non-significantly decreased the level of ALP. The 60 days administration of SCEE in the As-treated group significantly ($p < 0.0001$) recovered the altered level of hepatotoxicity (hepatic enzymes) in comparison to the As-pre-treated group. Non-significant changes were observed between control vs. *S.cumini* control (Figure 2 (a,b,c and d)).

3.2 Effect of *S. cumini* on Kidney Functional Test (KFT)

Arsenic-induced KFT parameters (urea, uric acid, creatinine and serum albumin) significantly ($p < 0.0001$) had severe variations compared to the control. As-treated

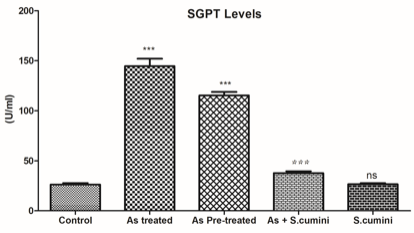


Figure 2 (a). SGPT levels are presented as mean ± SD. Data has been analysed by ANOVA; *** ($p < 0.0001$) significant compared to control vs. As-treated, As-treated vs. As-pre-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparisons in between control Vs. *S.cumini* control.

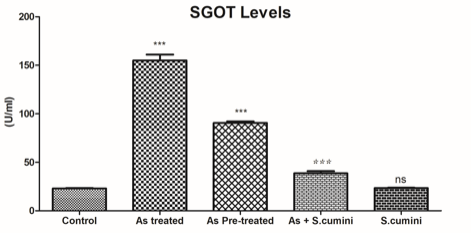


Figure 2 (b). SGOT levels are presented as mean ± SD. Data has been analysed by ANOVA; *** ($p < 0.0001$) significant compared to control vs. As-treated, As-treated vs. As-pre-treated, As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparison in between control vs. *S.cumini* control group.

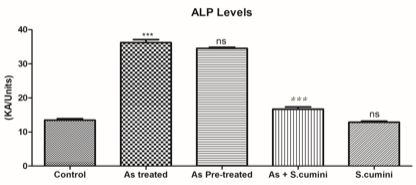


Figure 2 (c). ALP levels are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compared to control vs. As-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparisons in between As-treated vs. As-pre-treated and control vs. *S.cumini* control.

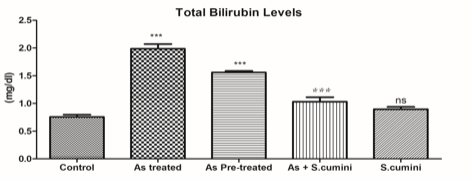


Figure 2 (d). Bilirubin level are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compared to control vs. As-treated, As-treated vs. As-pre-treated, As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparisons in between control vs. *S.cumini* control.

group vs. the As-pre-treated group (permitted to 60 days for auto recovery) significantly ($p < 0.0001$) revealed moderate restoration in the level of creatinine and uric acid in addition to non-significant in the level of urea and albumin. The 60 days SCEE administration upon the As-treated group normalised the KFT's alterations; significantly ($p < 0.0001$) restored the level of serum albumin, urea and creatinine but had a non-significant effect on uric acid in comparison to As-pre-treated group. The observation between *S. cumini* control vs. control was non-significant in serum albumin, urea and creatinine levels except significant ($p < 0.0001$) in uric acid level (Figure 3 (a, b, c and d))

3.3 Effect of *S.cumini* on Lipid Peroxidation (LPO)

The serum level LPO was significantly ($p < 0.0001$) elevated in the As-treated group compare to the control group. The As-pre-treated rats (left for 60 days auto recovery) had mild restoration significantly ($p < 0.0001$) compared to the As-treated group. However, 60 days of SCEE administration upon As-treated rats had a significant effect ($p < 0.0001$) reduce alteration in serum level of lipid peroxidation compared to the As-pre-treated group. Lonely SCEE treated group had non-significant changes compared to the control. Based on these outputs, *S. cumini* has been suggested as a remedial product by having antioxidant potential against arsenic-induced toxicity (Figure 4).

3.4 Effect of *S. cumini* on Glutathione (GSH) Level

The examination of arsenic-induced toxicity in both hepatic and renal tissue significantly ($p < 0.0001$) depleted the level of glutathione compared to the control. The As-pre-treated group (left for 60 days auto recovery) significantly ($p < 0.0001$) had mild restoration compared to the As-treated group. The SCEE administration for 60

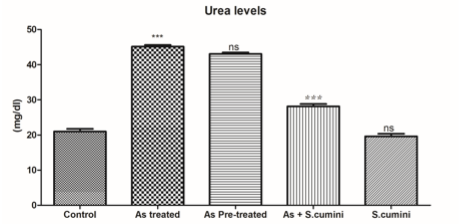


Figure 3 (a). Urea level are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compared to control vs. As-treated, As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicated comparison in between As-treated vs. As-pre-treated and Control vs. *S.cumini* control.

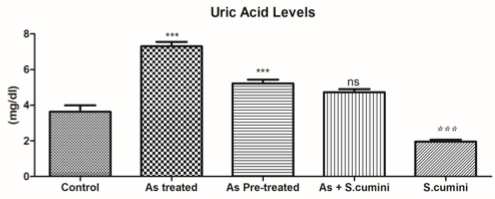


Figure 3 (b). Uric acid are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compared to control vs. As-treated, As-treated vs. As-pre-treated and control vs. *S.cumini*. ns (non-significant) indicates comparison between As-pre-treated vs. As-treated + *S.cumini*

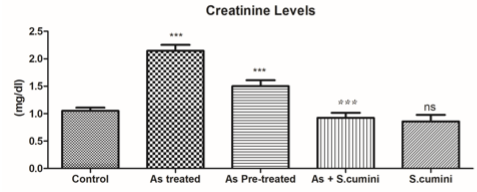


Figure 3 (c). Creatinine level are presented as mean ± SD. Data has been analysed by ANOVA. ***($p < 0.0001$) significant compared to control vs. As-treated, As-treated vs. As-pre-treated and As-pre-treated vs. As treated + *S.cumini* administration. ns (non-significant) indicates comparison between control Vs. *S.cumini*.

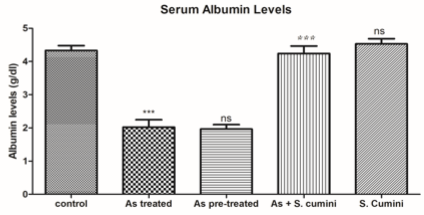


Figure 3 (d). Albumin level are presented as mean ± SD. Data analysed by ANOVA. *** ($p < 0.0001$) significant compared to control Vs. As-treated and As-pre-treated Vs. As-treated + *S.cumini* administration. ns (non-significant) indicated comparison between as-treated Vs. As-pre-treated and control Vs. *S.cumini*.

consecutive days in the As-treated group revealed a tremendous effect in the level of GSH that significantly (P < 0.0001) restored the arsenic-induced alterations compare to the As-pre-treated group. The SCEE-alone treated group had significant (p<0.0001) levels vs. the control group. (Figure 5 (a and b))

3.5 Effect of *S.cumini* Upon Superoxide Dismutase (SOD) Activity

Arsenic-induced toxicity in both liver and kidney tissue significantly (p < 0.0001) decreased the level of SOD in comparison to the control group. The As-pre-treated group (left after As-treatment for 60 days) exhibited substantially (p < 0.0001) modest restoration compared to the As-treated group. The 60 days of SCEE administration in the As-treated group significantly (p < 0.0001) restored the alteration of SOD activity in comparison to the As-pre-treated group. The non-significant alterations had been seen between the alonely *S.cumini* treated group vs. the control group (Figure 6 (a and b))

3.6 Effect of *S. cumini* Upon Catalase (CAT) Activity

The impact of arsenic on the As-treated group had significant (p < 0.0001) alterations in comparison to the control group. The auto-recovered As-pre-treated (left 60 days for self-restoration after arsenic treatment) had non-significant levels in comparison to the As-treated group.

Sixty days of SCEE administration upon the As-treated group therapeutically repaired the alteration significantly (p<0.0001) compared to the As-pre-treated group. Non-significant changes were observed in SCEE alone treated group compared to the control (Figure 7 (a and b)).

3.7 As (arsenic) Concentration and SCEE Phytoremedial Effect on Hepato-Nephro Tissues

Arsenic-induced concentrations on both hepatic and renal tissue were significantly (p < 0.0001) high in comparison to control. Moreover, the As-pre-treated group (left for 60 days for auto recovery) had significant (p < 0.0001) mild restoration compared to the As-treated group. But, sixty days of SCEE administration in the As-treated group had therapeutic changes significantly (p < 0.0001) compared to the As-pre-treated group. SCEE lonely-treated group had non-significant changes compared to the control (Figure 8).

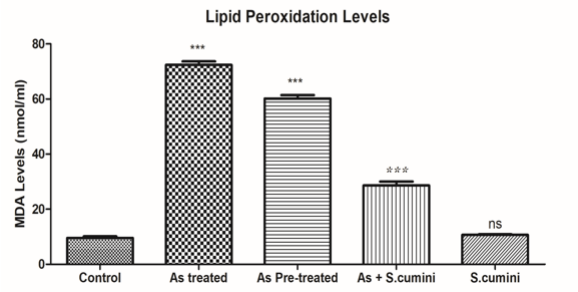


Figure 4. LPO levels are presented as mean ± SD. Data has been is analysed by ANOVA. *** (p < 0.0001) significant compared to control vs. As-treated, As-treated vs. As-pre-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparison between control vs. *S.cumini* control.

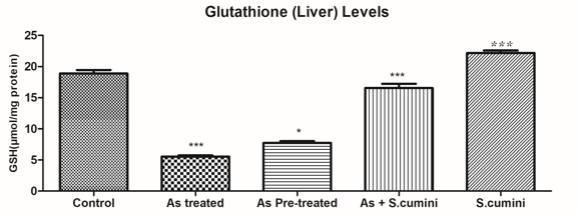


Figure 5 (a). GSH (Liver) concentration level are presented as mean ± SD. Data is analysed by ANOVA. *** (p < 0.0001) significant compared to control vs. As-treated, As-pre-treated vs. As-treated + *S.cumini* administration and control vs. *S.cumini* control. *(p < 0.0001) significant compared As-treated vs. As-pre-treated.

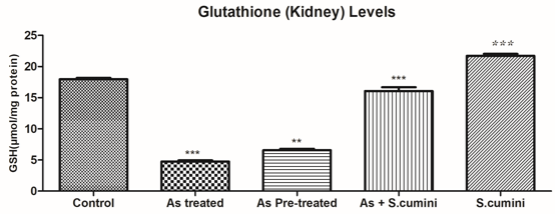


Figure 5 (b). GSH (Kidney) concentration level are presented as mean ± SD. Data is analysed by ANOVA. *** (p < 0.0001) significant compared to control vs. As-treated, As-pre-treated vs. As-treated + *S.cumini* administration and control vs. *S.cumini* control. ** (p < 0.0001) significant compared to As-treated vs. As-pre-treated.

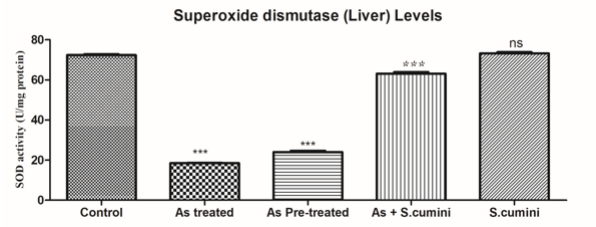


Figure 6 (a). SOD (Liver) concentration level are presented in mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compare to control vs. As-treated, As-treated vs. As-pre-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparison between control vs. *S.cumini* control.

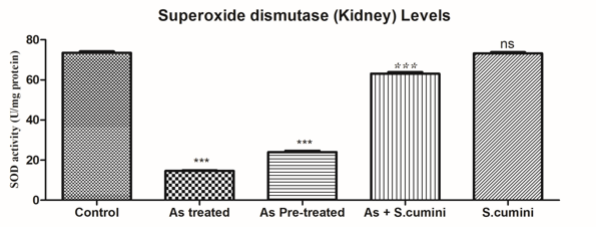


Figure 6 (b). SOD (Kidney) concentration level are presented in mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compare to control vs. As-pre-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicated comparison between control vs. *S.cumini* control.

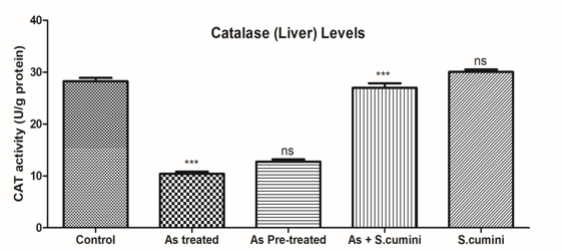


Figure 7 (a). Catalase (Liver) concentration level are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compare to control vs. As-treated, As-pre-treated vs. As-treated + *S.cumini* administration. ns (n0n-significant) indicated comparison in between As-treated vs. As-pre-treated and Control vs. *S.cumini* control.

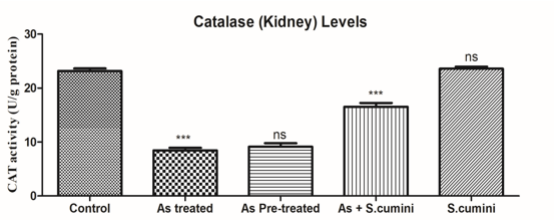


Figure 7 (b). Catalase (Kidney) concentration level are presented as mean ± SD. Data has been analysed by ANOVA. *** ($p < 0.0001$) significant compare to control vs. As-treated and As-pre-treated vs. As-treated + *S.cumini* administration. ns (non-significant) indicates comparison in between As-treated vs. As-pre-treated and control vs. *S.cumini* control.

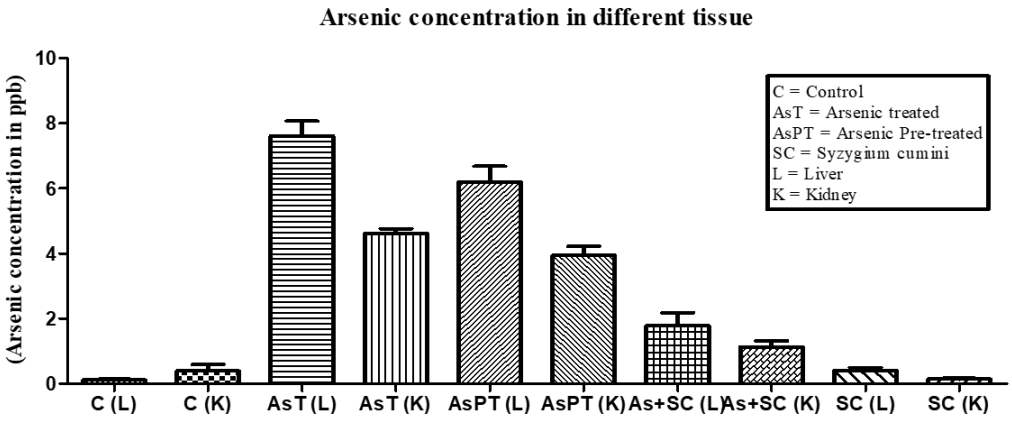


Figure 8. Arsenic accumulated Concentration (on both hepato-nephro tissues) levels are presented as mean ± SD. Data were analysed by ANOVA.

3.8 Hepatic Histopathological Interpretations

In this current investigation; the hepatic section of the control rats group showed normal histopathological architecture in hepatocytes. These hepatocytes are well arranged in the sinusoids along the central vein (Figure 9a). As-treated hepatic sections of rats revealed significant degradation, that was a significant increase in the Kupffer's cell numbers along with the degeneration in the central vein and vacuolations in the sinusoids in comparison to control (Figure 9b). The as-pre-treated group that was left for auto-recovery showed mild restoration in the hepatocytes (Figure 9c). The SCEE administration at 600 mg Kg^{-1} body weight upon arsenic exposed experimental rat's liver showed significant restoration in hepatocytes, central vein, portal vein and sinusoids (Figure 9d).

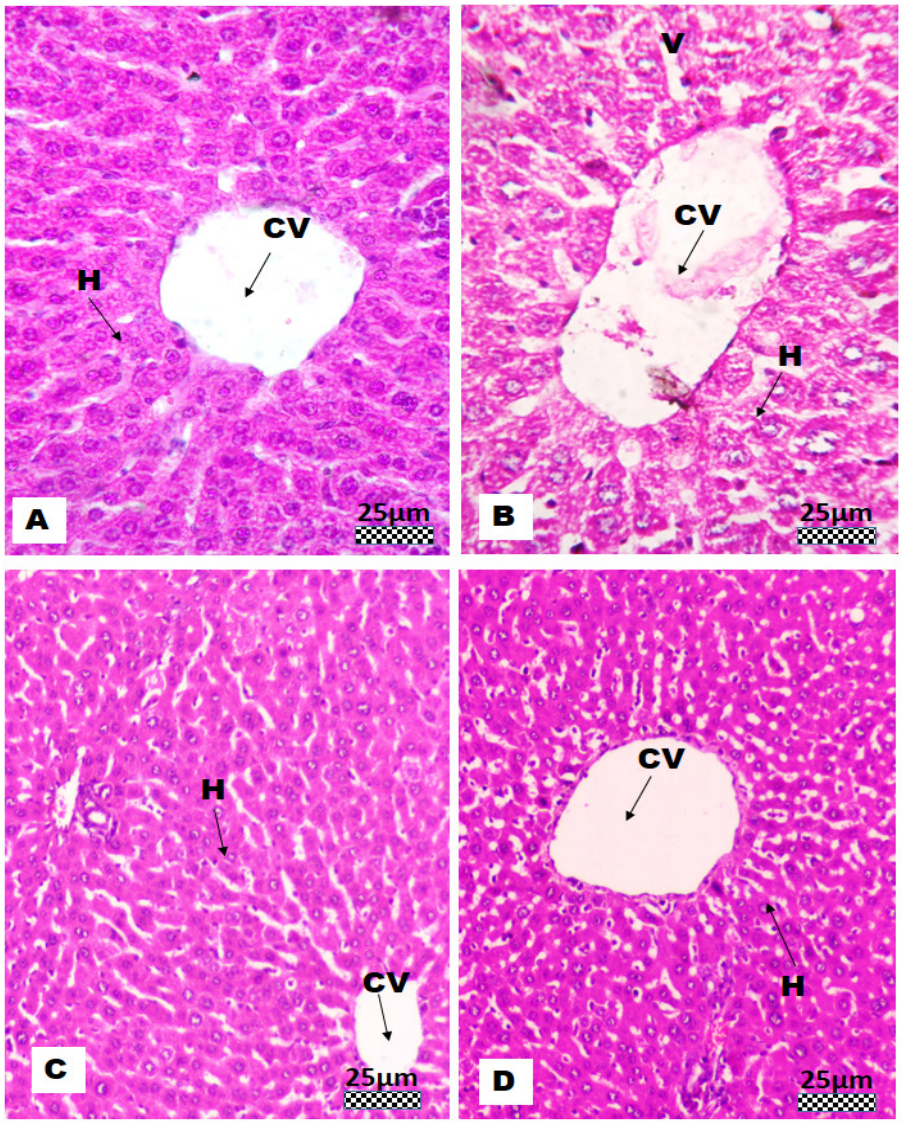


Figure 9. Microphotographs of the rat hepatic section were stained using haematoxylin and eosin (H&E × 500). **Section-A**; Control rat's hepatic section showing the normal architecture of hepatocytes, central vein, portal vein, sinusoids etc. (H&E × 500). **Section-B**; As-treated hepatic alterations showed significant degeneration in hepatocytes, the central vein with increased Kupffer's cells (H&E × 500). **Section-C** As-pre-treated control showed very mild recovery at the cellular level as degeneration in the portal vein with haemorrhage is still persistent (H&E × 500). **Section-D**; indicates almost full recovery in the hepatocytes in *S. cumini*-administered rats. In the liver's central vein, hepatocytes appear to have normal architecture (H&E × 500).

3.9 Renal Histopathological Interpretations

The renal section of the control group reveals the normal histopathological architecture of nephrons with precise proximal and distal convoluted tubules (Figure 10A). Compared to the control As-treated renal section reveals significant degeneration in nephrocytes and an elevated magnitude of degeneration in Bowman's capsule along with the glomerulus is observed (Figure 10B). However, the As-pre-treated group that was left for auto-recovery revealed mild restoration in the nephrocytes and glomerulus (Figure 10C). SCEE administration at 600mg Kg^{-1} body weight upon As-treated group rat's renal section reveals significant restoration in nephrocytes, glomerulus and well-defined distal and proximal tubules (Figure 10D).

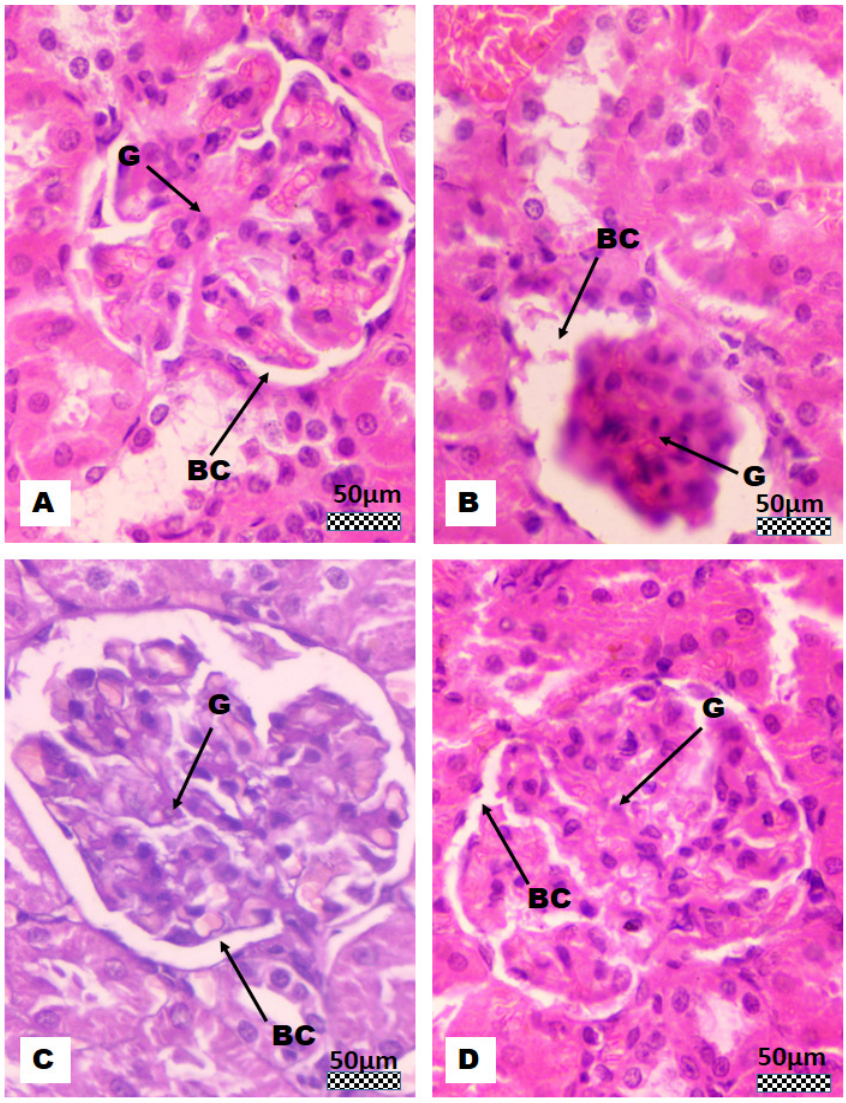


Figure 10. Renal microphotography of rat stained using haematoxylin and eosin (H & E). **Section-A**: A control group of rat's renal film showing the normal architecture of nephrocytes with glomerulus in Bowman's capsule (H&E × 800). **Section-B**: As-treated rat's renal film reveals a higher magnitude of degeneration in Bowman's capsule along with glomerulus (H&E × 800). **Section-C**: As-pre-treated group reveals the persistence of degeneration in the glomerulus and Bowman's capsule as there is severe haemorrhage in nephrocytes (H&E × 800). **Section-D**; SCEE administered group reveals significant restoration in the nephrocytes as the architecture of the glomerulus with Bowman's capsule appears to be normal. Moreover, the endothelial cells of convoluted tubules appear to be normal denoting normal functioning (H&E × 800).

4. Discussion

Arsenic in drinking water is injurious to health. Chronic exposure to arsenic is extensively known to produce toxins and affects multiple organ systems with complex mechanisms[23,25,28,30,59]. Consumption of this element is highly toxic causing oxidative damage, cellular degeneration, multi-system diseases like liver, kidney, neuro-disorder, low-rate infertility etc[60]. The toxic effect of entire heavy metals across permissible limits leads to increased hepatic impairment due to its metabolism[61,62]; whilst the liver plays a vital role in the kidney's ability to eliminate arsenic methylated species via urine[63]. Almost 70% of inorganic arsenic is eliminated by kidneys and excreted through urine. Through the intestines, inorganic arsenic that has been monomethylated is expelled[17,20,21,25,59,64].

Current research is used to investigate the therapeutic effect of *S.cumini* seeds against arsenic-induced hepatic and renal injury using biochemical and histological parameters. The used plant in this research is delicious and nutritious with a variety of vitamins and minerals that are easily available in the summer market[31,32,36]. It has been also consumed for the treatment of low blood pressure, blood sugar, oxidative stress, electrolyte balance and other conditions[33,34]. Pharmacological research indicates that the phenolic and flavonoid antioxidants found in the seed of *S.cumini* can be used to prevent oxidative damage in a variety of clinical conditions[34,35,37,38]. Phenolic antioxidants are consequences of secondary metabolism in plants, and their redox characteristics, may be important in the removal of transition metals and scavenging free radicals[39–44].

The findings of the current study revealed that As-treated rats' LFT levels were significantly higher compared to the control that was confirming hepatic injury and aberrant functions. These injuries confirmed that high doses of arsenic damage the liver by increasing the production of free radicals[64,65]. Arsenic-induced toxicity has been associated with oxidative damage, hepatocellular necrosis, and hepatic damage related to hepatocyte degeneration, sinusoids, vacuolization and portal vein haemorrhages. The outcomes of these experiments were parallel and reliable with previous research[25,27,63,64,65]. SCEE-administered therapy normalised the severity of arsenic-induced hepatic injury by which the LFT parameters (SGPT, SGOT, ALP and total bilirubin) significantly ($p < 0.0001$) normalised themselves and resolved the hepatic histopathological changes in comparison to As-treated architecture. The presence of flavonoids and other phenolic phytochemicals in *S.cumini* helps to restore the hepatic cells, as well as the antioxidant enzymes[33,35,37].

Arsenic-induced renal damage had large perfusion and an elevated concentration of expelled chemicals in renal tubular cells[25,26,30,65]. Acute arsenic toxicity increased KFT levels, resulting in renal damage that impacts tubular and glomerular degradation as well as decreased lysosomal latency as a sign of renal failure[26,30,63,65]. The arsenic-induced rats had significantly increased the serum level of urea, uric acid, and creatinine and decreased albumin levels, a consequence of nephrotoxicity, which is consistent with previous findings[26,63,65]. Changes in these indicators accelerate the onset of nephrotoxicity, acute inflammation and tubular necrosis[66,67]. The glomerular filtration rate decreased as a result of these critical changes and cellular growth[65,68]. SCEE administration revealed significant amelioration of altered KFT's biochemical parameters (urea, uric acid and creatinine along with albumin) in As-treated rats. These results were the remarkable restoration of renal activity, renal tubules and glomeruli[25,63,65,67,68].

Long-term exposure to arsenic leads to the generation of intracellular Reactive Oxygen Species (ROS), which can either directly oxidize molecules or affect signalling pathways and epigenetic modifications to mediate various changes in cell activity[25,65,67]. These exposures produce free radicals that resulted in significant changes in the serum Level Of Lipid Peroxidation (LPO). The current examination confirms that compared to the control group As-treated rats had significantly ($p < 0.0001$) increased serum levels of LPO which is the most important sign of oxidative damage occurring in the cell membrane[69]. The increased serum level of LPO was also earlier reported[25]. This accelerated lipid peroxidation is thought to result from oxidative stress, which occurs when the dynamic balance between antioxidant and antioxidant systems is perturbed[25,65,70] SCEE oral administration dose with 600mg Kg^{-1} body weight for 60 days upon As-treated group significantly reduced the altered LPO level and protected the cellular membrane injuries[38,45].

Arsenic-induced oxidative damage in the liver and kidney could be the consequence of hydrogen abstraction from membrane lipid molecules by H_2O_2-derived hydroxyl radical and the failure of antioxidants to

re-establish redox homeostasis[25,67,69]. Antioxidants both enzymatic and nonenzymatic are regarded to be the cell's front line of defence against oxidative damage[25,68,69]. A redox balance between prooxidants and antioxidants is essential for normal cellular function. The prooxidant-antioxidant balance is disturbed by arsenic's strong affinity for the sulfhydryl group in reduced Glutathione (GSH), which may affect the preservation of thiol-disulphide equilibrium[69,71]. This elevation might be the cause of lower concentration of SH-groups and antioxidant enzyme[25,71].

Superoxide Dismutase (SOD) is an antioxidant metal-loenzyme that transfers superoxide radicals to both oxygen and water molecules[72,73] Catalase (CAT) is an iron-containing enzyme (hem-enzyme) that catalyzes the decomposition of hydrogen peroxide into water and oxygen[73,74]. They are also recognized as an important enzyme in preventing oxidative cell injuries from reactive oxygen species. These concerned antioxidant enzymes (GSH, SOD and CAT) are known to be endogenous defence mechanisms that help in protection against free radical-induced damages[73,75].

The present study reveals that the As-treated group had significantly ($p < 0.0001$) reduced the level of GSH, SOD and CAT in comparison to the control group. The outcomes of this experiment rely on previous studies showing significant alterations in As-treated antioxidant enzymes (GSH, SOD, CAT) vs. the control group[71,73]. Non-probabilistic improvements were observed between the As-pre-treated group vs. the As-treated group[25,67,69,71]. SCEE administration effectively amended the cellular alteration in hepatocytes and nephrocytes as well as anti-oxidants enzyme activity through which the antioxidant enzymes (GSH, SOD, CAT) significantly ($p < 0.0001$) enhanced and restored the normal condition[70,71]. The presence of active constituents (myricetin, quercetin, fla-vonoids, kaempferol and polyphenol ellagic acid) in SCEE was performed to improve the levels and block the thiol depletion in addition to inhibiting SH-group oxidation[74]. SCEE administration eliminates the arsenic accumulation from tissues as there was a significant decrease in the con-tamination levels in the vital tissues of the treated rats[74,75].

5. Conclusion

Thus, the present study concludes that arsenic intoxication causes oxidative stress and serious damage to the hepatic and renal organs. Additionally, *S.cumini* administration produced significant improvements against arsenic-induced hepato-nephro toxicity. The study concludes that *S.cumini* has a plethora of mediating properties against arsenic toxicity, which can be used as an anti-drug against arsenic poisoning.

6. Acknowledgements

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7. Declarations

7.1 Consent to Participate

The co-authors have voluntarily agreed to participate in this study. I consent to the aforementioned and allow any-one to participate and be poised in this project.

7.2 Consent for Publish

Consent to publish this article has been accomplished by each co-author and the appropriate administration at the institute where the study was conducted before the work is submitted. Through the corresponding author, the pub-lication was given the author's unanimous approval.

7.3 Author Contributions

The entire experimental work was conceptualized by P.S., R.V.S. and A.K. The manuscript's principal author P.S. contributed the majority of writing activities, but support was also provided by R.V.S. and A.K., Literature search was done by P.S. Figures were developed by P.S. and A.K. The study design was carried out by P.S, R.V.S and A.K. The experimentation and data analysis were carried out by P.S. The figures were designed by P.S. and A.K. The sta-tistics and data interpretation was done by P.S. The final manuscript writing was done by P.S., R.V.S. and A.K. All authors read and approved the final manuscript.

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