Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45** Issue **5 Year 2024** Page 32**-44***

DOI: 10.53555/jaz.v45i5.4690

Therapeutic effect of *Citrullus lanatus* against Sodium arsenite induced reproductive toxicity in Charles Foster rats

Pintoo Kumar Niraj[1], Rana Vikram Singh[1], and Arun Kumar[2]

[1]University Department of Zoology, Jai Prakash University, Chapra, Bihar (India).
*[2]*Mahavir Cancer Sansthan and Research Centre, Patna, Bihar (India).*

****Corresponding Author:** Dr. Arun Kumar*
**Senior Research Scientist, Mahavir Cancer Sansthan & Research Centre Patna, Bihar- 801505. India,*
Email- drarunk31@gmail.com Cell No. +91-9334740800, Orcid I.D. https://orcid.org/0000-0002-8946-5909

Abstract

Arsenic contamination is a serious issue throughout the world and is substantial risk factor in most of the countries including India. Chronic arsenic exposure from drinking water to humans is causing major public health related issues. The present study aims to investigate the ameliorative effect of *Citrullus lanatus* (Watermelon) seeds against arsenic induced reproductive function and testicular toxicity in Charles Foster rats.

In the present study, twenty-four male Charles Foster rats (120±5gm). The study group includes group - I (n=6) as control, and group - II (n=18) treated with sodium arsenite (8 mg per Kg body weight per day) for 90 days. The group - II was furthermore divided into three sub-groups, Group – II-a, Group – II-b and Group – II-c. The group – II-a were sacrificed to see the effect of arsenic toxicity effect after arsenic exposure. Group – II-b was left with normal food and water for next 60 days to observe auto recovery. The group – II-c rats were administered with *Citrullus lanatus* (Watermelon) hydroxyl ethanolic seeds extract at the dose of 400 mg per Kg body weight per day for 60 days for 90 days upon pre-treated sodium arsenite treated group (8 mg per kg body weight). After completion of the experiment, all the treated group of animals were sacrificed for biochemical, hormonal assay, histopathological, testicular and reproductive functions study.

In the arsenic treated rats' group, there was significant ($p<0.001$) changes in serum levels of SGPT, SGOT, urea, uric acid and creatinine as well as in haematological parameters. There was decrease in the sperm counts and sperm motility accompanied by an increased incidence of sperm abnormalities and hormonal imbalance denotes infertility condition. In contrast, after the administration with *C.lanatus* (Watermelon) seeds hydroxy-ethanolic extract upon arsenic treated rats group, there was significant ($p<0.001$) improvement observed in the reproductive, hepatic and renal parameters. In the arsenic intoxicant rats' group, after administration with *C. lanatus* (Watermelon) seeds hydroxyl ethanolic extract, there was significant ($p<001$) reduction in the arsenic concentration in blood, liver, and kidney tissues as well as serum lipid peroxidation.

The histopathological study also showed the *C. lanatus* (Watermelon) seeds hydroxy ethanolic extract significantly restored the cellular integrity of testicular cells leading to normal functioning of it against arsenic induced toxicity.

Keywords: Sodium arsenite; Citrullus lanatus; Charles Foster rats; antidote effect, Therapeutic effect.

Introduction

Arsenic is indeed a naturally occurring metalloid that can be found widely distributed in water, food, and soil. In nature, it exists in two common forms: arsenite (As III) and arsenate (As V). Unfortunately, elevated levels of arsenic in groundwater have become a global health concern, impacting millions of people worldwide.

One of the most severely affected regions in Asia is the upper, middle, and lower Gangetic and Brahmaputra plains, primarily in India and Bangladesh. In India, states such as Bihar, Jharkhand, West Bengal, Uttar Pradesh, and Uttarakhand, along the Gangetic basin, have experienced significant arsenic contamination in groundwater. This issue has been documented in various studies (Tseng et al., 2002; Nickson et al., 2007).

In Bihar, an estimated 10 million populations are affected to groundwater arsenic poisoning, which has posed serious health hazards to the exposed population. The exposed population exhibit typical symptoms of arsenicosis with various types diseases such as skin manifestations, gastrointestinal disorders, cardiovascular disorders, loss of appetite, constipation, diarrhea, neurological disorders, reproductive disorders and cancer etc. (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).

The presence of a high level of arsenic in groundwater and the resulting serious health issues among the people of Bihar were initially reported in Semaria Ojha Patti village, which is located in the Bhojpur district of the state. This discovery was documented in studies conducted by (Chakraborti et al., 2002; Kumar et al., 2016). These findings marked the beginning of increased awareness and efforts to address the arsenic contamination problem in Bihar and other affected regions, emphasizing the need for comprehensive solutions to safeguard public health and access to safe drinking water.

Humans are exposed to a wide range of environmental toxins that are harmful to all living things and can cause a number of reproductive diseases and general health problems (Sutton et al., 2012). The detrimental effects of exposure to environmental hazardous chemicals on male reproductive have recently attracted a lot of attention (Skinner et al., 2010; Christou et al., 2017). Humans have experienced a progressive loss in sperm quality and quantity over the past 50 years (Mishra et al., 2018) if this trend continues, an individual would be unable to have children. (Kumar et al., 2019[a], 2015; Rahman et al., 2019). In a recent study (Kumar et al., 2019[b]) showed that chronic arsenic poisoning altered the neurobehavioral activity of school children in Simri village of Buxar district, Bihar, India, including low IQ and memory. Chronic arsenic exposure can result in malignancies of skin liver, kidney, lung, bladder, prostrate, and other organ in addition to the condition already stated (Hamza et al., 2022; Michael et al., 2011).

Metalloid arsenic, a known genotoxic carcinogen, is widely distributed. Several sources emit it into the environment. It is utilised in wood preservatives, glass, alloys, and semiconductors. Numerous research indicates that groundwater from various countries of the world, including India and Bangladesh, has a high level of inorganic arsenic (Chakraborti et al., 2004; Rango et al., 2013). Arsenic exposure is connected to male reproductive toxicity, according to epidemiologic research (da Silva et al., 2017).

Arsenic is found in drinking water in inorganic form, which is more hazardous than organic arsenic forms. Arsenite (As3+), the trivalent form of inorganic arsenic, is more active and poisonous than arsenate (As5+), the pentavalent form. The legal limit for arsenic in drinking water is 10 ppb (World Health Organisation). Acute arsenic poisoning can cause vomiting, severe diarrhea, and stomach pain (Ratnaike et al., 2003). Arsenic accumulates in tissues and essential organs from frequent consumption, which causes atherosclerosis and hypertension (Abhyankar et al., 2012; Simeconova et al., 2004). Testicular regression caused by arsenic intoxication can lead to infertility in males. In severe cases, it can also lead to testicular atrophy and permanent loss of fertility (Niraj et al., 2024).

Medicinal plants play a vital role in controlling the disease, as well as the normal functioning of the vital organs (Shankar et al., 2024; Shankar et al., 2023). The watermelon, scientifically known as *Citrullus lanatus*, is a member of the Cucurbitaceae family. Schipper (2000) found the fruit rich in vitamins A, B, and C, as its traditional uses include cleansing the kidneys and bladder, reducing hypertension, protecting against impotence, and treating jaundice and enlarged liver. The arginine found in the seeds is a common remedy for heart problems and hypertension. A total of 3.8 milligrams (mg) of vitamins B—including folate, thiamine, riboflavin, pantothenate, vitamin B_6, and niacin—are present in 31 grams of seeds, which is 19% of the recommended daily allowance. Among the most common elements are zinc, manganese, magnesium, iron, and calcium. Aside from its role in modulating carbohydrate metabolism, which impacts plasma sugar levels, magnesium controls blood pressure (Wahid and Saqib 2022; Logaraj 2011). The present study thus aims to find out the therapeutic effect of *Citrullus lanatus* seeds on arsenic treated rats.

Materials and methods Ethics Approval

The research study was approved by the Institutional Animal Ethics Committee (IAEC) with IAEC No. 2020/1C-27/08/20 dated 27/08/2020 (CPCSEA Regd. No. 1129/PO/ReBi/S/07/CPCSEA).

Chemical

Arsenic was used as sodium arsenite (assay 98%), manufactured by Loba Chemie, India (CAS No. 7784-46-5, Lot No. #SG59751302). A biochemical test kit carried out by the standard kit of Coral using a thermo-scientific spectrophotometer and an ELISA test kit of G. Biosciences (Code: ITEM00260, Batch No.: 2020711) using an ALEAR ELISA reader for hormonal analysis were purchased from the scientific store, Patna, Bihar, India.

Plant selected for study as antidote

The seeds of *Citrullus lanatus*, sometimes known as Watermelon, were bought from a nearby market in Patna, Bihar, India. The seeds were identified and certified by the Department of Botany, Jai Prakash University, Chapra Bihar (India). After rinsing with distilled water, the watermelon seeds were cleaned to remove any remaining soil and debris using running tap water. The seeds were dried at 37°C and thereafter grinded to a fine powder. For the study, hydroxyl-ethanolic extract, 400 mg of fine powder was sieved, weighed, and mixed with 10 ml of distilled water that had previously been mixed with 5% alcohol. The mixture was then vigorously vortexed for two hours to ensure that all of the components were completely mixed and the solution was ready to be given to the treated animals.

Animals

Twenty-four healthy male Charles Forster rats, 8 weeks old, 120±5gm g weight, were obtained from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, India. The rats were acclimatized under 12-hour light and dark cycles (room temperature at 22±2 ℃) for 7 days before the start of the treatment in a laboratory house. These experimental rats were housed in conventional polypropylene cages with stainless steel grills and were provided with a diet (prepared by the laboratory itself) and water *ad libitum.*

Dose Selection

Sodium arsenite was used to make the arsenic model. The dose selection of sodium arsenite was calculated based on LD_{50} (Zhao et al., 2018). The final dose was selected at 8mg/kg body weight. The sodium arsenite dose was dissolved in 10 ml of distilled water and administered intragastrically by gavage method.
For *Citrullus lanatus* (Watermelon) extract, the dose was calculated after LD_{50} estimation. The dose was finally titrated to $1/8^{th}$ dose of 400 mg/kg body weight. For this 400mg of *C. lanatus* seeds extract were dissolved in 5% hydroxyethanol and then delivered to the rats in their respective experimental groups.

Experimental design

Rats were randomly divided into four groups (n=6)

Group I: Vehicle control- Rats were intragastrically administered distilled water for 150 days. **Group II a** - Rats were dissected after 90 days' sodium arsenite treatment for arsenic control. **Group II b**- Rats were left for auto recovery. **Group II c**- *C.lanatus* seeds extract treated- Rats were administered orally 400 mg/kg body weight/day for 60 days upon 90 days pre-treated sodium arsenite group at the dose of 8 mg/kg body weight per day.

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.

Biochemical assay

Biochemical analysis was performed through the serum by the standard kit process (Coral crest) on a UV-Vis spectrophotometer (UV-10, Thermo Scientific, USA).

Determination of Liver Function Test

In liver function tests (LFT), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were measured according to the method of Reitman and Frankel (1957), alkaline phosphatase (ALP) by the method of Kind and King (1954), and total bilirubin activity by the method of Jendrassik and Grof's (1938).

Determination of Kidney Function Test

The kidney function test (KFT) was analyzed through urea by the method of Fawcett and Scott (1960); creatinine by the method of Bones and Tausky (1945); and uric acid by the method of Fossati and Prencipe (1980).

Determination of Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were estimated by the double heating method (Draper & Hadley, 1990). This method 90ºC.is a spectrophotometric measurement of the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 mL of 100 g/L trichloroacetic acid (TCA) solution was added to 0.5 mL of serum in a centrifuge tube and incubated for 15 min at 90ºC. After cooling in tap water, the mixture was centrifuged at 3000 g for 10 min, and 2 ml of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube and again incubated for 15 min at 90ºC. The solution was then cooled in tap water, and its absorbance was measured using a Thermo Scientific UV-10 (UV-Vis) spectrophotometer (USA) at 532 nm.

Sperm Counts

The cauda epididymis of the rat was dissected out and cleaned thoroughly in normal saline (0.85%). The cauda epididymis was incised and punctured at several places, and sperm were released in 1mL of distilled water in a watch glass. Then two drops of eosin Y were mixed with sperm. A drop of the above preparation was taken into Neubauer's chamber to be observed at 800x magnification.

Histopathological study

Small pieces of liver, kidney and testis tissues were fixed in 10% formalin for 24 h. Thereafter, the tissues were dehydrated with a graded ethanol concentration and embedded in paraffin. The tissue sections were grossed at 5µm thickness through a digital rotary microtome (Microm HM 340E, Thermo Scientific USA) and stained with haematoxylin and eosin (H&E) for the investigation of histopathological changes under a light microscope. Four microscopic slides per animal were examined for assessment of histological changes in liver, kidney and testis tissues respectively. The 20 random microscopic fields of microscopic slides were examined to check for various histological changes such as degenerations, vacuolizations, haemorrhages etc. Hepatocytes and tubular degeneration were assessed in each rat by counting the degeneration among 100 hepatic cells and tubules.

Hormonal Assay

The testosterone and Luteinizing hormone ELISA kit was equilibrated at room temperature; 50µl of standard working solution and 50 µl of sample were added to each well, and immediately 50µl of biotinylated-antigen working solution was added to each well, mixed and incubated for 1h. I discarded the liquid in the plate, added 200 µl wash buffer to each well, and washed the plate three times. After drying, add 100µl of Streptavidin-HRP working solution to each well and incubate at 37
°C for 60 minutes. I discarded the liquid in the plate, added 200µl wash buffer to each well, and washed the plate five times. After spin-drying, add 90µl TMB to each well and incubate at 37 °C for 20 minutes. Finally, I added 50µl stop solution to each well, read the plate at 450 nm immediately, and calculated the result.

Statistical Analysis

The result was expressed as mean ± SEM; n = 6 animals in each group; * p<0.001: statistically significant from the control. Statistical analysis was carried out using Graph Pad, version 5.0, and PRISM software. One-way ANOVA was used, followed by Bonferroni multiple comparison tests; arsenic-treated rats were compared with control rats and *C. lanatus* pre-arsenic treated were compared with the arsenic-treated group.

Results

Effects on Body weight

Compared to control the body weight of As-treated rat's group significantly had (p<0.001) reduction. Mild weight gain was observed in As-pre-treated group in comparison with As treated group. *C. lanatus* administration upon As-treated group shown its good effect where significant (P<0.001) weight gain was observed in comparison with As Control group **[Table-1].**

Parameter	Control	As Treated	As Control	*C. lanatus* treated upon As treated
Body weight examination	305.0 ± 4.164	178.0 ± 6.367	265.5 ± 4.724	384.2 ± 2.607

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

Biochemical study

Compared to control group the As treated rats showed significant (p<0.001) changes in serum level of urea, uric acid and creatinine. However, compared to As treated group vs. As control group which were left for auto recovery had significant (p<0.001) restoration in the level of serum creatinine and uric acid. Non-significant changes were observed in the level of urea. Compared to As-control group vs. *C. lanatus* administration upon As treated group had significant (p<0.001) restoration in the serum level of urea, and creatinine, while non-significant changes were observed in uric acid.

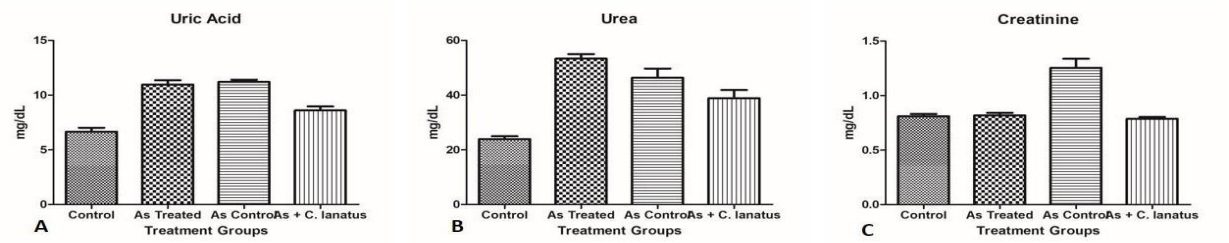


Figure 1. Graphs showing biochemical parameters –Kidney function test parameters – with levels of Urea, Uric Acid, and Creatinine in control, arsenic treated, arsenic control and *C. lanatus* treated groups. All data values are expressed as Mean ±SE.

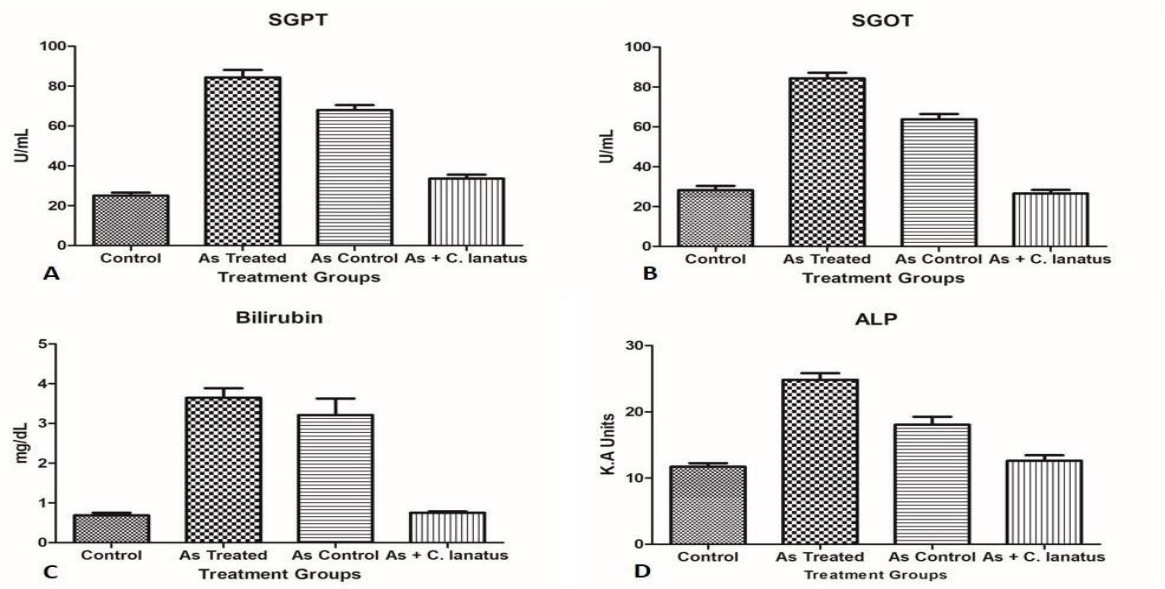


Figure 2. Graphs showing biochemical parameters – Liver function test parameters – with levels of SGPT, SGOT, alkaline phosphatase and bilirubin in control, arsenic treated, arsenic control and *C. lanatus* treated groups. All data values are expressed as Mean ±SE.

Hormonal Study

The testosterone level shows significant ($p<0.001$) decrease in level in comparison to control. Moreover, there was mild restoration in As control group. However, *C. lanatus* administration upon arsenic treated group showed significant ($p<0.001$) restoration in testosterone level compared to arsenic treated group. The LH level shows significant ($p<0.001$) increase in the Luteinising hormone levels in comparison to control. Moreover, there was very mild restoration in the arsenic control group. But, in *C. lanatus* administered rat group (on arsenic pre-treated group) there was significant normalisation in the LH level.

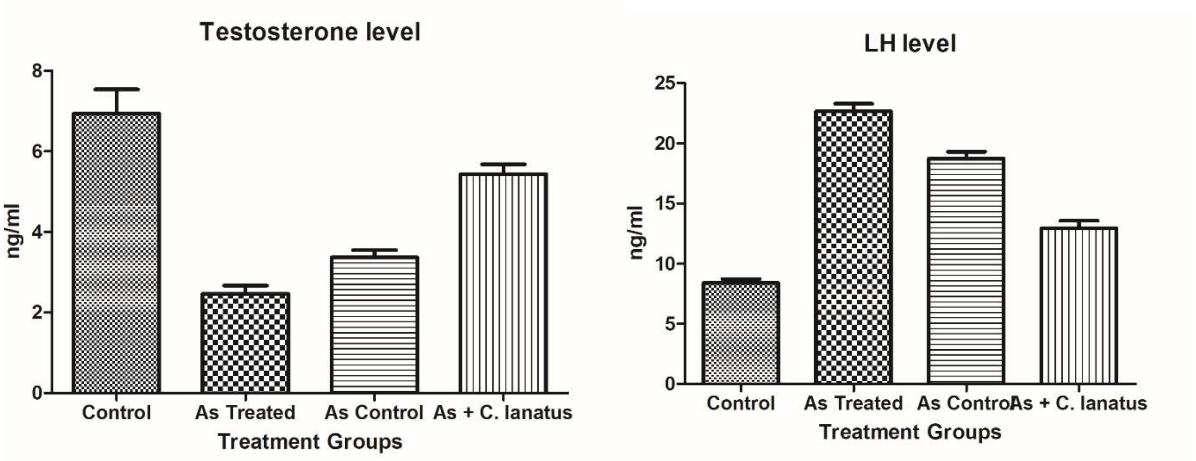


Figure 3. Graph Showing Testosterone and LH levels of rat in control, arsenic treated, arsenic control and *C. lanatus* treated groups. All data values are expressed as Mean ±SE.

Sperm Counts

The sperm count levels show significant ($p<0.001$) decrease in the levels in comparison to control. Moreover, there was mild restoration in arsenic control group. However, *C. lanatus* administration upon arsenic treated group showed significant ($p<0.001$) restoration in sperm counts level compared to arsenic treated group.

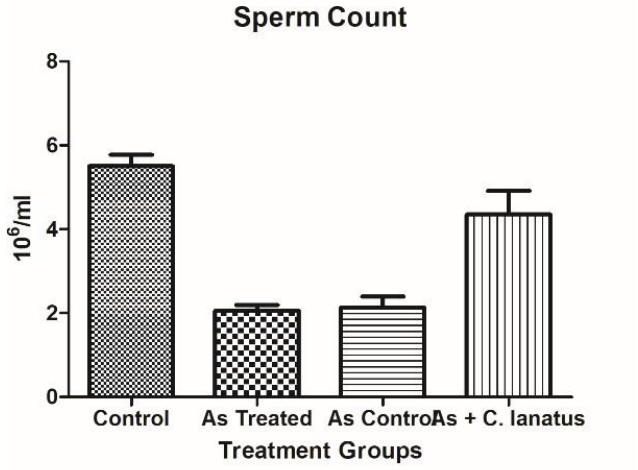


Figure 4. Graph Showing Sperm counts level of rat in control, arsenic treated, arsenic control and *C. lanatus* treated groups. All data values are expressed as Mean ±SE.

Lipid Peroxidation study

The serum level of MDA was significantly ($p<0.001$) elevated in the arsenic treated rats in comparison to control group. The arsenic pre-treated rats which were left for auto recovery without any treatment for 60 days had mild restoration. However, *C. lanatus* administration upon arsenic pre- treated rats had significant ($p<0.001$) reduction in comparison to arsenic pre-treated rats.

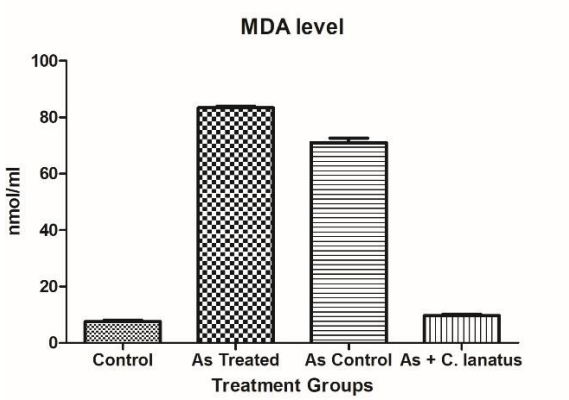
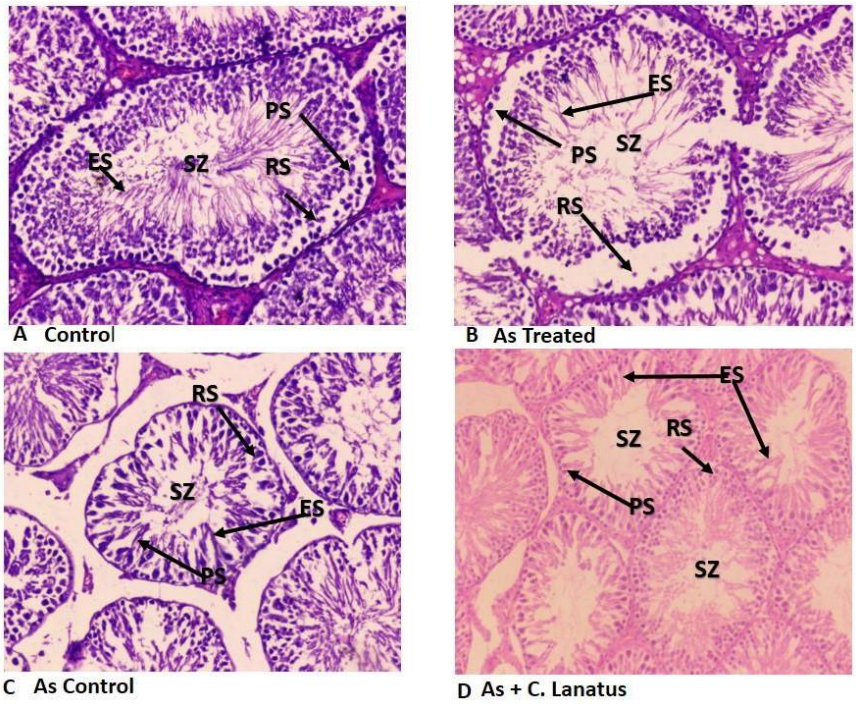


Figure 5. Graph Showing MDA level in control, arsenic treated, arsenic control and *C.lanatus* treated groups. All data values are expressed as Mean ±SE

Histopathological Analysis

The control testis showed normal architecture of seminiferous tubules with arranged spermatogenetic stages primary spermatocytes, spermatogonia, spermatids and spermatozoa. The leydig cells aligning the inter-seminiferous tubules were normal showing the normal functioning of the spermatogenesis. However, in arsenic treated testicular cells showed severe damage in the seminiferous tubules denotes abnormal functioning of the testicular cells. The leydig cells also appeared to be in very highly degenerative condition as haemorrhage was seen. The arsenic treated group and the arsenic recovery group of rat's similar testicular degeneration was observed. However, after administration of *C.lanatus* there was immense amelioration, as restoration in the spermatogenetic stages could be observed. The primary spermatocytes, Spermatogonia, spermatids, and spermatozoa all well- arranged denoting the significant normalization in the function of the testicular cells. The leydig cells also showed amelioration denotes the normalization in its function.



RS = Round Spermatids, ES = Elongating Spermatids, PS = Pachytene Spermatids, SZ=Spermatozoa.

Figure-6: Microphotograph of testis section stained with haematoxylin and eosin. **(A).** Testis of control rat showing normal histopathological structure of active mature functioning of seminiferous tubules associated with complete spermatogenic series (H&E×500). **(B).** Testis of arsenic treated rats, showing marked degeneration of most seminiferous tubules with absence of spermatogenic series in tubular lumen (H&E×500). **(C).** Section of arsenic pre-treated testis showing persistence of degeneration due to arsenic toxicity RS, PS and SZ (H&E×500). **(D).** Section of *C.lanatus* administrated upon arsenic pre-treated group shows restoration in testicular toxicity (H&E×500).

Discussion

The biochemical parameters are the essential part of the diagnosis to know the level of changes occurring at the organ system levels. In the liver function tests, the SGPT and the SGOT are the glucose biomarkers, while alkaline phosphatase and the bilirubin are enzyme markers of the liver. In the present study, there was significant increase ($p<0.001$) in the SGPT, SGOT, ALP and bilirubin levels in comparison to the control. There was non-significant normalisation in the levels. But, after the administration of *C.lanatus,* there was significant restoration in the levels denotes that *C.lanatus* has hepatoprotective properties (Bayrami et al., 2022; Delwatta et al., 2018; Munglue et al., 2014).

The kidney function test are the enzyme markers which helps in the elimination of toxicants from the body via urine or sweat or other fluids. In the present study, there was significant increase in the urea, uric acid and creatine levels in the arsenic treated rat group in comparison to the control and arsenic control. But, there was significant normalization in these levels after the administration of *C.lanatus* denotes nephroprotective properties (Khushboo et al., 2018; Aruldhas et al., 2005).

Hormones are the essential part of the system which regulates the major functions of body. Testosterone and the luteinising hormones are the important hormones of the male reproductive system (Darbandi et al., 2018). In the present study, there was significant ($p<0.001$) decrease in the testosterone levels while significant increase ($p<0.001$) in the luteinising hormone was observed in the arsenic treated groups in comparison to the control group of rats. There were very mild changes in hormone levels were observed in the arsenic control group of rats. But, after the administration with *C.lanatus,* there was significant normalization in the levels of the hormones, denotes that *C.lanatus* possesses properties which regulates and controls the proper hormone functions of the body.

Similarly, arsenic toxicity causes severe damage to the lipids of the membranes by depleting it through lipid peroxidation activity (Barai et al., 2017; Jahan et al., 2016). This causes loss of membrane activity and cell integrity. In the present study also, there was significant increase in the levels of lipid peroxidation in the arsenic treated group of rats in comparison to the control and arsenic control levels (Zargari et al., 2022). But, after the administration of *C.lanatus* there was significant ($p<0.001$) normalization in the levels of the lipid peroxidation denotes that its medicinal properties possesses antioxidant activities (Hosseini et al., 2022; Fakunle et al., 2024).

Histopathological studies are the essential part of any study where, the vital organs pathological changes can be observed to know the level of changes occurring at the cellular level (Greaves et al., 2011). In the present study, the histopathological study of male reproductive system as testis was studied (Nejatbakhsh et al., 2017; Malini et al., 1985). In the arsenic treated group, there was significant degeneration observed in the primary spermatocytes, secondary spermatocytes, spermatids, spermatozoa and Sertoli cells denotes that it has damaging effect. Moreover, there was no significant auto-restoration observed in the arsenic control group of rats. But, after the administration with *C.lanatus,* there were significant restoration observed in the testis (Gul et al., 2020; Rahman MS et al., 2019[a,b]), as there was immense increase in the number of spermatozoa in the lumen of the testis denotes the normal testicular function. Similarly, in the arsenic treated rats there was significant reduction in the sperm counts in comparison to the control and arsenic control treated rat groups. But, after the administration of *C.lanatus,* there was significant normalization in the sperm counts (Rotimi et al., 2023; Monton et al., 2015)

The active principle of *C.lanatus* possesses ingredients such as 59.46% oil, 30.4% carbohydrate, and 3.89% protein, the seed oil is edible and nonrancid with free fatty acid value of 1.94%, peroxide value 8.0 mEq/kg, iodine value 10.5, saponification value of 193 mEq/kg (Adekunle & Oluwo, 2008). Mariod and Matthaeus 2008) trans-anethol, fenchone, estragole, which probably play the vital role in controlling arsenic induced toxicity. It also possesses the properties which regulates the normal hormonal functions (Shahat et al., 2011; Alam et al., 2019). It finally plays the important role as antioxidant and membrane repairing functions, normalizing the integrity of the cell thence its function as well (Nadeem et al., 2022).

Conclusion

In the present study *C.lanatus* seeds extract indicates that it has therapeutic effect in controlling the damage caused by the arsenic. There was significant restoration at the haematological level, biochemical level, hormonal level and histological level. The active ingredients present in the seeds of the *C.lanatus* proves to be having trans-anethol, fenchone, estragole as important molecules having therapeutic effect. Moreover, further studies are recommended to be carried out for the clinical study to validate its therapeutic drug efficacy.

Acknowledgements

The authors provide his gratitude to University Department of Zoology, Jai Prakash University, Chapra, (Bihar) India; for infrastructural facilities and Mahavir Cancer Sansthan and Research Centre, Patna (Bihar) India for experimental rats, ethical approval and laboratory facilities.

Declarations

Consent for participate
The co-authors have voluntarily agreed to participate in this study. All the authors give their consent for participation of the work.

Consent for publish

Consent to publish this article has been accomplished from 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 publication was given the author's unanimous approval.

Author contributions

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

Funding
No funding was received for this work.

Competing Interest
In relation to this article authors affirmed that they have no any conflict of interest.

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.

References:

1. Abhyankar, L. N., Jones, M. R., Guallar, E., & Navas-Acien, A. (2012). Arsenic exposure and hypertension: a systematic review. *Environmental health perspectives*, *120*(4), 494–500. https://doi.org/10.1289/ehp.1103988
2. Alam, P., Abdel-Kader, M. S., Alqarni, M. H., Zaatout, H. H., Ahamad, S. R., & Shakeel, F. (2019). Chemical composition of fennel seed extract and determination of fenchone in commercial formulations by GC-MS method. *Journal of food science and technology*, *56*(5), 2395–2403. https://doi.org/10.1007/s13197-019-03695-9.
3. Aruldhas MM, Subramanian S, Sekar P, Vengatesh G, Chandrahasan G, Govindarajulu P, et al. Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (Macaca radiate Geoffroy). Human Reprod. 2005;20(10):2801–2813.
4. Barai, M., Ahsan, N., Paul, N., Hossain, K., Abdur Rashid, M., Kato, M., Ohgami, N., & Azim Akhand, A. (2017). Amelioration of arsenic-induced toxic effects in mice by dietary supplementation of Syzygium *cumini* leaf extract. *Nagoya journal of medical science*, *79*(2), 167–177. https://doi.org/10.18999/nagjms.79.2.167
5. Bayrami, A., Shirdel, A., Rahim Pouran, S., Mahmoudi, F., Habibi-Yangjeh, A., Singh, R., & Abdul Raman, A. A. (2020). Co-regulative effects of chitosan-fennel seed extract system on the hormonal and biochemical factors involved in the polycystic ovarian syndrome. *Materials science & engineering. C, Materials for biological applications*, *117*, 111351.

https://doi.org/10.1016/j.msec.2020.111351.
6. Chakraborti, D., Mukherjee, S. C., Pati, S., Sengupta, M. K., Rahman, M. M., Chowdhury, U. K., Lodh, D., Chanda, C. R., Chakraborti, A. K., & Basu, G. K. (2003). Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger?. Environmental health perspectives, 111(9), 1194–1201. https://doi.org/10.1289/ehp.5966.
7. Chakraborti, D., Rahman, M. M., Ahamed, S., Dutta, R. N., Pati, S., & Mukherjee, S. C. (2016). Arsenic contamination of groundwater and its induced health effects in Shahpur block, Bhojpur district, Bihar state, India: risk evaluation. Environmental science and pollution research international, 23(10), 9492–9504. https://doi.org/10.1007/s11356-016-6149-8.
8. Christou, M. A., Christou, P. A., Markozannes, G., Tsatsoulis, A., Mastorakos, G., & Tigas, S. (2017). Effects of Anabolic Androgenic Steroids on the Reproductive System of Athletes and Recreational Users: A Systematic Review and Meta-Analysis. *Sports medicine (Auckland, N.Z.)*, *47*(9), 1869–1883. https://doi.org/10.1007/s40279-017-0709-z
9. Da Silva, S. N., Gimenez, T., Souza, R. C., Mello-Moura, A. C. V., Raggio, D. P., Morimoto, S., Lara, J. S., Soares, G. C., & Tedesco, T. K. (2017). Oral health status of children and young adults with autism spectrum disorders: systematic review and meta-analysis. *International journal of paediatric dentistry*, *27*(5), 388–398. https://doi.org/10.1111/ipd.12274.
10. Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R., & Sadeghi, M. R. (2018). Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology*, *16*, 1-14.
11. Delwatta, S. L., Gunatilake, M., Baumans, V., Seneviratne, M. D., Dissanayaka, M. L. B., Batagoda, S. S., Udagedara, A. H., & Walpola, P. B. (2018). Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. *Animal models and experimental medicine*, *1*(4), 250–254. https://doi.org/10.1002/ame2.12041.
12. Fakunle, P. B., Abijo, A. Z., Ehiremen, S. E., Akanji, O. D., Odubela, O. K., Olamoyero, B., ... & Dawodu, A. J. (2024). Subacute Use of Aqueous Extract of Water Melon (Citrullus lanatus) Seeds Mitigates against Pyramidal Neuronal Toxicity of Lead Acetate in Adult Wistar Rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, *13*(2), 1-12.
13. Greaves, P. (2011). *Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation*. Academic Press.
14. Gul, M., Hildorf, S., Dong, L., Thorup, J., Hoffmann, E. R., Jensen, C. F. S., Sønksen, J., Cortes, D., Fedder, J., Andersen, C. Y., & Goossens, E. (2020). Review of injection techniques for spermatogonial stem cell transplantation. *Human reproduction update*, *26*(3), 368–391. https://doi.org/10.1093/humupd/dmaa003.
15. Hamza, M., Alam, S., Rizwan, M., & Naz, A. (2022). Health risks associated with arsenic contamination and its biotransformation mechanisms in environment: a review. *Hazardous Environmental Micro-pollutants, Health Impacts and Allied Treatment Technologies*, 241-288.
16. Hosseini, E., Majidi, M. M., Saeidnia, F., & Ehtemam, M. H. (2022). Genetic analysis and physiological relationships of drought response in fennel: Interaction with mating system. *PloS one*, *17*(11), e0277926. https://doi.org/10.1371/journal.pone.0277926.
17. Jahan, S., Rehman, S., Ullah, H., Munawar, A., Ain, Q. U., & Iqbal, T. (2016). Ameliorative effect of quercetin against arsenic-induced sperm DNA damage and daily sperm production in adult male rats. *Drug and chemical toxicology*, *39*(3), 290–296. https://doi.org/10.3109/01480545.2015.1101772.
18. Khushboo, M., Murthy, M. K., Devi, M. S., Sanjeev, S., Ibrahim, K. S., Kumar, N. S., Roy, V. K., & Gurusubramanian, G. (2018). Testicular toxicity and sperm quality following copper exposure in Wistar albino rats: ameliorative potentials of L-carnitine. *Environmental science and pollution research international*, *25*(2), 1837–1862. https://doi.org/10.1007/s11356-017-0624-8
19. Kumar A, Ali M, Kumar R, Rahman MS, Srivastava A, Chayal NK, Sagar V, Kumari R, Parween S, Kumar R, Niraj PK, Anand G, Singh SK, Ghosh AK (2020a): High Arsenic Concentration in Blood Samples of People of Village Gyaspur Mahaji, Patna, Bihar Drinking Arsenic-Contaminated Water. Springer Nature Journal Exposure and Health, 12, 131–140 (published print version 2020). https://doi.org/10.1007/s12403-018-00294-5.
20. Kumar A, Ali Md, Rahman S Md, Iqubal A Md, Anand G, Niraj P.K, Shankar P and Kumar R (2015a): Ground Water Arsenic Poisoning in "Tilak Rai Ka Hatta" Village of Buxar District, Bihar, India Causing Severe Health Hazards and Hormonal Imbalance. J Environ Anal Toxicol 5:290. https://doi.org/10.4172/2161-0525.1000290.

21. Kumar A, Kumar R, Rahman MS, Iqubal M, Ali M, Niraj PK, Anand G, Prabhat K., Abhinav & Ghosh A.K. (2016) : Ground water arsenic contamination: A local survey in India. Int J Prev Med ;7:100. https://doi.org/10.4103/2008-7802.188085.
22. Kumar A, Rahman MS, Kumar R, Ali M, Niraj PK, Srivastava A, Singh SK and Ghosh AK. (2019a) Arsenic contamination in groundwater causing impaired memory and intelligence in school children of Simri village of Buxar district of Bihar. J Mental Health Hum Behav;24:132-8. https://doi.org/10.4103/jmhhb.jmhhb_31_18.
23. Kumar A., Ghosh A.K. (2021d) Assessment of Arsenic Contamination in Groundwater and Affected Population of Bihar. In: Kumar N. (eds) Arsenic Toxicity: Challenges and Solutions. Springer, Singapore. https://doi.org/10.1007/978-981-33-6068-6_7.
24. Kumar, A., & Ghosh, A. K. (2019b). Arsenic and Cancer. In P. Papadopoulou, C. Marouli, & A. Misseyanni (Ed.), Environmental Exposures and Human Health Challenges (pp. 106-132). IGI Global. https://doi.org/10.4018/978-1-5225-7635-8.ch005.
25. Kumar, A., Ali, M., Kumar, R., Kumar, M., Sagar, P., Pandey, R. K., Akhouri, V., Kumar, V., Anand, G., Niraj, P. K., Rani, R., Kumar, S., Kumar, D., Bishwapriya, A., & Ghosh, A. K. (2021a). Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk. Scientific reports, 11(1), 2376. https://doi.org/10.1038/s41598-021-81579-9.
26. Kumar, A., Ali, M., Raj V, Kumari A, Rachamalla M, Niyogi S, Kumar D, Sharma A, Saxena A, Panjawani G, Jain P, Vidyarthi A, Kumar N, Kumar M, Niraj PK, Rahman MS, Bishwapriya A, Kumar R, Sakamoto M, Kumar S, Singh M, Ghosh AK. (2023). Arsenic causing gallbladder cancer disease in Bihar. Scientific reports, 13(1), 4259. https://doi.org/10.1038/s41598-023-30898-0.
27. Kumar, A., Kumar, R, Rahman, MS., Iqubal,A., Anand,G., Niraj,P.K. &. Ali, M. (2015[b]) : Phytoremedial effect of *Withania somnifera* against arsenic-induced testicular toxicity in Charles Foster Rats. Avicenna Journal of Phytomedicine, 5 (4) : 355-364.
28. Kumar, A., Kumar, R., Rahman, M. S., Ali, M., Kumar, R., Nupur, N., Gaurav, A., Raj, V., Anand, G., Niraj, P. K., Kumar, N., Srivastava, A., Biswapriya, A., Chand, G. B., Kumar, D., Rashmi, T., Kumar, S., Sakamoto, M., & Ghosh, A. K. (2021b). Assessment of arsenic exposure in the population of Sabalpur village of Saran District of Bihar with mitigation approach. Environmental science and pollution research international, 10.1007/s11356-021- 13521-5. Advance online publication. https://doi.org/10.1007/s11356-021-13521-5.
29. Kumar, A., Kumar, V., Akhouri, V., Kumar R., Ali., M, Rashmi T., Chand G.B., Singh S.K., Ghosh A.K. (2022d) Protective efficacy of Coriandrum sativum seeds against arsenic induced toxicity in Swiss albino mice. Toxicol Res. (2022). https://doi.org/10.1007/s43188-022- 00123-7.
30. Kumar, A., Rahman, M. S., Ali, M., Salaun, P., Gourain, A., Kumar, S., Kumar, R., Niraj, P. K., Kumar, M., Kumar, D., Bishwapriya, A., Singh, S., Murti, K., Dhingra, S., Sakamoto, M., & Ghosh, A. K. (2022a). Assessment of disease burden in the arsenic exposed population of Chapar village of Samastipur district, Bihar, India, and related mitigation initiative. Environmental science and pollution research international, 29(18), 27443–27459. https://doi.org/10.1007/s11356-021-18207-6.
31. Kumar, A., Rahman, M.S., Ali, M., Kumar, R., Niraj, P.K., Akhouri, V., Singh, S.K., Kumar, D., Rashmi, T., Bishwapriya, A., Chand G.B., Sakamoto, M., Ghosh, A.K., (2021c). Assessment of arsenic exposure and its mitigation intervention in severely exposed population of Buxar district of Bihar, India. Toxicol. Environ. Health Sci. https://doi.org/10.1007/s13530-021-00086-6.
32. Kumar, A., Raj, V., Srivastava, A., Ali, M., Ghosh, A. K., Rachamalla, M., & Kumar, D. (2022c). Autophagy in arsenic exposed population and cancer patients. In Autophagy and Metabolism (pp. 141-161). Academic Press. https://doi.org/10.1016/B978-0-323-99879- 6.00010-9.
33. Kumar, A., Ravi, C., Dhingra, S., Krishna Murti, M. A., & Ghosh, A. K. (2022b). Arsenic Causing Gallbladder Cancer Disease near the Himalayan bound Rivers in Bihar: A Case study of Gallbladder Cancer. Journal of Cancer Science and Clinical Therapeutics, 6, 388-391. https://doi.org/10.26502/jcsct.5079178.
34. Kumar, V., Akhouri, V., Singh, S. K., & Kumar, A. (2020b). Phytoremedial effect of Tinospora cordifolia against arsenic induced toxicity in Charles Foster rats. Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine, 33(6), 379–396. https://doi.org/10.1007/s10534-020-00256-y.
35. Logaraj, T. V. (2011). Watermelon (*Citrullus lanatus* (Thunb.) Matsumura and Nakai) seed oils and their use in health. In Nuts and seeds in health and disease prevention (pp. 1149- 1157). Academic Press. https://doi.org/10.1016/B978-0-12-375688-6.10136-7.
36. Malini, T., Vanithakumari, G., Megala, N., Anusya, S., Devi, K., & Elango, V. (1985). Effect of

Foeniculum vulgare Mill. seed extract on the genital organs of male and female rats. *Indian journal of physiology and pharmacology*, *29*(1), 21–26.

37. Michael F. Hughes, Barbara D. Beck, Yu Chen, Ari S. Lewis, David J. Thomas, Arsenic Exposure and Toxicology: A Historical Perspective, *Toxicological Sciences*, Volume 123, Issue 2, October 2011, Pages 305–332, https://doi.org/10.1093/toxsci/kfr184
38. Mishra, P., Negi, M. P. S., Srivastava, M., Singh, K., & Rajender, S. (2018). Decline in seminal quality in Indian men over the last 37 years. *Reproductive biology and endocrinology: RB&E*, *16*(1), 103. https://doi.org/10.1186/s12958-018-0425-z
39. Monton, A., Gil, L., Malo, C., Olaciregui, M., Gonzalez, N., & de Blas, I. (2015). Sage (Salvia officinalis) and fennel (Foeniculum vulgare) improve cryopreserved boar epididymal semen quality study. Cryo letters, 36(2), 83–90.
40. Munglue, P., Kupittayanant, S., & Kupittayanant, P. (2014). Effect of watermelon (Citrullus lanatus) flesh extract on sexual behavior of male rats. *Chiang Mai University Journal of Natural Sciences*, *13*(1), 519.
41. Nadeem, M., Navida, M., Ameer, K., Iqbal, A., Malik, F., Nadeem, M. A., ... & Din, A. (2022). A comprehensive review on the watermelon phytochemical profile and their bioactive and therapeutic effects. *Food Science and Preservation*, *29*(4), 546-576.
42. Najafi, A., Daghigh Kia, H., Mehdipour, M., Shamsollahi, M., & Miller, D. J. (2019). Does fennel extract ameliorate oxidative stress frozen-thawed ram sperm?. *Cryobiology*, *87*, 47–51. https://doi.org/10.1016/j.cryobiol.2019.02.006.
43. Nejatbakhsh, R., Riyahi, S., Farrokhi, A., Rostamkhani, S., Mahmazi, S., Yazdinezhad, A., Kazemi, M., & Shokri, S. (2017). Ameliorating effects of fennel and cumin extracts on sperm quality and spermatogenic cells apoptosis by inducing weight loss and reducing leptin concentration in diet-induced obese rats. *Andrologia*, *49*(8), 10.1111/and.12748. https://doi.org/10.1111/and.12748.
44. Nickson, R., Sengupta, C., Mitra, P., Dave, S. N., Banerjee, A. K., Bhattacharya, A., Deverill, P. (2007). Current knowledge on the distribution of arsenic in groundwater in five states of India. *Journal of Environmental Science and Health, Part A*, *42*(12), 1707–1718. https://doi.org/10.1080/10934520701564194.
45. Niraj, P. K., Singh, R. V., Shankar, P., Ghosh, A. K., & Kumar, A. (2024). Protective And Antidote Effect Of Foeniculum vulgare Against Sodium Arsenite Induced Hepatotoxicity And Testicular Toxicity In Charles Foster Rats. Journal of Advanced Zoology, 45(3). https://doi.org/10.53555/jaz.v45i3.4270.
46. Rahman MS, Kumar A, Kumar R, Ali M, Ghosh AK, Singh SK. (2019[a]): Comparative quantification study of arsenic in the groundwater and biological samples of Simri village of Buxar District, Bihar, India. Indian J Occup Environ Med;23: 126-32.
47. Rahman SMD, Kumar A, Kumar R, Ali M, Singh S.K and Ghosh AK, (2019[b]) Hematological and Free Radicals Changes among People of Arsenic Endemic Region of Buxar District of Bihar, India. Int J Pub Health Safe 4: 178.
48. Rango, T., Vengosh, A., Dwyer, G., & Bianchini, G. (2013). Mobilization of arsenic and other naturally occurring contaminants in groundwater of the Main Ethiopian Rift aquifers. *Water research*, *47*(15), 5801–5818. https://doi.org/10.1016/j.watres.2013.07.002
49. Ratnaike R. N. (2003). Acute and chronic arsenic toxicity. *Postgraduate medical journal*, *79*(933), 391–396. https://doi.org/10.1136/pmj.79.933.391
50. Rotimi, D. E., & Asaleye, R. M. (2023). Impact of Watermelon (Citrallus lanatus) on Male Fertility. *JBRA assisted reproduction*, *27*(4), 702–708. Advance online publication. https://doi.org/10.5935/1518-0557.20220075.
51. Shahat, A. A., Ibrahim, A. Y., Hendawy, S. F., Omer, E. A., Hammouda, F. M., Abdel- Rahman, F. H., & Saleh, M. A. (2011). Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. *Molecules (Basel, Switzerland)*, *16*(2), 1366–1377. https://doi.org/10.3390/molecules16021366.
52. Shankar, P., Singh, R. V., & Kumar, A. (2023). Therapeutic Protection of Arsenic-Induced Oxidative Stress and Hepato-Nephro Toxicity by Syzygium cumini (Seed) Ethanolic Extract (SCEE) in Charles Foster Rats. Toxicology International (Formerly Indian Journal of Toxicology), 207-224. https://doi.org/10.18311/ti/2023/v30i2/32429.
53. Shankar, P., Singh, R. V., & Kumar, A. (2024). Bio-Remedial Impact of Elaeocarpus sphaericus Seed Extract (ESSE) Against Sodium Arsenite (As)-Induced Nephrotoxicity in Charles Foster Rats. Journal of Advanced Zoology, 45(3) https://doi.org/10.53555/jaz.v45i3.3974.
54. Simeonova, D. D., Lièvremont, D., Lagarde, F., Muller, D. A., Groudeva, V. I., & Lett, M. C. (2004).

Microplate screening assay for the detection of arsenite-oxidizing and arsenate-reducing bacteria. *FEMS microbiology letters*, *237*(2), 249–253. https://doi.org/10.1016/j.femsle.2004.06.040

55. Skinner, M. K., Manikkam, M., & Guerrero-Bosagna, C. (2010). Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends in endocrinology and metabolism: TEM*, *21*(4), 214–222. https://doi.org/10.1016/j.tem.2009.12.007.
56. Sutton, P., Woodruff, T. J., Perron, J., Stotland, N., Conry, J. A., Miller, M. D., & Giudice, L. C. (2012). Toxic environmental chemicals: the role of reproductive health professionals in preventing harmful exposures. *American journal of obstetrics and gynecology*, *207*(3), 164–173. https://doi.org/10.1016/j.ajog.2012.01.034.
57. Tseng, W., & Newton, F. (2002). International students' strategies for wellbeing. College Student Journal, 36, 591-597
58. Wahid, M., & Saqib, F. (2022). Scientific basis for medicinal use of *Citrullus lanatus* (Thunb.) in diarrhea and asthma: In vitro, in vivo and in silico studies. Phytomedicine: international journal of phytotherapy and phytopharmacology, 98, 153978. https://doi.org/10.1016/j.phymed.2022.153978.
59. Yakut, H. I., Koyuncu, E., Cakir, U., Tayman, C., Koyuncu, İ., Taskin Turkmenoglu, T., Cakir, E., Ozyazici, A., Aydogan, S., & Zenciroglu, A. (2020). Preventative and therapeutic effects of fennel (Foeniculum vulgare) seed extracts against necrotizing enterocolitis. Journal of food biochemistry, 44(8), e13284. https://doi.org/10.1111/jfbc.13284.
60. Zargari, F., Rahaman, M. S., KazemPour, R., & Hajirostamlou, M. (2022). Arsenic, Oxidative Stress and Reproductive System. *Journal of xenobiotics*, *12*(3), 214–222. https://doi.org/10.3390/jox12030016.
61. Zhao, H. P., Gao, Y. F., Xia, D., Zhao, Z. Q., Wu, S., Wang, X. H., Liu, H. X., Xiao, C., Xing, X. M., & He, Y. (2018). *Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]*, *52*(5), 538–544. https://doi.org/10.3760/cma.j.issn.0253-9624.2018.05.014.