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Protective And Antidote Effect Of *Foeniculum vulgare* Against Sodium Arsenite Induced Hepatotoxicity And Testicular Toxicity In Charles Foster Rats

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Abstract:

Arsenic poisoning in groundwater is the most common environmental pollutant, which is leading to serious pollution worldwide. Chronic arsenic exposure from drinking water to humans causes major public health-related issues. The present study was conducted to investigate the antidote effects of *Foeniculum vulgare* (Fennel) against arsenic-induced hepatotoxicity and testicular toxicity in Charles Foster rats.

In the present study, twenty-four male Charles Foster rats (120±5gm) were divided into four Groups (n=6), where control Group-I received a normal diet and water; Group - II and Group - III received sodium arsenite (8 mg per kg body weight per day) for 90 days. Group III was left with a normal diet and water for the next 60 days for auto-recovery. The group IV rats were administered *Foeniculum vulgare* (Funnel) hydroxyl ethanolic seed extract at a dose of 150 mg per kg body weight for 60 days in a 90-day pre-treated sodium arsenite group (8 mg per kg body weight). After complete dose duration, all the treated animals were sacrificed the same day for haematological, biochemical, hormonal, and histopathological studies.

In the arsenic treated rats, there were significant ($p<0.001$) changes in serum levels of SGPT, SGOT, urea, uric acid and creatinine as well as in haematological parameters. And there was also decease in the sperm count and sperm motility, accompanied by an increased incidence of sperm abnormalities and hormonal imbalances leading to infertility. In contrast, after the administration of *F. vulgare* seeds hydroxy-ethanolic extract to arsenic-treated rats, significant ($p<0.001$) improvements were observed in hepatic and renal parameters as well as haematological parameters. In the arsenic-intoxicant rat, after administration of *F. vulgare* seeds hydroxyl ethanolic extract, there was a significant ($p<001$) reduction in the arsenic concentration in blood, liver, and kidney tissues as well as serum LPO.

The histopathological study also showed the *F. vulgare* seeds hydroxy-ethanolic extract significantly restored the cellular integrity of testicular cells, leading to their normal functioning against arsenic-induced toxicity.

Key words: Sodium arsenite; Foeniculum vulgare; Charles Foster rats; antidote effect, protective effect.

1. Introduction

Arsenic (As), a naturally occurring metalloid, has been a major concern for the environment due to its adverse effects on human health. This metalloid is also known as a chronic poison and a non-threshold carcinogen. These toxicants are ubiquitously present all around the world. Globally, 300 million people from around 50 countries are exposed to arsenic through drinking arsenic-contaminated groundwater (Kumar et al., 2021). The contamination of groundwater with arsenic occurs from natural geological sources and also from anthropogenic activities (Hassan 2018; Kumar et al., 2022a; Richards 2022, 2021, 2020). Anthropogenic activities include the combustion of fossil fuels, mining, utilisation of arsenical pesticides, herbicides, and agricultural condiments for livestock, which are responsible for enhancing arsenic in groundwater and soil (Das, et al.; 2015).

Arsenic has two forms: organic and inorganic, and scientifically, the inorganic arsenic AsIII is the more toxic species than AsV (Mukherjee, et., al, 2006). Moreover, it has been reported that this contamination is a world-wide serious issue and a substantial risk factor in most of the countries, including China, the U.S.A., India, Pakistan, Bangladesh, Mexico and Argentina. Human revelation to arsenic is through oral routes involving food and water or through inhalation of agricultural pesticides (Rahman, et al., 2019; Landrigan et al., 2018; Shahid et al., 2018). Exposure to arsenic causes several harmful effects, such as keratosis and melanosis in the skin, liver, kidneys, lungs, bladder and lymphatic system (Mukherjee et al., 2006). In Bihar, out of 38 districts, presently 22 districts are severely affected by groundwater arsenic poisoning, which has posed health hazards to the exposed population (Chakraborti et al., 2003 & 2016; Kumar 2022a).

Chronic arsenic exposure is reported to cause cardiovascular, respiratory, hepatic, haematological, neurological, diabetes, cancer and reproductive effects in humans (Kumar et al., 2021[a,b,c,d], 2019[a,]b; Rahman et al., 2019[a,b]). Apart from this, the long-term arsenic exposure in the Gangetic plains of Bihar, in the present time has caused eruptions of cancer incidences such as skin cancer, liver cancer, bladder cancer, colorectal cancer, breast cancer and gallbladder cancer (Kumar et al., 2022[b,c]; 2021[a,b,c,d]; 2020[a,b]; 2016; 2015). Prenatal exposure to inorganic arsenic causes adverse pregnancy outcomes and children's health problems. Some epidemiological studies have reported that arsenic exposure causes premature delivery, spontaneous abortion, and stillbirth. In animal studies, inorganic arsenic also causes fetal malformation, growth retardation, and fetal death. In males, inorganic arsenic causes reproductive dysfunctions, including reductions of the testis weights, accessory sex organ weights, and epididymal sperm counts (Minas et al., 2018; Khatun et al., 2018; Kumar et al., 2015).

In addition, inorganic arsenic exposure also induces alterations of spermatogenesis, reductions of testosterone and gonadotrophins, and disruptions of steroidogenesis. However, the reproductive and developmental problems following arsenic exposure are poorly understood, and the molecular mechanism of arsenic-induced reproductive toxicity remains unclear. The scarcity of treatment options to manage this affected population has made the situation much worse (Barai et al., 2017).

Foeniculum vulgare (F. vulgare), common name fennel, sweet fennel, family Umbelliferae is a common herb that grows in many countries, especially in the Mediterranean region. It has been known as a diuretic, an emmenagogue, as well as an antibacterial (Said, 1973; Mukenstrum et al., 1997; Kaur et al., 2009). The morphology, ethnomedicinal applications, phytochemistry, pharmacology, and toxicology of *F. vulgare* were extensively reviewed (Badgujar et al., 2014). Recently, the ripe fruit of *F. vulgare* has been widely utilized in Arabian folk medicine systems as a stimulant, digestive, appetizer, diuretic, and infantile febrifuge.

Several studies have shown the importance of *F. vulgare* as a folk medicine in the Arabian Peninsula. However, the toxic effect of the plant is poorly studied (Shah et al., 1991). Hence, the present study aims to observe the antidote effect of arsenic in rats using *F. vulgare.*

2. Materials and methods

2.1. Ethical 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).

2.2 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.

2.3. Plant selected for study as antidote

Foeniculum vulgare (Fennel) seeds were purchased from a local market in Patna, Bihar, India. The seeds of *F. vulgare* were identified by Dr. Ashok Kumar Ghosh (Botanist), MCSRC. The plant seeds were washed through running tap water to remove the soil and dirt, then rinsed with distilled water. The seeds were dried at 37°C and thereafter grinded to a fine powder. Then fine powder was sieved and weighed 150mg, mixed in 10 ml of distilled water (pre-mixed in 5% alcohol) to make hydroxyl-ethanolic extract and vortexed rigorously for 2 hours for complete mixing up of the compounds in the solution to make it suitable for delivery to the treated animals.

2.4. Animals

Twenty-four healthy male Charles Forster rats, weighing 120±5gm g at 8 weeks old, were obtained from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, India. The rats were acclimatized in a laboratory house under 12-hour light and dark cycles (room temperature at 22±2 °C) for 7 days before the start of the treatment. 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.*

2.5. 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 8 mg/kg body weight. The sodium arsenite dose was dissolved in 10 ml of distilled water and administered intragastrically by gavage method.

For *Foeniculum vulgare* seeds (*F. vulgare*) extract, the dose was calculated after LD_{50} estimation. The dose was finally titrated to $1/8^{th}$ dose of 150 mg/kg body weight. For this, 150 mg of *F. vulgare* seed extract were dissolved in 5% hydroxyethanol and then delivered to the rats in their respective experimental groups.

2.6. 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: Arsenic treated – Rats were intragastrically induced with (gavage method) sodium arsenite 8 mg/kg body weight/day for 90 days.

Group III- Arsenic treated – Rats were intragastrically induced with (gavage method) sodium arsenite 8 mg/kg body weight/day for 90 days and were left for auto recovery as arsenic control.

Group IV- *F. vulgare* seeds extract treated- Rats were administered orally 150 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.

After the completion of the entire treatment, all groups and subgroups of rats were anaesthetized and sacrificed. Blood samples were collected through the orbital puncture in EDTA-coated and plain vacutainers. Serum was separated for the liver function test, kidney function test, lipid peroxidation estimation and ELISA test. Tissues from the liver and kidney were also dissected for histopathological studies and arsenic concentration determination.

3. Haematological assay:

The haematological indices like haemoglobin (Hb) were measured by the micro-haematocrit method using capillary tubes called Sahli̇s method (1962), while red blood corpuscles (RBCs) (1969) and white blood corpuscles (WBCs) (1969) were counted manually using an improved Neubauer counting chamber (Delwatta et al., 2018).

4. 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).

4.1. 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 Grofs (1938).

4.2. Determination of Kidney function Test

The Kidney function tests (KFT) was analyzed through urea by the method of Fawcett and Scott (1960); Berthelot (1859); creatinine by the method of Bones and Tausky (1945); and uric acid by the method of Fossati and Prencipe (1980), while albumin levels were measured according to Doumas and Watson (1971).

4.3. 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 (UVVis) spectrophotometer (USA) at 532 nm.

4.4. Hormonal Assay

4.5. 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 1 ml 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.

5. 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 5 µm 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.

6. Hormonal Assay

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

7. 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 arsenic + *F.vulgare* were compared with the arsenic-treated group.

8. Results

8.1 Haematological study

Table 1. Showing Comparative levels of haematological parameters in control and treatment groups

Haematological Parameters	**Control**	**Arsenic Treated**	**Arsenic Treated Control**	***F.vulgare* Treated**
RBC Counts (10^6 /mm^3)	4.618 ± 0.1432***	2.493 ± 0.1947***	2.998 ±0.2183	6.400 ± 0.3642***
WBC Counts (10^3 /mm^3)	4865±366.8	12996 ±518.1	11767±328.3	7583±311.3
Haemoglobin Percentage	13.23±0.2616	6.617±0.3198	7.583±0.1956	12.18±0.2651

Haematological parameters in various groups of rats (n=6), values are expressed as mean ±SE
*** ($p<0.001$); *($p<0.05$); ns (non-significant) compared to control group (Bonferroni multiple comparison)

7.2 Biochemical study

The SGPT level shows significant ($p<0.001$) increase in the levels of SGPT, SGOT, ALP and bilirubin levels in comparison to control. Moreover, there was very mild restoration in the arsenic control group. But, in *F.vulgare* administered rat group (on arsenic pretreated group) there was significant normalization in the levels (Figure 1).

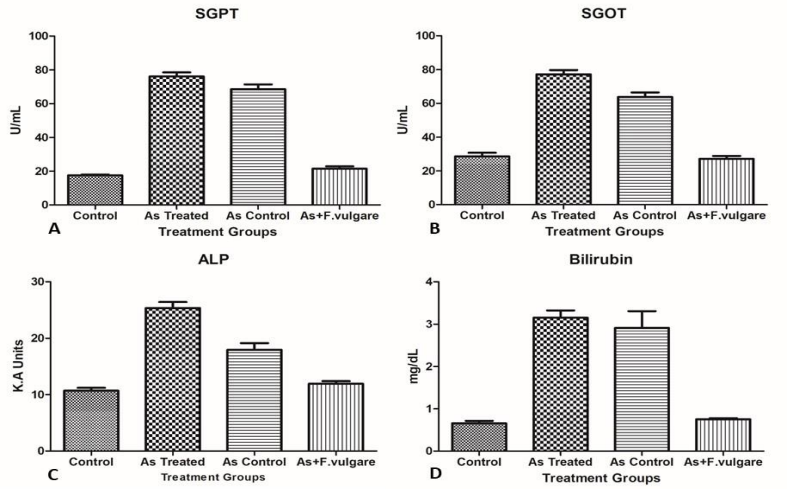


Figure 1. Graphs showing biochemical parameters – Liver function test parameters – with levels of SGPT, SGOT, alkaline phosphatase and bilirubin in control, arsenic treated, arsenic control and *F. vulgare* treated groups. All data values are expressed as Mean ±SE.

7.3 Sperm Counts

The sperm count levels show a significant ($p<0.001$) decrease in levels in comparison to the control. Moreover, there was a mild restoration of arsenic control. However, *F.vulgare* administration in the arsenic-treated group showed significant ($p<0.001$) restoration in sperm count levels compared to the arsenic-treated group (Figure 2).

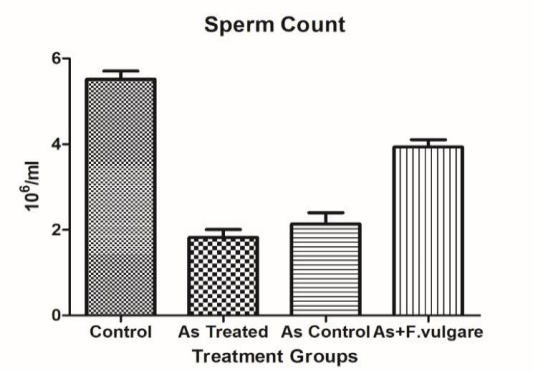


Figure 2. Graph Showing Sperm level of rat in control, arsenic treated, arsenic control and *F.vulgare* treated groups. All data values are expressed as Mean ±SE.

7.4. Hormonal Study

The testosterone level shows a significant ($p<0.001$) decrease in level in comparison to the control. Moreover, there was mild restoration in As Control. However, *F. vulgare* administration in the arsenic treated group showed significant ($p<0.001$) restoration in testosterone levels compared to the arsenic-treated group (Figure 3).

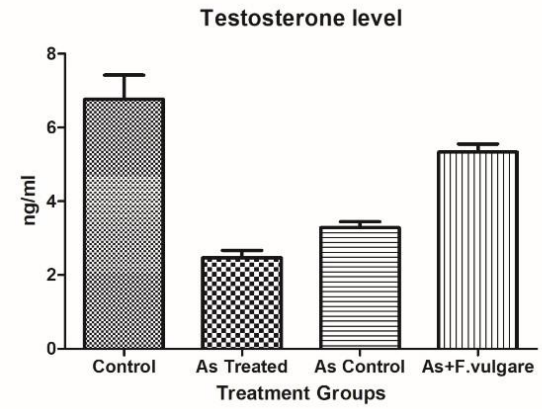


Figure 3. Graph Showing Testosterone level of rat in control, arsenic treated, arsenic control and *F. vulgare* treated groups. All data values are expressed as Mean ±SE.

The LH level shows a significant ($p<0.001$) increase in luteinizing hormone levels in comparison to the control. Moreover, there was a very mild restoration in the arsenic control group. But, in the *F.vulgare*-administered rat group (on arsenic-pretreated group), there was significant normalization in the LH level (Figure 4).

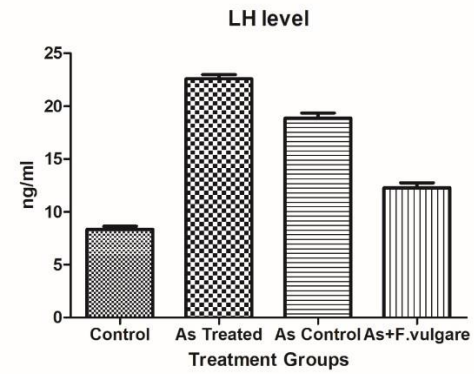


Figure 4. Graph Showing LH level of rat in control, arsenic treated, arsenic control and *F.vulgare* treated groups. All data values are expressed as Mean ±SE.

7.5. Lipid Peroxidation study

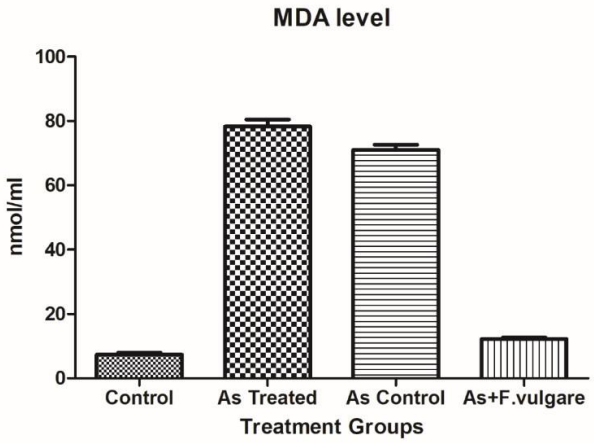
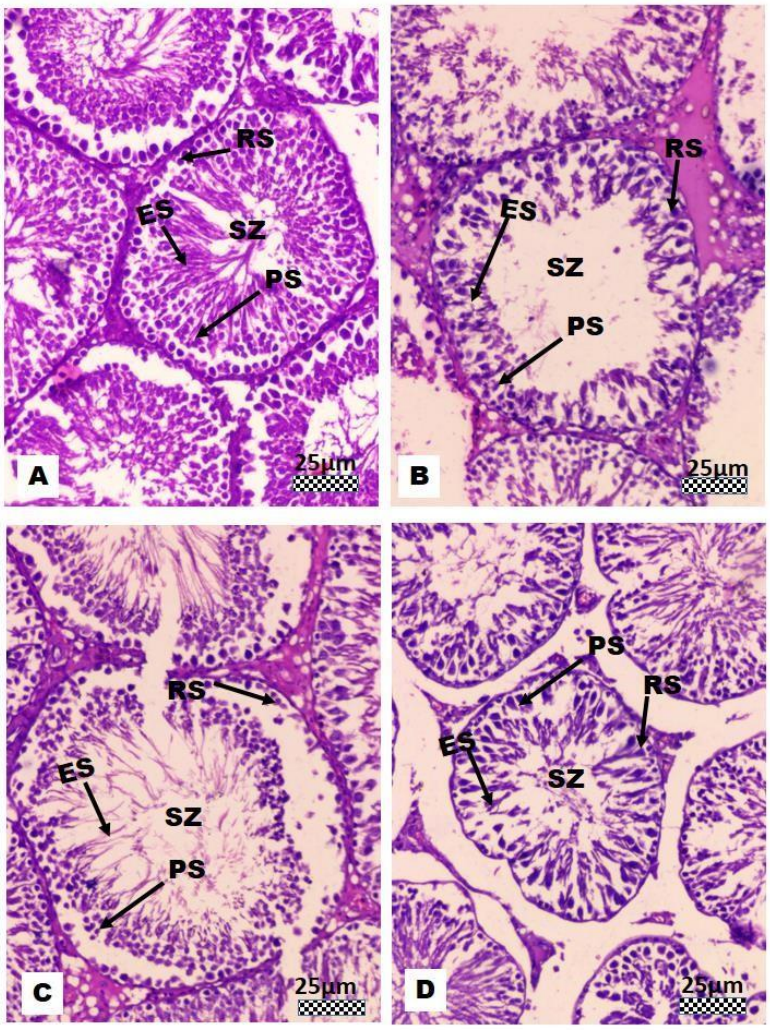


Figure 5. Comparative level of malondialdehyde in various group of rats.

The serum level of MDA was significantly ($p<0.001$) elevated in the arsenic treated rats compared to the control group. The arsenic-pretreated rats, which were left for auto recovery without any treatment for 60 days, had mild restoration. However, *F.vulgare* administration in arsenic-pretreated rats had a significant ($p<0.001$) reduction compared to arsenic-pretreated rats (Figure 5).

7.6. 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, arsenic-treated testicular cells showed severe damage in the seminiferous tubules, which indicates abnormal functioning of the testicular cells. The leydig cells also appeared to be in a very highly degenerative condition, as haemorrhage was seen. In arsenic-controlled testicular damage, the same as arsenic-treated cells, the Leydig cells appeared to have a degenerative condition. However, after administration of *Foeniculum vulgare***,** there was immense amelioration, as restoration in the spermatogenetic stages could be observed. The primary spermatocytes, spermatogonia, spermatids, and spermatozoa are all well-arranged, denoting the significant normalisation in the function of the testicular cells. The Leydig cells also showed amelioration, which denotes normalisation in their function (Figure. 6).



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

Figure-6: Microphotograph of a testis section stained with haematoxylin and eosin (A). Testis of the control rat show 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 the absence of spermatogenic series in the tubular lumen (H&E×500). (C). Section of arsenic pretreated testis sowing persistence of degeneration due to arsenic toxicity RS, PS and SZ (H&E×500). (D). A section of *F.vulgare* administered to the arsenic-pretreated group shows restoration in testicular toxicity (H&E×500).

Discussion:

Haematological parameters are the biological indicators of the body, which, in terms of intoxication, inflammation, or disease, are being reflected on them. In the present study, in the arsenic treated rats, there was a significant decrease ($p<0.001$) in the haematological parameters such as RBC counts, WBC counts and haemoglobin percentage. But, after the administration of *F.vulgare*, there was significant restoration ($p<0.001$) in the haematological parameters (Khan et al., 2022; Dai et al., 2020).

The biochemical parameters are an essential part of the diagnosis to determine the level of changes occurring at the organ system level. In the liver function tests, the SGPT and the SGOT are the glucose biomarkers, while alkaline phosphatase and bilirubin are enzyme markers of the liver. In the present study, there was a significant increase ($p<0.001$) in the SGPT, SGOT, ALP and bilirubin levels in comparison to the control (Bayrami et al., 2022). There was a nonsignificant normalization of the levels. But, after the administration of *F.vulgare*, there was significant normalization in the levels, which indicates that *F.vulgare* has hepatoprotective properties (Zhang et al., 2017)

The kidney function test measures the enzyme markers that help in the elimination of toxicants from the body via urine, sweat, or other fluids. In the present study, there was a significant increase in urea, uric acid and creatinine levels in the arsenic-treated rat group in comparison to the control and arsenic control groups. But

there was significant normalization in these levels after the administration of *F.vulgare*, which denotes nephroprotective properties (Alghamdi S. et al., 2020).

Hormones are an essential part of the system that regulates the major functions of the body. Testosterone and the luteinizing hormones are the important hormones of the male reproductive system (Abbas et al., 2020). In the present study, there was a significant ($p<0.001$) decrease in the testosterone levels, while a significant increase ($p<0.001$) in the luteinizing hormone was observed in the arsenic-treated groups in comparison to the control group of rats. There were very mild changes in hormone levels observed in the arsenic control group of rats. But, after the administration of *F.vulgare*, there was significant normalization in the levels of the hormones, which indicates that *F.vulgare* possesses properties that regulate and control the proper hormone functions of the body.

Similarly, arsenic toxicity causes severe damage to the lipids of the membranes by depleting them through lipid peroxidation activity (Barai et al., 2017; Jahan et al., 2016). This causes a loss of membrane activity and cell integrity. In the present study also, there was a 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

F.vulgare, there was a significant ($p<0.001$) normalization in the levels of lipid peroxidation, which indicates that its medicinal properties possess antioxidant activities (Hosseini et al., 2022; Najafi et al., 2019).

Histopathological studies are an 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. In the present study, the histopathological study of the 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, which indicates that it has a damaging effect. Moreover, there was no significant auto-restoration observed in the arsenic control group of rats. But, after the administration of *F.vulgare*, there was significant restoration observed in the testis (Gul et al., 2020), as there was an immense increase in the number of spermatozoa in the lumen of the testis, which denotes normal testicular function. Similarly, in the arsenictreated rats, there was a significant reduction in the sperm counts in comparison to the control and arsenic-control-treated rat groups. But, after the administration of *F.vulgare*, there was significant normalization in the sperm counts (Monton et al., 2015; Malo et al., 2012)

The active principle of *F.vulgare* possesses ingredients such as trans-anethol, fenchone, estragole, and limonene, which probably play a vital role in controlling arsenic-induced toxicity. It also possesses properties that regulate normal hormonal functions (Shahat et al., 2011; Alam et al., 2019). It finally plays the important role of antioxidant and membrane repairing functions, normalizing the integrity of the cell and its function as well (Yakut et al., 2020; Singh & Kale 2008)

Conclusion:

The entire study concludes that *F.vulgare* possesses ingredients such as transanethol, fenchone, estragole, and limonene, which play a vital role in combating the arsenic induced toxicity in rats by controlling the normal functions of the system at the haematological, biochemical, hormonal and cellular levels. Moreover, *F.vulgare* also plays the vital role in normalizing the testicular functions damaged due to arsenic exposure. Therefore, *F.vulgare* has therapeutic properties that can combat arsenic-induced toxicity and can be recommended for further drug discovery.

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Declarations

Consent for participate

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

Consent for publish

Consent to publish this article has been obtained 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., P.S. and A.K., and a 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., P.S., R.V.S., A.K.G., 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., A.K.G., and A.K. All authors read and approved the final manuscript.

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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.

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