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## Fertility booster effect of *Asparagus racemosus* against arsenic induced reproductive toxicity in Charles Foster rats


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***Abstract***

In Bihar, an estimated 10 million are exposed to arsenic poisoning through drinking of water. The entire Gangetic plain region people are exposed to arsenic poisoning which is causing lot of health hazards including the problem of infertility. Usually, the infertility problem is associated with the hormonal imbalances and arsenic is a xenoestrogen toxicant which plays major role in causing the imbalance. Moreover, this reproductive disorder has become a major problem in the society causing social stigma in the exposed population. Therefore, there is indeed need for search of novel drug which may play major role in combating the present problem. The present study therefore aims to discover fertility booster against the arsenic induced male reproductive toxicity in animal models.

The current study was conducted on Charles Foster Rats after approval by the Animal Ethics Committee of the Institution. A total of n=18 rats (12 weeks of age) with a mean weight of 160±20 g was used in this study. The study group consisted of n=6 controls and n=12 orally treated with sodium arsenite at a dose of 8mg/Kg b.w per day for 40 days. The n= 6 animals were dissected and rest n=6 was administered orally with *Asparagus racemosus* root ethanolic extract at the dose of 400mg/Kg b.w per day for 40 days. At the end of the entire experiment, all the animals were dissected out and their reproductive organs were taken out especially epididymis for sperm counts, sperm motility, sperm mortality, sperm morphology. The blood samples were taken for hormonal determination (testosterone and luteinizing hormone), as well as for haematological and biochemical analysis.

The study showed a severe degeneration in the reproductive organs of the arsenic-treated rats. There were degenerative changes in sperm count, sperm motility, sperm mortality, sperm morphology, in the hormonal parameters, as well as in the hematological and biochemical parameters in the arsenic treated rats. But, in the *Asparagus racemosus* root ethanolic extract treated group, there was significant normalization in all these parameters. Therefore, in the present study, the *Asparagus racemosus* root ethanolic extract at the dose of 400mg/Kg/ body weight per day shows significant fertility boost effect on arsenic induced Charles Foster rats.



***Keywords: Sodium arsenite, Charles Foster rats, ethanolic root extract of Asparagus racemosus, male reproductive toxicity, fertility booster.***

## 1. Introduction

The most hazardous substance that has so far had an effect on humanity is arsenic. Because it occurs naturally, it may be found in water, food, and air, exposing individuals all over the world to minute dosages. Scientists have lately expressed alarm about the level of arsenic in groundwater, which is a major supply of drinking water for many countries. An estimated 300 million people are exposed to arsenic worldwide and in India about 70 million people, while in Bihar about 10 million people are exposed to arsenic groundwater poisoning (Shaji et al., 2021; Hassan, 2018; Kumar et al., 2022[a]). Millions of people are poisoned every day by inorganic arsenic in their water supply. Consequently, water supply authorities in a number of South and East Asian countries are facing a major difficulty. Alluvial sediments containing arsenic were deposited into the Bay of Bengal in the late Quaternary (Holocene) by the Ganges, Brahmaputra, Meghna, and other minor rivers that run over the Bengal Delta Plain. Arsenic seeps into groundwater in the Bengal Delta Plain because biogeochemical processes breakdown oxyhydroxides in the area's reducing environment. (Richards 2022, 2021, 2020; Mukherjee and Bhattacharya 2001; Mukherjee et al., 2006).

Groundwater arsenic poisoning has been a major health concern in Bihar, affecting a significant proportion of the population. Common arsenicosis symptoms in exposed people include skin problems, gastrointestinal issues, cardiovascular problems, lack of appetite, constipation, diarrhea, neurological problems, reproductive problems, 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).

By binding to sulfhydryl-containing enzymes, arsenite affects their activity. Additionally, it inhibits a wide variety of enzymes involved in cellular functions, such as fatty acid oxidation, product-ion of glutathione, glucose absorption, and gluconeogenesis, by attaching to sulfhydryl groups. Phosphate is unable to bind to arsenite because the latter has a preference for binding to dithiol groups, which weakens the stability of the phosphorus anion in phosphate. As(V) anion-catalyzed rapid hydrolysis of ATP and other molecules "uncouples" mitochondrial respiration by releasing high-energy phosphate bonds (Cantoni et al., 2021; Dover et al., 2018; Guidarelli et al., 2017 & 2020; Fiorani et al., 2018; Aposhian and Aposhian 2006).

The second main kind of arsenic poisoning is called "arsenolysis." It occurs when arsenic reacts with ADP to form ADP arsenate, which leads to a permanent decrease in cellular energy and the inability to perform oxidative phosphorylation. Thus, reactive oxygen species (ROSs) may damage DNA, lipids, and proteins, which may interfere with cell signaling. (Yang and Frenkel 2002; Qian et al., 2003; Singh et al., 2011).

Prolonged arsenic exposure in humans increases the likelihood of cancer, cardiovascular disease, and developmental problems. The reproductive and developmental toxicity of arsenic has been the subject of little epidemiological study (Moore et al., 2019; Tchounwou et al., 2019; Wang et al., 2006). According to epidemiological studies, prenatal arsenic exposure is associated with an elevated incidence of stillbirth, low birth weight, spontaneous abortion, and other complications (Ahmad et al., 2001; Milton et al., 2005). Unfortunately, there is a lack of data on potential confounding factors and accurate maternal arsenic exposure in these studies. These factors include, but are not limited to, smoking, maternal age, and exposure to other metals. To examine the developmental and reproductive toxicity of arsenic, animal models are essential because they provide more rigorous experimental controls, including the aforementioned confounders and prenatal observations of developmental changes. Furthermore, it permits the use of animals in pre-human drug screenings.

Toxic effects of arsenic on rats were countered in this research by administering *Asparagus racemosus*. It contains a broad variety of phytochemicals, including isoflavones, flavonoids, steroid saponins, oligospirostanoside, the alkaloid aspargamine, and the Indian name Shatavari. According to some research, it may have both hepatoprotective and renal- protective effects (Laddha et al., 2024; Goyal et al., 2003; Palanisamy and Manian, 2012; Acharya et al., 2012; Bansode et al., 2015; Qazi and Raza 2021).

Considering that in regard, the purpose of this research is to determine that an extract from the roots of *Asparagus racemosus* had any protective effects against the reproductive toxicity caused by the effect of sodium arsenite in rats. Thus, the present study deciphers the fertility booster effect of *Asparagus racemosus* against arsenic induced testicular toxicity.

## 2. Materials & Methods:

**2.1** ***Test Chemical:*** Loba Chemie, India-manufactured Sodium Arsenite AR (98.0%), (CAS No.7784-46-5, Lot No. 20/21-28a-45-60-61) was obtained from the Patna Scientific store.

**2.2** ***Ethics approval:*** Approval for this research was granted by the Institutional Animal Ethics Committee

of Mahavir Cancer Sansthan and Research Centre at Phulwarisharif, Patna, Bihar, India.

**2.2 *Animals:*** Thirty male Charles Foster rats ranging in weight from 160 to 180 grams and 8 weeks old were supplied by the animal laboratory of the Mahavir Cancer Institute and Research Centre in Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The study was approved by the Institutional Animal Ethics Committee (IAEC). Rats were given free access to food and water, with some of the food being made in the lab. Traditional polypropylene cages were used to house the experimental animals, which were kept in pairs. Putting rats into treatment and control groups at random. The experimental animal compartment had a 12-hour light/dark cycle and was maintained at a temperature of 22±2 degrees Celsius.

**2.4 *Preparation of plant ethanolic extract:*** The dried Asparagus racemosus roots used in this investigation were acquired from the Haridwar Medicinal Store in Haridwar, Uttarakhand, India. A botanist from A.N. College in Patna, Bihar, India, subsequently identified these roots as a remedy for rats ingested with arsenic. A fine powder was made from the collected *Asparagus racemosus* roots after it was shade-dried. Following a 48-hour soaking in 70% ethanol, the powder was dried at 37 degrees Celsius after being extracted with 100% ethanol using a Soxhlet apparatus for 6 to 8 hours. By determining the LD50 at a dosage of 400 mg/kg body weight per day, the ethanolic extract dose was established.

**2.5 *Experimental Design:*** For this study, male Charles Foster rats (n = 24) were orally dosed with 8 mg Kg-1 body weight per day of sodium arsenite, a form of arsenic, for 45 days. For contrast, six male rats served as controls. Following a 40-day pre-treatment with sodium arsenite, a group of participants were daily dosed 400 mg Kg-1 body weight with an ethanolic extract of the roots of *Asparagus racemosus* (Shatavari). All the animals had free access to food and water. Following the completion of the experiment, the rats were sacrificed, their blood was collected to perform biochemical tests hormonal tests such as luteinizing hormone, and testosterone hormones, and their tissues -testes were preserved in neutral formalin for histological examination.

**2.6 *Sperm counts:*** After removing the cauda epididymis, it was thoroughly washed with 0.85% normal saline. In order to facilitate the release of sperm, Cauda's epididymis was punctured and minced using a watch glass immersed in1mL of distilled water. Subsequently, sperm were completely mixed with two drops of Eosin Y. Sperm counts were performed and seen at a 450x magnification using an improved Neubauer's chamber and a drop of the aforementioned solution.

**2.7 *Sperm motility:*** A surgically excised and ruptured cauda epididymis was seen on a microscope slide. After the spermatozoa were covered, their motility was examined.

**2.8 *Hormonal Assay:*** The levels of testosterone and luteinizing hormone (LH) were determined by the use of an ELISA kit procured from LILAC Medicare (P) Ltd., Mumbai. A volume of 25 microlitres of serum was transferred to the microwell plates after the reference range had been established. A hundred microliters of enzyme conjugate were added to each well. Then, for one hour, the mixture was left to incubate at 37 degrees Celsius. The next three times, the wells were drained and replaced with 300 microliters of distilled water. Next, 100 microliters of TMB solution were added to each well plate as a substrate, and the plates were incubated for 15 minutes to allow the color to develop. Each well was treated with 100microliters of stop solution to end the reaction. A 630 nm measurement in ng/ml was obtained using the Merck ELISA reader.

**2.9 *Lipid Peroxidation:*** Thiobarbituric acid reactive substances (TBARS), a marker for LPO, can be determined using the double heating procedure. Spectrophotometric measurements were taken of the color change that occurred when thiobarbituric acid (TBA) was added to malondialdehyde (MDA). This was achieved by heating 2.5 ml of a 100 g/l trichloroacetic acid (TCA) solution to 90 degrees Celsius for 15 minutes while mixing 0.5 ml of serum in a centrifuge tube. The mixture was re-incubated for 15 minutes at 90 degrees Celsius after being centrifuged at 3000 g for 10 minutes after cooling in tap water. Then, 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube. Thermo Scientific's UV- 10 (UV - Vis) spectrophotometer (USA) was used to detect the absorbance at 532 nm. Water from the faucet was used to chill the mixture.

**2.10 *Histopathology:*** All of the rats were sacrificed after the stipulated experimental treatment completed. After removing each testicle from a rat using a midsagittal incision, they were preserved in 10% neutral formalin. The sections were viewed under a light microscope using slides that were stained with haemotoxylin and eosin.

**2.11 *Statistical Analysis:*** The data sets' averages and standard deviations were determined using one-way analysis of variance (ANOVA). To determine whether the mean difference was statistically significant, Dunnett's test was used. All of the calculations were performed using Graph Pad Prism, which is a program developed by Graph Pad software, Inc. and located in San Diego, California, USA. The $p < 0.05$ threshold was established as the level of statistical significance.

## 3. Results:

**3.1** ***Morbidity & mortality:*** The rats exposed to arsenic showed toxic consequences such as vomiting, nosebleeds, loss of coordination (24% of the rats suffered symptoms similar to paralysis), blackening of the tongue and paws, and overall weakening after 40 days.

**3.2** ***Sperm counts:*** The sperm count of rats treated with sodium arsenite was much lower than that of normal rats. A rise in sperm count after treatment with *Asparagus racemosus* may indicate a recovery of testicular function. (Fig. 1).

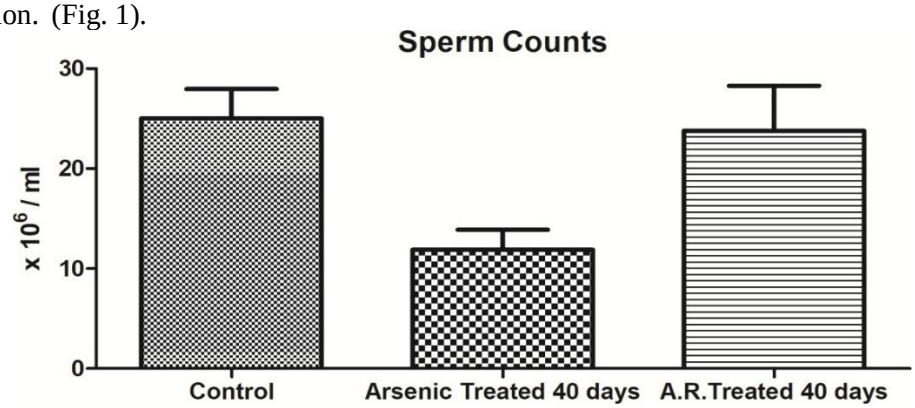


**Fig. 1. Graph figure showing sperm counts in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

**3.3** ***Sperm mortality & motility:*** Sperm motility was significantly lower in rats fed sodium arsenite compared to controls. Sodium arsenite poisoning was associated with serious sperm abnormalities like tail loss, coiling, etc. *Asparagus racemosus* significantly increased the motility of the sperm, indicating that the spermatozoa were revived. (Fig. 2A, 2B).

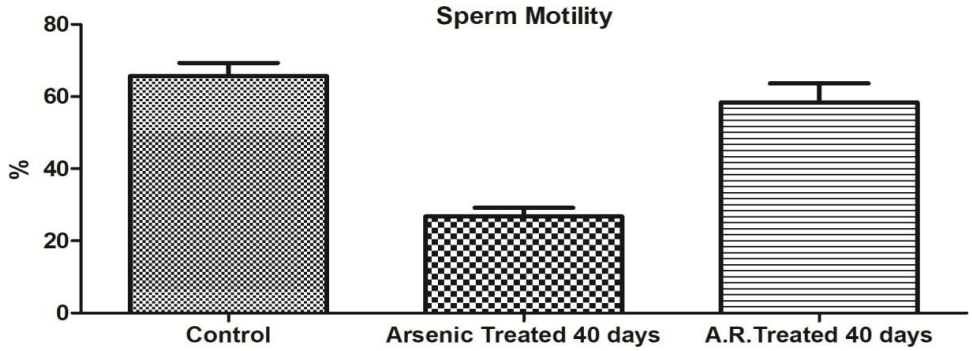


**Fig. 2A. Graph figure showing sperm motility in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

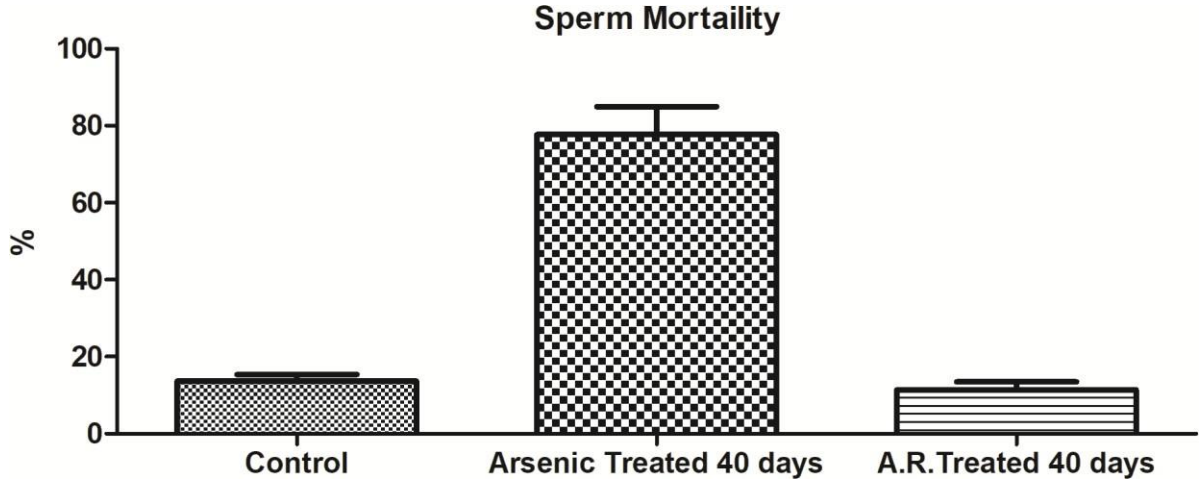


**Fig. 2B. Graph figure showing sperm mortality in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

**3.4** ***Hormonal assay:*** Arsenic exposure significantly reduced serum testosterone levels compared to the control group, indicating endocrine disruption; treatment with *Asparagus racemosus*, on the other hand, restored normal endocrine function, as seen by higher serum testosterone levels (Fig. 3A). The levels of LH rise compared to the control group after arsenic exposure, but they decrease significantly ($p<0.05$) following treatment with *Asparagus racemosus* (Fig. 3B).

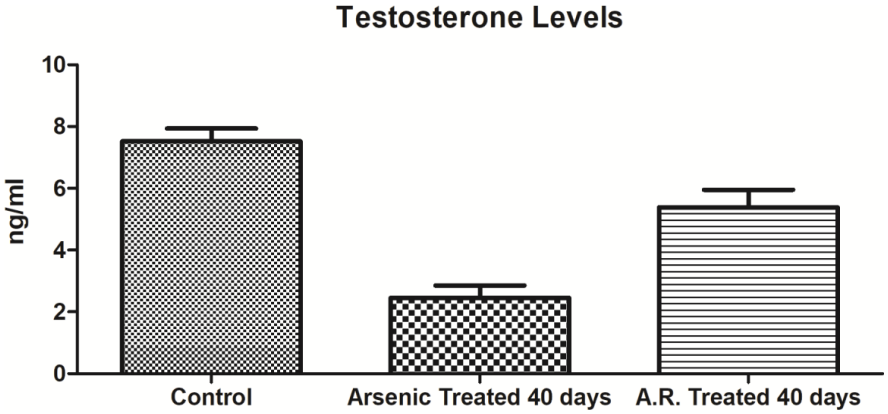


**Fig. 3A. Graph figure showing hormone testosterone levels in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

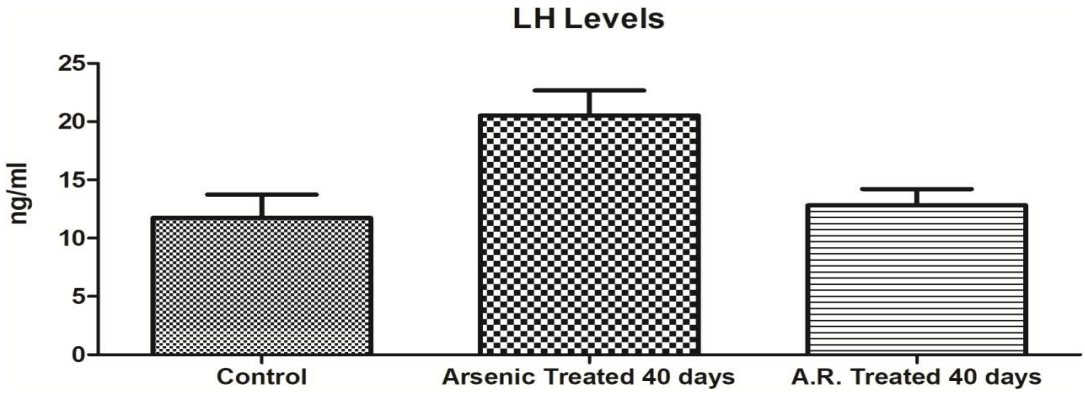


**Fig. 3B. Graph figure showing Luteinizing hormone levels in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

**3.5** ***Lipid peroxidation assay:*** Lipid peroxidation (LPO) levels were shown to rise in response to arsenic treatment compared to controls, suggesting cellular oxidative stress. In contrast, the antioxidant action of *Asparagus racemosus* was proven by a significant decrease in LPO levels **(Fig. 4).**

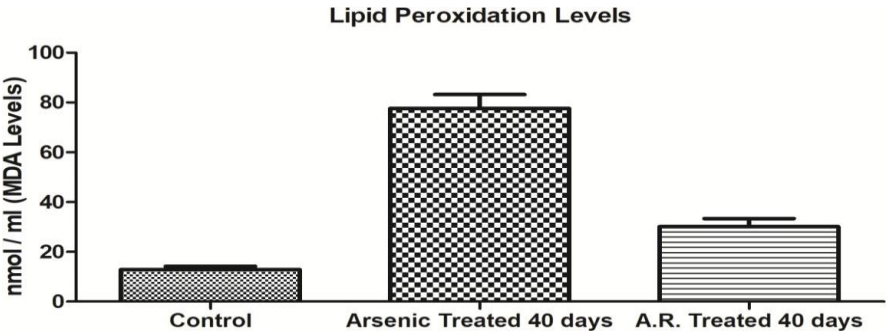


**Fig. 4. Graph figure showing Lipid Peroxidation levels in different treatment groups** ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

**3.6** ***Histopathological study*****:** The major spermatocytes (PS), spermatogonia (SG), spermatids (SPM), and spermatozoa are all found in the seminiferous tubules (ST) of a healthy testis. Fig. 5A shows that during normal spermatogenesis, the leydig cells that align the inter- seminiferous tubules are functioning precisely. Sodium arsenite-treated testicular sections have seminiferous tubules, but there are either no spermatogenetic phases or just 22% of the normal activity. Figure 5B shows hemorrhaging in the leydig cells, which indicates

that they are likewise in a severely degenerative state. However, after the administration of Asparagus racemosus, an enormous improvement was seen, and the spermatogenetic stages restored to their normal state. When testes include spermatozoa, spermatids (SPM), SG, and effectively generates primary spermatocytes (PS), it means that cellular activity has restored to normal. As seen in Figure 5C, normal leydig cell function will be reinstated after their condition improves. Fig. 5D depicts the normal architecture of spermatozoa, as shown in the sperm morphology research. Rat sperm treated with arsenic exhibit a coiled tail and other morphological abnormalities (Fig. 5E), while sperm treated with *Asparagus racemosus* show considerable restoration in the spermatozoa (Fig. 5F).

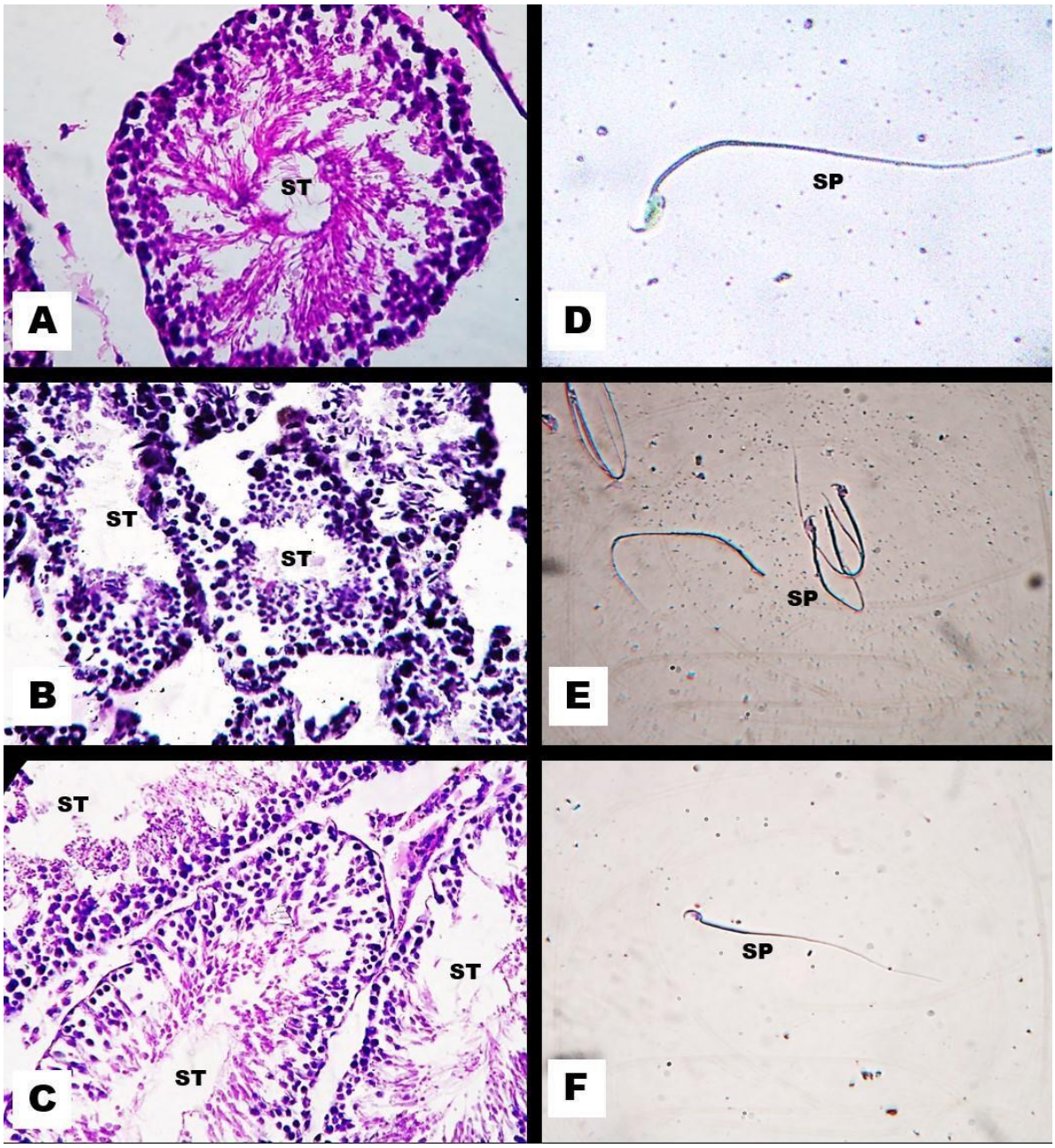


**Figure 5,** sections stained with haematoxylin and eosin in a microphotograph of [A] A section of the testis of a control rat demonstrating the typical arrangement of the spermatogenetic stages (ST). [B] Testis of arsenic-treated rats exhibiting significantly reduced spermatogenetic stages (ST) and aberrant architecture of seminiferous tubules. Testis of rats given *Asparagus racemosus* restored in function during spermatogenetic stages (ST) [C]. 500x. [D] Typical sperm morphological architecture (SP) [E] The morphology of arsenic-treated rat sperm reveals folded tails (SP). The sperm morphology (SP) X 500 stained with Giemsa stain demonstrates a notable restoration in rat sperm treated with *Asparagus racemosus*.

## 4. Discussion:

Arsenic exposure at 8 milligrams per kilogram of body weight significantly reduced male fertility in this investigation. The organs and tissues of the body are impacted by the toxic effect of AsIII (sodium arsenite). The effects of arsenic on sperm motility, testosterone levels, lipid peroxidation, and sperm counts suggest that the endocrine system is disrupted, which in turn causes an overproduction of luteinizing hormone (LH) and deficient leydig cell activity. Histopathological analysis showed that spermatogenesis had been interrupted due to unusually low testosterone levels, since there were few sperms in the lumen of seminiferous tubules. Because arsenic inhibits sperm growth in the testes, it is known to cause infertility.

Previous research has shown that male reproductive cells are negatively affected by arsenite exposure, whether it is by drinking water or intraperitoneally. One way that AsIII interferes with spermatogenesis is by changing the way spermatogenetic enzymes work. Furthermore, AsIII lowers levels of gonadotrophins and testosterone. Arsenic has several potential effects, including on the pituitary gland and brain as well as on germ cells. Male mice that were administered sodium arsenite in their drinking water showed reproductive impairment without any noticeable symptoms. (Chinoy et al., 2004; Pant et al., 2004 & 2001; Sarkar et al., 2003).

Research on mice has also shown that AsIII influenced cholesterol metabolism and testicular testosterone synthesis. Male Swiss mice treated with arsenic trioxide (As2O3) had their cholesterol levels up and their testicular protein levels decreased. Degeneration was discovered in the testicles' tubules and germinal epithelial cells. Sperm were also absent from the seminiferous tubule lumen. Both serum testosterone and the activities of 3- and 17-HSD in the testes decreased. Cholesterol is an ingredient in the testis' interstitial tissue that is used to make testosterone. (Chinoy et al. 2004; Kabbaj et al., 2003).

Sodium arsenite has been demonstrated in another study to decrease testicular weight, accessory sex organ weight, and epididymal sperm counts in rats. The concentrations of LH, FSH, and testosterone in the plasma all moved in the same direction. We measured spermatogenesis quantitatively by counting the proportion of different sperm cell types at stage VII of the seminiferous epithelium cycle. Testosterone acts most effectively at this phase of spermatogenesis. By stage 7, there was severe deterioration in all of the germ cells. The researchers also suggested that arsenic-induced low levels of luteinizing hormone and follicle-stimulating hormone might be the reason for lower testosterone synthesis. When testosterone levels were low, sperm quality started to drop faster. Potentially, AsIII binds to thiol and inhibits germ cells directly; alternatively, it may operate on the pituitary or brain to lower LH and FSH levels. Another possible cause of low serum LH, FSH, and testosterone levels is high blood corticosterone levels. As shown in rats treated with arsenate, elevated corticosterone levels may reduce serum concentrations of gonadotrophins and testosterone. On the other hand, LH was found to be increased when tested in blood serum (Hardy; 2005; Wang et al., 2006; Ali et al., 2013; Kumar et al., 2015; Reddy et al., 2010; Ahmad et al., 2010).

According to Wiboonpun et al. (2004), Asparagmine A seems to have an important role in restoring the structure of cells and modifying their proper function. In addition, arsenic causes serious histological damage to the seminiferous tubules of testis as primary spermatocytes, spermatogonia are severely damaged. Significant cellular restoration, however, was seen in the group treated with asparagus extract. The cellular activity has likely been restored by Asparagmine A. It may scavenge free radicals due to its potent active antioxidant capabilities. The enzyme also regulates proper cellular activity by catalyzing the ATP-dependent interaction between aspartate and glutamine, which results in asparagine and glutamate. Asparagmine A, a component of the root extract, is therefore more important than the other components in protecting against arsenic-induced toxicity. In addition, the current research has also shown that *Asparagus racemosus* has a highly promising function compared to other plant components. Moreover, at acts like fertility booster in arsenic induced toxicity in the present study (Hamdi et al., 2024;2021; 2017; Guo et al., 2023; Kohli et al., 2023; Acharya et al., 2012; Palanisamy and Manian 2012; Singh 2016; Bopana and Saxena 2007; Lomelino et al., 2017).

## Conclusion:

The present investigation found that sodium arsenite led to infertility by reducing sperm counts, sperm motility, sperm morphological deterioration, and spermatogenetic stage arrest. On the other hand, the testicular function was reversed and the spermatogenetic phases returned to normal when *Asparagus racemosus* was administered. All it means is that the testicular cells are revitalized by Asparagus racemosus. These findings demonstrate that *Asparagus racemosus* is a one-of-a-kind medicinal herb with rejuvenating and antioxidant capabilities, as well as a role in maintaining normal function and health of testicular cells. Among other medications, it protects reproductive organs from the toxic effects of arsenic. It is a significant fertility booster against the arsenic induced toxicity.

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**Declarations**

**Competing interests**
The authors declare that they have no conflicts of interest.

**Consent for publish**

All the authors provide their consent to publish this article.

**Author contributions**

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

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**Availability of data and materials**

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

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