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Ameliorative Effect Of *Curcuma longa* Against Arsenic Induced Reproductive Toxicity In Charles Foster Rats

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Abstract:

The present study aims to develop the antidote against arsenic induced male reproductive toxicity in animal models. The study was carried out on Charles Foster rats after the approval from Institutional Animal Ethics Committee. A total of n=18, rats (12 weeks old) of an average weight of 160 ± 20 g were used for the study. The study group included n=6 control and n= 12 treated with sodium arsenite orally at the dose of 8mg/Kg body weight daily for 40 days. The n= 6 animals were dissected and rest n=6 was administered orally with *Curcuma longa* rhizome ethanolic extract at the dose of 600mg/Kg body weight 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 collected for the hormonal assay (testosterone and luteinizing hormone), as well as for hematological and biochemical analysis.

The study showed, high magnitude of degeneration in the reproductive organs of the rats in the arsenic treated group. There were degenerative fluctuations in the sperm counts, sperm motility, sperm mortality, sperm morphology and in the hormonal parameters, as well as in the hematological and biochemical parameters in the arsenic treated rats. But, after the administration of Curcuma longa, there was significant amelioration in all these parameters. Therefore, the present study shows that *Curcuma longa* plays vital role to combat arsenic induced male reproductive toxicity.

Keywords: Sodium arsenite, Charles Foster rats, ethanolic rhizome extract of Curcuma longa, male reproductive toxicity, amelioration.

1. Introduction

Arsenic is the most hazardous element or compound known to have had a significant impact on human history. It's naturally occurring, so people everywhere are exposed to minute doses through water, food, and air. Ground water is the primary source of drinking water in many nations; however, scientists have recently been concerned about the amount of arsenic in this water. High quantities of inorganic arsenic are present in the drinking water of millions of people. As a result, it has become a significant challenge for water supply authorities in several nations in South and East Asia. The Ganges, Brahmaputra, Meghna, and other smaller

rivers that flow across the Bengal Delta Plain and into the Bay of Bengal are thought to have deposited arsenic-containing alluvial sediments during the late Quaternary age (Holocene age). Due to the reducing environment, the oxyhydroxides in the Bengal Delta Plain are dissolved by biogeochemical processes, releasing the arsenic into the groundwater (Shaji et al., 2021; Hassan, 2018; Kumar et al., 2022[a]; Richards 2022, 2021, 2020; Mukherjee and Bhattacharya 2001; Mukherjee et al., 2006).

In Bihar, an estimated 10 million population 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, diarrhoea, 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).

Arsenite affects sulfhydryl-containing enzymes by binding to them. Through sulfhydryl group binding, it also inhibits many other cellular enzymes involved in processes including glucose absorption, gluconeogenesis, fatty acid oxidation, and product - ion of glutathione. Phosphate is not a competitor for arsenite because arsenite preferentially binds to dithiol groups, making the phosphorus anion in phosphate less stable. Fast hydrolysis of molecules like ATP by the As(V) anion results in the loss of high-energy phosphate bonds and "uncouples" mitochondrial respiration (Cantoni et al., 2021; Dover et al., 2018; Guidarelli et al., 2017 & 2020; Fiorani et al., 2018; Aposhian and Aposhian 2006).

'Arsenolysis' refers to the second primary type of arsenic poisoning, in which arsenic interacts with ADP to create ADP arsenate, causing irreversible loss of energy in cells and preventing oxidative phosphorylation from taking place. Therefore, ROSs may disrupt cell signalling by destroying DNA, lipids, and proteins (Yang and Frenkel 2002; Qian et al., 2003; Singh et al., 2011).

Humans who are exposed to arsenic over extended periods of time are at risk for developmental consequences, cancer, and cardiovascular disease. Few epidemiological research has been conducted on the developmental and reproductive toxicity of arsenic (Moore et al., 2019; Tchounwou et al., 2019; Wang et al., 2006). Arsenic exposure during pregnancy has been linked to an increased risk of spontaneous abortion, stillbirth, and low birth weight, according to epidemiological research (Ahmad et al., 2001; Milton et al., 2005). These studies, however, do not provide enough information on confounders (such as other metal exposure, smoking, maternal age, etc.) and accurate maternal arsenic exposure. Since animal models provide more stringent experimental controls, including the aforementioned confounders, and prenatal observations of developmental alterations, they are crucial for studying arsenic's developmental and reproductive toxicity. Additionally, it allows for the testing of therapies on animals before they are used on people.

One of the earliest types of cultivated spice plants, Curcuma longa L. (turmeric) is a member of the ginger family (Zingiberaceae). The rhizome of this plant, which has been used medicinally for centuries, has been shown to be both safe and effective in the treatment of a wide range of chronic conditions, including diabetes mellitus (DM). Curcumin (diferuloylmethane), turmeric's key ingredient, has been shown to provide a wide variety of health benefits. Historically, people have used (*Curcuma longa*) dating back nearly 4000 years to the Vedic culture in India, turmeric has been used both as a culinary spice and as a medicinal remedy for a wide range of conditions, including but not limited to: enhancing digestion and intestinal flora; relieving gas and eliminating intestinal worms; reducing swelling; fortifying the liver; applying topically to sprains, burns, cuts, bruises, insect bites and itches; and alleviating coughs, asthma, and general feelings Classical Unani texts describe a wide range of pharmacological activities and applications for Curcuma longa, including those in the fields of gastrointestinal, cosmetology, and pulmonary medicine. Numerous investigations, both animal and human, have been conducted on it because of its many potential pharmaceutical applications (Moghadamtousi et al., 2014; Jennings & Parks 2020; Aggarwal et al., 2007; Hosseini & Hosseinzadeh et al., 2018; Rai et al., 2020; Prasad & Tyagi 2015).

Thus, present study deciphers the ameliorative effect of *Curcuma longa* 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:*** The Institutional Animal Ethics Committee of Mahavir Cancer Sansthan and Research Centre in Phulwarisharif, Patna, Bihar, India granted its approval for this study.

2.2 *Animals:* The animal laboratory of the Mahavir Cancer Institute and Research Centre in Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA) provided 30 male Charles Foster rats weighting between 160g and 180g and aged 8 weeks. IAEC (Institutional Animal Ethics Committee) approval was obtained for the research. Food and water were provided ad libitum to rodents (with food prepared by the laboratory itself). The experimental animals were confined in small groups of two in conventional polypropylene enclosures. Random assignment of rodents to control and treatment groups. The experimental animal chamber was maintained at 22.2 degrees Celsius with a 12-hour light/dark cycle.

2.4 *Preparation of plant ethanolic extract:* The desiccated rhizome of *Curcuma longa* was purchased from the Haridwar Medicinal Store in Haridwar, Uttarakhand, India, for this study and later recognized by a Botanist at A.N. College, Patna, Bihar, India, as an antidote for arsenic-induced poisoning in rats. The collected rhizome of *Curcuma longa* was shade-dried and then ground into a fine powder. The powder was then immersed for 48 hours in 70% ethanol, extracted with absolute ethanol using a Soxhlet apparatus for 6 to 8 hours, and the residue was concentrated and desiccated at 37 degrees Celsius. The ethanolic extract dose was determined by calculating the LD_{50} at the dose of 600 mg/kg body weight per day.

2.5 *Experimental Design:* In the current investigation, male Charles Foster rats (n = 24) were orally given dosage with 8 mg Kg-1 body weight per day of arsenic in the form of sodium arsenite for 45 days, with control male rats (n = 6) used for comparison. Then, a group that had been pre-treated with sodium arsenite for 40 days was given an ethanolic extract of the rhizome of *Curcuma longa* (Turmeric) at a dosage of 600 mg Kg-1 body weight each day. The animal had free access to food and water at all times. After the experiment was complete, the rats were sacrificed, their serum was extracted for a lipid peroxidation study, luteinizing hormone and testosterone hormone assays, and their testes were fixed in neutral formalin for histopathological analysis.

2.6 *Sperm counts:* The cauda epididymis was dissected out and extensively cleansed in 0.85% normal saline. The epididymis of Cauda was incised and pierced multiple times in 1 ml of distilled water in a watch glass so that sperm could escape. After that, two droplets of Eosin Y were thoroughly combined with sperm. Using an enhanced Neubauer's chamber and a drop of the above preparation, sperm counts were conducted and observed at 450x magnification.

2.7 *Sperm motility:* On a microscope slide, a cauda epididymis was surgically removed and ruptured The spermatozoa's motility was analyzed after being placed under a cover slip.

2.8 *Hormonal Assay:* The luteinizing hormone (LH) and testosterone levels were measured using an ELISA kit from LILAC Medicare (P) Ltd., Mumbai. After determining the reference range, 25 l of serum was withdrawn into the microwell plates. Each well had 100 l of enzyme conjugate added to it. The mixture was then allowed to incubate at 37 degrees Celsius for an hour. After that, at least three 300 l changes of distilled water were made, and the wells were wiped dry each time. The substrate, 100 l of TMB solution, was then applied to each well plate and incubated for 15 minutes to develop colour. To terminate the reaction, 100 l of stop solution was added to each well. The Merck ELISA reader was used to get a 630nm reading in ng/ml.

2.9 *Lipid Peroxidation:* The double heating technique [26] was used to identify thiobarbituric acid reactive compounds (TBARS), a marker for LPO. The color change caused by adding thiobarbituric acid (TBA) to malondialdehyde (MDA) was measured spectrophotometrically. This was accomplished by incubating 0.5 ml of serum in a centrifuge tube with 2.5 ml of a 100 g/l trichloroacetic acid (TCA) solution for 15 minutes at 90 degrees Celsius. Centrifuged at 3000 g for 10 minutes after chilling in tap water, 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test-tube and the combination was re-incubated for 15 minutes at 90C. The absorbance at 532 nm was then measured using a Thermo Scientific UV-10 (UV -Vis) spectrophotometer (USA). The solution was cooled in tap water.

2.10 *Histopathology:* After the specified period of time, every rat was euthanized. All of the rats' testicles were removed by a midsagittal incision and then preserved in 10% neutral formalin. Haemotoxylin and eosin-stained slides were made for the light microscopic research, and the sections were examined.

2.11 *Statistical Analysis:* One-way analysis of variance (ANOVA) was used to find the average and standard deviation of the data sets. Dunnett's test was used to examine the significance of the mean difference. Graph

Pad Prism (Graph Pad software, Inc., San Diego, California, United States) was used for all of the calculations. Statistical significance was determined to be at the P< 0.05 level.

3. Results:

3.1 ***Morbidity & Mortality:*** Toxic effects such as vomiting, nosebleeds, loss of coordination (24% of rats experienced paralysis-like symptoms), blackening of the tongue and paws, and general weakness were seen in arsenic-exposed rats after 40 days.

3.2 ***Sperm counts:*** Compared to normal rats, those given sodium arsenite had significantly less sperm counts. Treatment with *Curcuma longa* resulted in an increase in sperm count, suggesting that testicular function had been restored (Fig. 1).

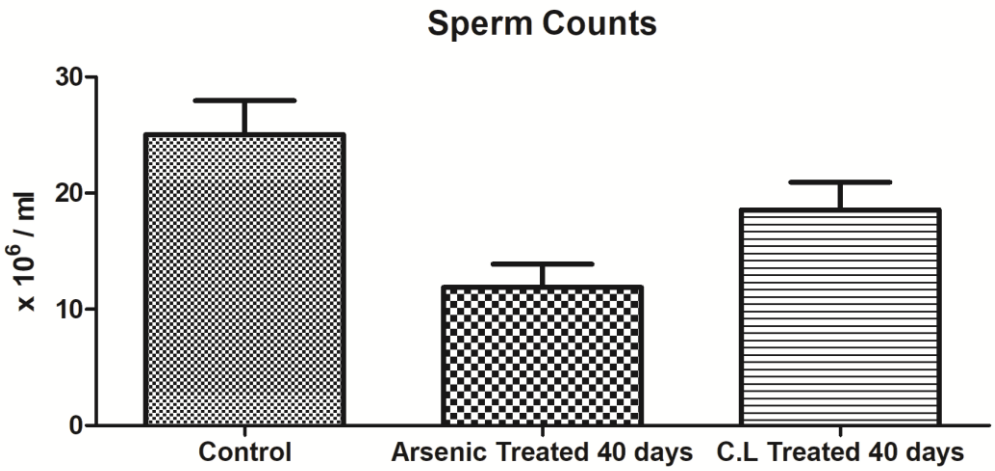

Fig. 1. Graph figure showing sperm counts in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

3.3 ***Sperm mortality & motility:*** Rats given sodium arsenite had much less sperm motility than controls Significant sperm abnormalities, such as loss of sperm tails, sperm tails coiling, etc., were seen in cases with sodium arsenite poisoning. The sperm's motility improved dramatically after consuming *Curcuma longa*, showing that the spermatozoa had been revitalized (Fig. 2A, 2B).

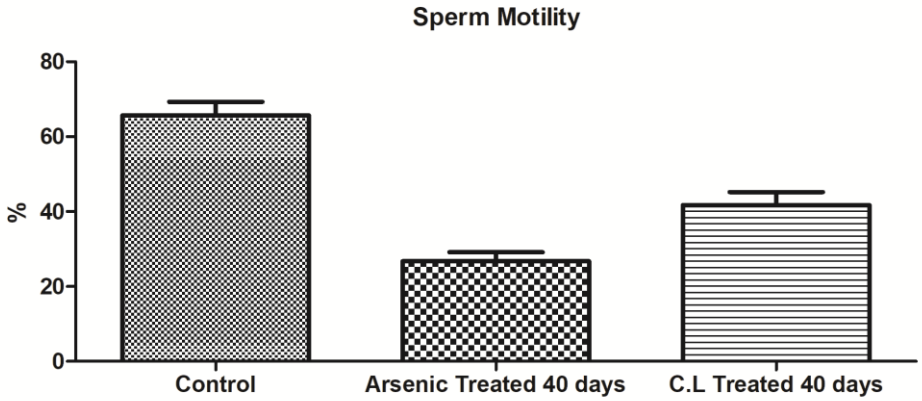

Fig. 2A. Graph figure showing sperm motility in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

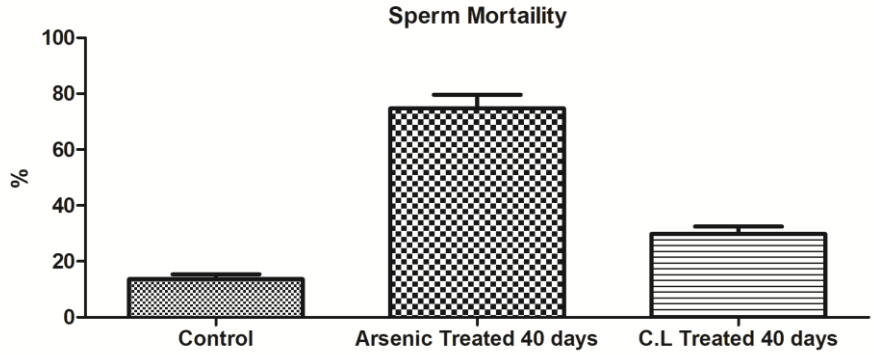

Fig. 2B. Graph figure showing sperm mortality in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

3.4 ***Hormonal assay:*** Serum testosterone levels were considerably lower in the arsenic-exposed group compared to the control group, suggesting endocrine disruption; however, they were greater in the *Curcuma longa*-treated group, suggesting restoration of normal endocrine function **(Fig. 3A).** After arsenic exposure, LH levels increases relative to the control group, but fall considerably ($p<0.05$) after treatment *with Curcuma longa* **(Fig. 3B).**

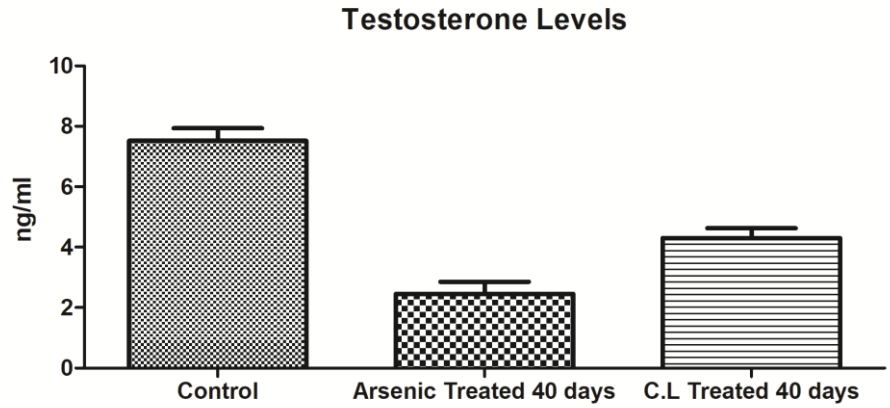

Fig. 3A. Graph figure showing hormone testosterone levels in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

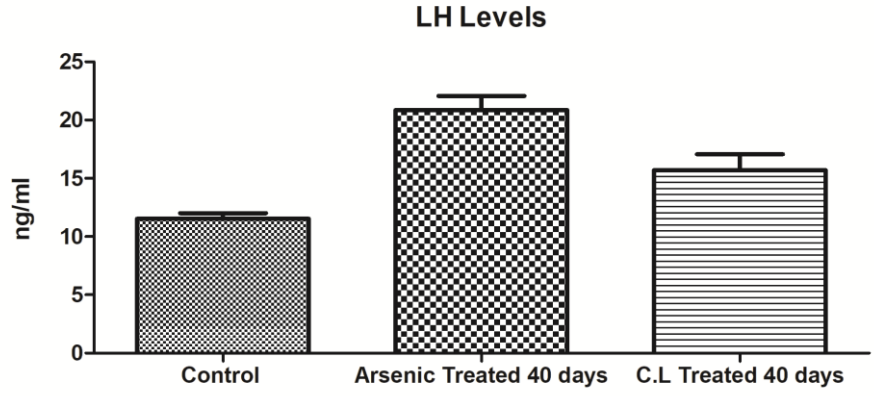

Fig. 3B. Graph figure showing Luteinising hormone levels in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

3.5 ***Lipid peroxidation assay:*** While arsenic treatment increases lipid peroxidation (LPO) levels relative to controls, this is an indication of cellular oxidative stress; with Curcuma longa administration, however, considerably reduced LPO levels, demonstrating its antioxidant effect **(Fig. 4).**

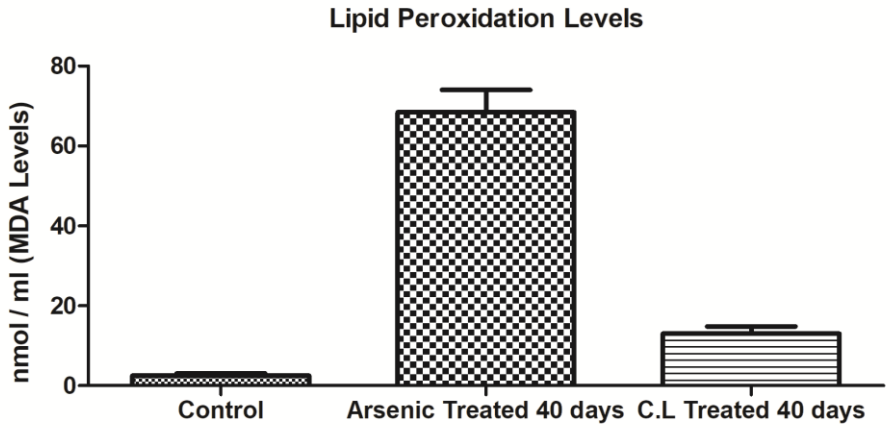

Fig. 4. Graph figure showing Lipid Peroxidation levels in different treatment groups ***(One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)***

3.6 ***Histopathological study*****:** In a normal testis, the seminiferous tubules (ST) contain the main spermatocytes (PS), spermatogonia (SG), spermatids (SPM), and spermatozoa. The Leydig cells that direct the inter - seminiferous tubules into alignment are shown in **(Fig. 5A)** to be functioning normally during normal spermatogenesis. Testicular sections treated with sodium arsenite show seminiferous tubules, but no spermatogenetic phases or just 22% of typical activity. The Leydig cells **(Fig. 5B)** show signs of haemorrhaging, suggesting that they are also in a very degenerative condition. Once *Curcuma longa* was administered however, things drastically improved, and the spermatogenetic stages restored to normal. The presence of properly formed primary spermatocytes (PS), spermatogonia (SG), spermatids (SPM), and spermatozoa indicates that cellular activity in the testes has returned to normal. Recovery of normal Leydig cell function is anticipated when the Leydig cells' state improves **(Fig. 5C)**. The sperm morphology study shows in control the normal architecture of spermatozoa **(Fig. 5D)**. In arsenic treated rat sperm morphology showing distorted sperms with coiled tail **(Fig. 5E)**, while in *Curcuma longa* treated rat sperm shows significant restoration in the spermatozoa **(Fig. 5F)**.

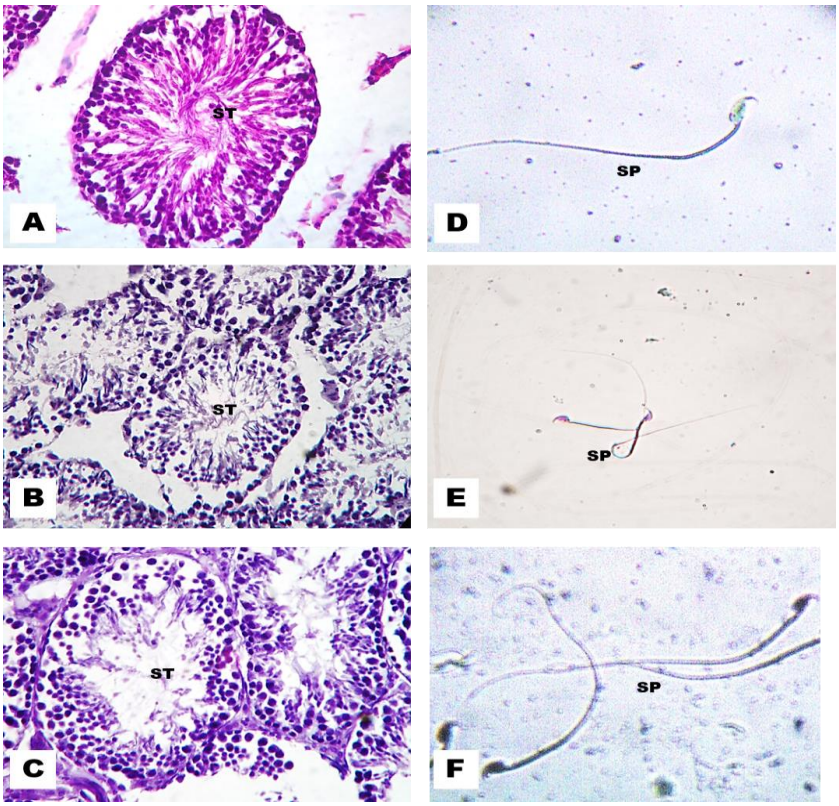

Fig 5. Microphotograph sections stained with haematoxylin & eosin of **[A]** Control rat testis section showing normal architecture of spermatogenetic phases (ST). **[B]** Arsenic treated rat testis showing abnormal

architecture of seminiferous tubules with severe loss of spermatogenetic stages (ST). **[C]** *Curcuma longa* treated rat testis showing restoration in the function of the spermatogenetic phases (ST). X 500. **[D]** Normal architecture of sperm morphology (SP) **[E]** Arsenic treated rat sperm morphology showing distorted sperms with coiled tail (SP). **[F]** *Curcuma longa* treated rat sperm shows significant restoration in the sperm morphology (SP) X 500 stained with Giemsa stain.

4. Discussion:

In this study, arsenic exposure at 8mg per Kg body weight severely impaired male reproductive capacity. Sodium arsenite's AsIII is toxic to the body's organs and tissues. Arsenic's effects on sperm counts, sperm motility, testosterone levels, and lipid peroxidation indicate endocrine disruption leading to excessive luteinizing hormone (LH) secretion and consequently defective Leydig cell function. Histopathological examinations revealed few sperm in the lumen of seminiferous tubules, indicating that the abnormally low testosterone levels had disrupted spermatogenesis. Arsenic has been shown to induce infertility by stopping the development of sperm in the testes.

Arsenite exposure, either by drinking water or intraperitoneally, has been shown to be harmful to male reproductive cells in earlier investigations Altering the activity of spermatogenetic enzymes, AsIII disrupts spermatogenesis [28]. In addition, testosterone and gonadotrophin levels are reduced by AsIII. In addition to acting directly on the germ cells, arsenic may also affect the brain and pituitary gland [29]. Reproductive damage without clinical symptoms was seen in male mice given sodium arsenite in drinking water (Chinoy et al., 2004; Pant et al., 2004 & 2001; Sarkar et al., 2003).

Cholesterol metabolism and testosterone production in the testicles were also affected by AsIII in the mouse model research. Arsenic trioxide (As2O3) treatment of male Swiss mice raised cholesterol levels and reduced testicular protein levels. The tubules and germinal epithelial cells in the testicles were found to be degenerating. The lumen of the seminiferous tubules likewise lacked sperm. There was also a reduction in serum testosterone and testicular 3- and 17-HSD activity. Testosterone is synthesized from cholesterol found in the testis' interstitial tissue (Chinoy et al. 2004; Kabbaj et al., 2003).

Testicular weight, accessory sex organ weight, and epididymal sperm counts were all shown to be reduced in rats treated with Sodium arsenite, according to another research. LH, FSH, and testosterone plasma concentrations all followed the same pattern. We conducted quantitative study of spermatogenesis by counting the relative amount of each kind of germ cells at stage VII of the seminiferous epithelium cycle. This stage of spermatogenesis is particularly sensitive to testosterone. All the germ cells showed massive degeneration by stage 7. Low levels of LH and FSH, which were reported to be caused by arsenic, were also proposed as a possible cause of reduced testosterone production by these authors. The decline of sperm quality was accelerated by low testosterone levels. It is possible that AsIII acts on the brain or pituitary to reduce LH and FSH levels, but it is also possible that AsIII directly inhibits germ cells by binding to thiol. High blood corticosterone levels are another probable reason of low serum LH, FSH, and testosterone. Serum levels of gonadotrophins and testosterone may be lowered by high corticosterone as shown in AsIII-treated rats. When measured in blood serum, however, LH was shown to be elevated (Hardy; 2005; Wang et al., 2006; Ali et al., 2013; Kumar et al., 2015; Reddy et al., 2010; Ahmad et al., 2010).

The most intriguing discovery of this research is that *Curcuma longa*, an antioxidant rich in Curcumin which improves male reproductive function by lowering lipid peroxidation levels Plant-based hormone precursors occupy receptor sites on cell membranes in a manner that prevents the real hormone from attaching and exerting its action during times of high hormonal activity as a result, both LH and Testosterone levels have returned to normal (El-Sherbiny et al., 2022; Nasr et al., 2022; Muratoğlu et al., 2019; Tsao et al., 2022; Prasad and Tyagi 2015).

Conclusion:

In the current study, sodium arsenite caused infertility due to low sperm counts, sperm motility, sperm degeneration in morphology, and complete arrest of spermatogenetic stages. However, after administration of Curcuma longa, there was a dramatic reversal in the testicular function, with the spermatogenetic stages returning to normal. This indicates that *Curcuma longa* has a revitalizing effect on testicular cells. From these results, it is clear that *Curcuma longa* is a unique medicine that not only has antioxidant and rejuvenating properties, but also helps keep testicular cells healthy and operating normally. An antidote to the reproductive damage caused by arsenic, among other drugs.

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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 the majority of 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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