

## IMPACT OF ARSENIC ON TESTOSTERONE SYNTHESIS PATHWAY AND SPERM PRODUCTION IN MICE.

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### ABSTRACT

Arsenic is found in biological systems as inorganic oxy forms (oxides, arsenites and arsenates) and as organic arsenic compounds. Arsenic found in soil and drinking water comes from geophysical as well as anthropogenic sources. The most common route of exposure for the general public is food and drinking water. Public health concerns have centered on carcinogenic, cardiovascular and nervous system effects seen in populations exposed to arsenic in drinking water. In geographical areas that do not have high levels of As contamination in drinking water, dietary intake is the major exposure route. Thus, consumption of contaminated foods or their processed products are often major contributors to As exposure and subsequent human-health effects.

To evaluate possible toxicity on the male reproductive system during arsenic therapy, male mice were used as a model. In the present investigation, the dose of 3 mg/Kg b.w & 4 mg/Kg b.w of Arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) was continuously administered to male mice for 8 weeks. The mice were sacrificed on 2<sup>nd</sup> , 4<sup>th</sup> ,6<sup>th</sup> , & 8<sup>th</sup> week to observe the sperm quality(sperm count & sperm motility) and level of Testosterone and Leutinizing hormone (LH) in the serum.

After treatment, major changes were observed in androgenic activity of male mice with reduced accumulation of spermatozoa and imbalance hormonal level. Significant changes were observed in sperm count, and motility (p<0.001) and declination in the level of Testosterone and inclination in the level of LH were observed which signify the testicular dysfunctions and leading to inhibit testosterone synthesis pathway, finally causes male infertility.

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### INTRODUCTION

Arsenic is an element that raises much concern from both environmental and human health standpoints. Human may encounter arsenic in water from wells drilled into arsenic-rich ground strata or in water contaminated by industrial or agrochemical waste [1]. They may eat food contaminated with arsenical grown with arsenic-contaminated water or in arsenic-rich soil.[2,3]. Today, arsenic compounds are still widely used in industry and agriculture. However, arsenic has been identified as a human carcinogen by the International Agency for Research on Cancer [4]. Arsenic exposure can result in both chronic and acute toxicity in humans. Chronic arsenic poisoning is a global health problem affecting millions of people, especially in India, Bangladesh, and China [5-7]. The main cause of the widespread chronic arsenicosis is the consumption of

underground drinking water naturally contaminated by arsenic. Arsenic contamination of drinking water may also result from mining and other industrial processes.

Arsenic exposure has been associated with an increased risk of dermatitis along with hyperkeratosis, gangrene and tumors of skin, bladder, liver, kidney, lung, prostate and other tissues [8-13]. Arsenic affects the mitochondrial enzymes, impairs the cellular respiration and causes cellular toxicity. It can also substitute phosphate intermediates, which could theoretically slow down the rate of metabolism and interrupt the production of energy [14]. Male infertility is reflected by low sperm count, low sperm motility and bad quality of sperms [14]. Sodium arsenite has been found to have an inhibitory effect on the activity of testicular steroidogenic enzyme D5-3b- hydroxysteroid

dehydrogenase (D5-3b-HSD) and 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) and to reduce the weight of testes and accessory sex glands [14] in rat. High Arsenic level may suppress the sensitivity of gonadotroph cells to GnRH as well as gonadotropin secretion by elevating plasma levels of glucocorticoids. These ultimately lead to the development of gonadal toxicity [14-15].

Adverse effects of mercury, manganese, chromium and arsenic on semen quality and altered serum hormone are less well documented. There is no clear evidence that Arsenic exposure may impair reproductive health in men. Only a few studies have investigated reproductive effects of concomitant exposure to several metals and controlled for potential confounders. Thus our present study has been done to test the causal relationship between testosterone synthesis and production of sperm through arsenic generated toxicity using mouse as a model animal.

#### **MATERIALS & METHODS :**

In the present investigation, experiments were performed on 10-12 weeks old healthy male Swiss albino mice, *Mus musculus*. Animals were maintained in polypropylene cages lined with paddy husk under a well regulated light and dark (12h:12h) schedule at 23 $\pm$ 1 $^{\circ}$ C. Animals were given food and water *ad libitum* in the animal house, Mahavir cancer Institute & Research centre, patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) and was duly approved by the IAEC . For experiments, mice were randomly selected into three groups consisting six mice in each: group A, control; group B, arsenic-treated @ 3mg / kg b.w. and group C, arsenic-treated @ 4 mg / kg b.w. Accordingly, animals of groups B, and C were orally

treated with aqueous solution of arsenic trioxide, 3 mg/kg body wt/day & 4 mg/kg body wt/day respectively for 8 weeks. A vehicle of control group of mice was served with equal volume of distilled water by gavage method. Arsenic trioxide was purchased from Merck company, Mumbai (India). Experiment was approved by Institutional Animal Ethics Committee (IAEC)

The treated and control group were sacrificed on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> & 8<sup>th</sup> week of treatment. The sperm were taken through cauda epididymis for sperm count and motility. Hormonal assessment were done through blood.

#### **SPERM COUNT**

The Cauda epididymis was dissected out and washed thoroughly in normal Saline (0.85 %).

Cauda epididymis was incised and made puncture at several places in 1 ml distilled water so as to allow the sperm to ooze out. After that, two drops of Eosin Y is mixed well with sperm.

Sperm counts was made using an improved Neubauer's chamber taking a drop of above preparation in it & observed at 450x magnification.

#### **SPERM MOTILITY**

Cauda epididymis was dissected out and ruptured on microscopic slide. After covering it with a cover slip, the motility of the spermatozoa was examined.

#### **Hormonal Assessment :**

Following hormonal assays were done

(I). Testosterone

(II).Luteinizing Hormone

#### **Collection of Blood :**

The blood from the control and treated mice were collected for the data. Mice were anaesthetized and blood samples were obtained from mice by orbital sinus puncture through Hematocrit tube which is one of the most effective method, which causes least stress to the animal. Serum was obtained from the blood for the estimation of Testosterone and LH.

Blood of individual mice of each treated group were taken in separate titration tube. After separation of the serum it was finally transferred in eppendorf for hormonal assessment

#### **ESTIMATION OF TESTOSTERONE & LH LEVEL IN SERUM OF MICE : BY ELISA TECHNIQUE**

Blood sample were collected after each sacrifice and their serum were isolated. Using the ELISA method Testosterone kit of LILAC Medicare (P) Ltd., Mumbai was utilized for the experiment.

**Method:** The normal range was calibrated and then 25  $\mu$ l serum samples were taken in the well plates. 100  $\mu$ l of enzyme conjugate was added in each well. After that, it was left for incubation at 37 $^{\circ}$ C in incubator for 1 hour. Then, the wells were washed with 300  $\mu$ l distilled water for at least 3 times and blotted. Then, 100  $\mu$ l TMB solution was added as substrate in each well plate and was again left for the incubation for 15 minutes for the colour. Finally, 100  $\mu$ l stop solution was added in each well to stop the reaction. Reading was taken at 630nm through Merck ELISA reader in ng/ml value

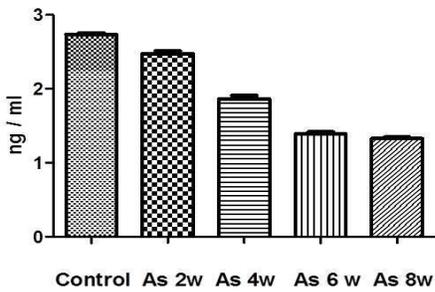
**Statistical Analysis of the Data:** The data were presented as mean  $\pm$  SEM. Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett's test, Graph Pad Prism 5.0.

#### **RESULTS**

The present study indicates that oral administration of sublethal dose of Arsenic trioxide ( 3.0 mg/ Kg b.w & 4.0 mg/ Kg b.w ) brings about various significant changes in the level of testosterone, sperm count and sperm motility that shows damaging effect on various stages of spermatogenesis. ELISA observation for serum Testosterone and luteinizing hormone have been done for control and Arsenic trioxide treated mice. The observed serum testosterone and luteinizing hormone level of control and Arsenic trioxide treated have been graphically represented in graphical plates. The legend mentioned in the graph clearly indicates the testosterone fluctuation in mice due to Arsenic trioxide. (Graphical plate I & II ). LH level indicated by Graphical plate III & IV. Declination in the level of testosterone and inclination in the level of luteinizing hormone indicates the abnormal condition of Leydig cells, hence inhibition of testosterone synthesis pathway can be observed.

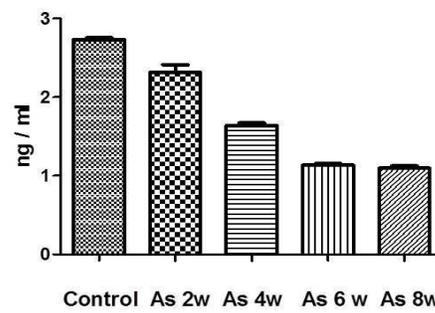
In the present investigation regarding sperm count and sperm motility, it has been observed that on both the doses 3.0 mg/ Kg b.w & 4.0 mg/ Kg b.w respectively marked reduction in sperm count as well as sperm motility as compared to control. All the values of sperm count and sperm motility mentioned have been graphically represented in graphical plates (V - VIII). The legend mentioned in each of the graph clearly indicates the fluctuation in sperm count and sperm motility due to Arsenic trioxide.

Testosterone Level in Arsenic Treated @ 3mg/Kg b.w



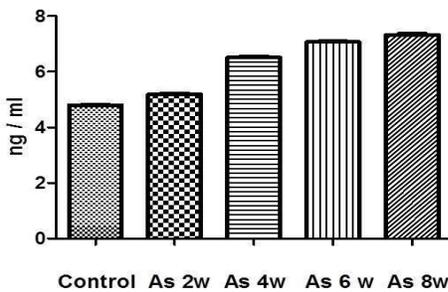
Graph - I

Testosterone Level in Arsenic Treated @ 4mg/Kg b.w



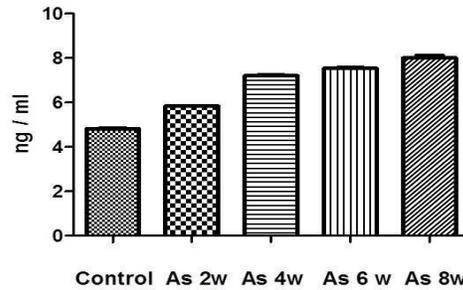
Graph - II

LH Level in Arsenic Treated @ 3mg/Kg b.w



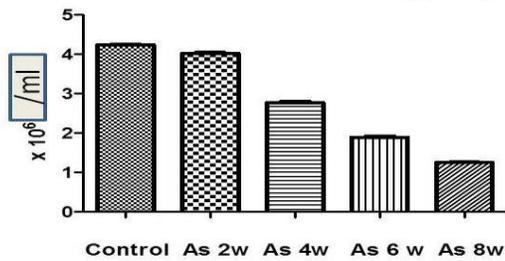
Graph - III

LH Level in Arsenic Treated @ 4mg/Kg b.w



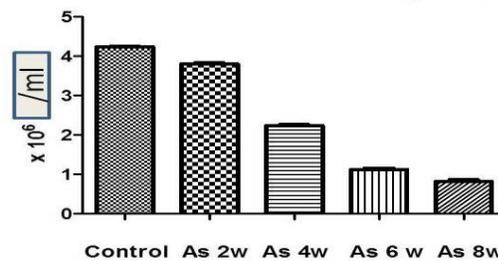
Graph - IV

Sperm Count of Arsenic Treated @ 3mg/Kg b.w



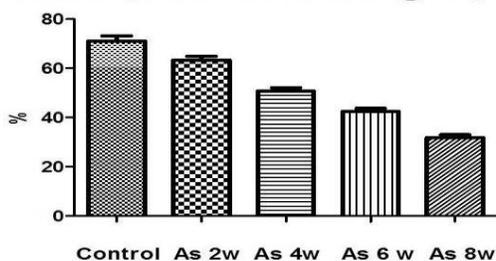
Graph - V

Sperm Count of Arsenic Treated @ 4mg/Kg b.w



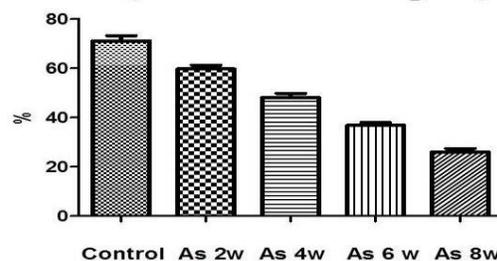
Graph - VI

Sperm motility in Arsenic Treated @ 3mg/Kg b.w



Graph - VII

Sperm motility in Arsenic Treated @ 4mg/Kg b.w



Graph - VIII

## DISCUSSION

The present study was aimed to determine the toxic effect of arsenic on male reproductive system. In the present study reproductive potential of sperm of male mice was measured using parameters like sperm count, sperm motility, testosterone and luteinizing hormone. The arsenic dose selected in the present study showed toxic symptoms in mice by significantly decreasing the sperm count and inhibiting the synthesis of testosterone.

Significant decrease in sperm counts was also observed in arsenic exposed in rats [16]. Mammalian sperm

contain large amounts of thiol-rich proteins in the flagellum which maintains sperm motility and stability. Arsenic is a well known thiol-inhibiting metalloid [17]. The decrease in sperm motility in the present study may be due to accumulation of arsenic in epididymis where the sperm matures and acquires motility. It might be possible that electrophilic nature of the arsenic it binds to sulphhydryl groups on proteins and thereby inhibits enzyme activity [18].

Increase in the luminal areas of the seminiferous tubules associated with decreased spermatozoal mass might be due to low levels of gonadotropins in arsenic treated rats, and these low levels are responsible for the decreased production of steroidogenic enzymes [14]. It has been established that arsenic administration leads to decrease in ovarian steroidogenic enzymes synthesis [14]. Thus the low levels of testosterone and increased level of LH might be responsible for the decrease in the spermatozoal mass in the lumen which provides support towards low production of sperm count and motility. The decrease in serum testosterone could be due to diminished responsiveness of Leydig cells to leutinizing hormone and/or the direct inhibition of testosterone steroidogenesis. In steroidogenesis,  $\Delta 5$ , 3- $\beta$ HSD and 17- $\beta$ HSD are the key regulatory enzymes [19]. A significant decrease in the activity levels of these steroidogenic enzymes in testis of experimental mice indicate decreased steroidogenesis, which in turn may suppress the reproductive activities in the male mice. This is in agreement with the findings where arsenic treatment was associated with inhibition of testicular steroidogenesis in rat [20]. This alteration in steroidogenic enzyme activity in experimental mice may be the result of changes in the levels of plasma FSH and LH, since these are the regulators of HSD activities [21]. The elevated levels of LH with lowered circulatory testosterone levels in experimental mice are indicative of intact pituitary-testicular axis. Testosterone plays an important role in attachment of the germ cells in seminiferous tubules. Low levels of intra-testicular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate cell apoptosis [22]. The increase in the levels of serum LH could be due to the impairment of spermatogenesis by the arsenic on the spermatogenic compartment or through the inhibition of testosterone production. Thus, the increase in the levels of serum FSH reflect the germ cell loss in the spermatogenic compartment or damage to the sertoli cells, thereby affecting the feed back regulation of FSH secretion as described [23]. The study has also shown that exposure to arsenic produces a contraception-like effect at pituitary level due to decreased production of testosterone by the testis. The decreased levels of testosterone might affect the status of reproductive potential of these mice.

## CONCLUSION

The present study indicates that toxicity of arsenic may affect the male reproductive health in a way to decrease the level of testosterone. Decreased level of testosterone & increased level of luteinizing hormone observation confirms the impairment of leydig cells and checks spermatogenesis. Inhibition in testosterone synthesis pathway responsible for low production of sperm & finally causes infertility in male mice.

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