

## Original Research Article

### Molybdenum induced histopathological and histomorphometric alterations in testis of male wistar rats

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#### ABSTRACT

#### Keywords

Ammonium molybdate, Epididymis, Histopathology, Rat, Spermatogenesis, Testis

The aim of present study was to elucidate the toxic effects of ammonium molybdate on histomorphologic and histologic picture of testis in male rats. Group A served as control while Group B, C and D received 50, 100 and 150 mg/kg b. wt./day ammonium molybdate orally respectively for 60 days. Group E rats received 150 mg/kg b. wt./day for 60 days and thereafter left for recovery study for 60 days. Ammonium molybdate treatment resulted in a significant decrease in the relative weight of testis. Histomorphometric observations revealed a reduction in the germ cell population in stage VIII of seminiferous cycle as well as diminution in seminiferous tubular diameter, Leydig cell nuclear diameter and cauda epididymal cell height. Histopathology of testis indicates marked degeneration in spermatogenic and Leydig cells, shrunk seminiferous tubules, thinner germinal epithelium, exfoliation of germ cells and depletion of spermatozoa from lumen of seminiferous tubule. After 60 days of the cessation of treatment in highest dose group of ammonium molybdate (150 mg/kg b.wt./day), testicular histoarchitecture represented almost normal appearance. The findings suggest that ammonium molybdate had significant adverse impact on male rat reproduction endpoints including fertility however significant recovery is possible after dose cessation.

#### Introduction

During the last few decades, exposure of various metals has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are considered to be major sources of metal pollution. Metals are widely distributed in the environment and some of them occur in food, water, air and tissues even in the absence of occupational exposure. Men are

inevitably exposed to metals due to their ubiquity in nature, wide use in industries and long-term persistence in the environment. Toxicity of metals has been raised as a global problem that might be responsible for the rising threat to humanity. Various recent experimental studies suggest that many metals have adverse impact on the male reproductive function (Pizent *et al.*, 2012; Mruk and Cheng, 2011; Mathur *et al.*, 2010)

Molybdenum (Mo), with an atomic number of 42, is presently considered an essential trace element that participates in a number of enzymatic reactions in many microorganisms and mammals. The estimated safe and adequate daily dietary intake (ESADDI) is 40–80 µg/day for infants, 50-100 µg/day for young children and 150-500 µg/day for teenagers and adults (Pennington and Jones, 1987). It is widely used as an alloying element in steel, cast iron, and super alloys and in the electronics industries (Mills and Davis, 1987). Molybdenum trioxide and molybdenum-oxygen compounds are added to steel and corrosion-resistant alloys. They are used in industrial catalysts, corrosion inhibitors, pigments, glass, ceramics, enamels, flame retardant for polyester and polyvinyl chloride resins, as crop nutrients in agriculture, and as reagents in chemical analyses. In addition to being a cofactor of enzymes related to purine and pyrimidines detoxification, it also has therapeutic potential as it is being used in the treatment of diabetes, Wilson disease, cancer and delayed amyotrophic lateral sclerosis onset (Pandey *et al.*, 2012a; Brewer, 2009; Waern and Harding, 2004; Tokuda *et al.*, 2008). Molybdenum has been claimed to reduce the incidence and severity of dental caries. It has also been reported to be beneficial to various groups of individuals with sulfite sensitivity, asthmatics with elevated urinary ratios of sulfites to sulfates, and those intolerant to intravenous sulfur containing amino acids (Mills and Davis, 1987).

Biologically, Mo belongs to the group of trace elements, i.e. the organism needs it only in minute amounts. If, however, an organism takes up too high amounts of Mo, toxicity symptoms are observed. Excessive dietary intake of grains, seeds, and legumes rich in molybdenum can cause deposits in soft tissues and joints, and trigger arthritic

symptoms. Other symptoms include gout, severe diarrhea, growth depression, and anemia (typical symptoms of copper deficiency) (Turnlund, 2002; Underwood, 1977). Molybdate ingestion in excessive amount might be associated with damage of testicular tissue of experimental animals. Molybdenum administered orally by capsule for 129 days to two male Holstein calves at doses between 4.1 and 7.8 mg/kg of body weight per day caused a degeneration of seminiferous tubules, gradual disappearance of the spermatogenic and interstitial tissue (Thomas and Moss, 1951).

With the persistent use of molybdate for industrial, therapeutic and agricultural purposes, Mo may enter the food chain with unforeseen consequences to the health of man and animals. Indeed, there are only few studies available on the histopathological and hisomorphological effects of ammonium molybdate on rat testis and none studies represent recovery studies after withdrawal of treatment. In this regard, aim of the present study was to investigate the effects of ammonium molybdate exposure on male reproductive organs of rats, with emphasis on the testis and epididymis, by evaluation of histological, hormonal and semen parameters.

## **Materials and Methods**

### **Animals**

Adult male Wistar rats (n=40), weighing 180–200 g, were used for the study. Animals were housed in polypropylene cages. Rats were maintained under controlled temperature (23±1°C) and lighting conditions (12:12-h photoperiod). Standard laboratory chow (Aashirwad Food Industries, Chandigarh, India) and water was provided ad libitum. The experimental study was approved by the ethical committee of

the Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur. Standard Guidelines were followed for maintenance and use of the experimental animals.

### **Treatment regimen**

Rats were divided into five groups (n=8 each). The compound was dissolved in 0.5 ml distilled water and administered orally to the rats every morning for 60 consecutive days. Treatment duration was selected according to the duration of one seminiferous cycle, which is 58–60 days in albino rats.

Group A: Control rats received 0.5 ml/day of the vehicle, i.e., distilled water.

Group B: Rats were treated with Ammonium molybdate ((NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O) at 50 mg/kg b.wt./day.

Group C: Rats were treated with Ammonium molybdate ((NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O) at 100 mg/kg/b.wt./day.

Group D: Rats were treated with Ammonium molybdate ((NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O) at 150 mg/kg/ b.wt./day.

Group E: Recovery of 60 days after withdrawal of Ammonium molybdate ((NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O) treatment similar to group D.

### **Autopsy schedule**

The rats were sacrificed within 24 h of the last administration of the compound. The testes, epididymides, seminal vesicles, prostate, kidney, and liver were excised, dissected, and freed of fat/blood vessels and weighed. Blood samples were collected from the cardiac puncture under light ether

anesthesia; serum was separated by centrifugation at 2000 rpm for 20 min at 4°C and stored at -20°C for hormone assays.

### **Body and organ weights**

Initial and final body weights were recorded. Weights of testes were recorded in every autopsy schedule and reported as relative weights (organ weight/body weight\*100).

### **Histopathological examination**

For the histological examination, the testis tissue was fixed in Bouin's fluid, dehydrated, and embedded in paraffin wax before sectioning at 5 µm and stained with Harris hematoxylin and eosin.

### **Histomorphometry**

#### **Seminiferous tubule diameter:**

Measurements were taken from at least 40 tubular profiles per animal using a light microscope equipped with ocular micrometer calibrated with stage micrometer. Only round tubules were selected randomly in various areas of the section. Two diameters perpendicular to each other were measured at X 100 magnification and averaged and expressed as seminiferous tubule diameter.

### **Quantitative evaluation of germ cells**

Quantitative evaluation of germ cells were made using 50 round tubules per group selected randomly at X 400 according to method described by Leblond and Clermont (1952). Quantitative evaluation of spermatogonia, preleptotene, pachytene spermatocytes and round spermatids were made in stage VII of seminiferous tubule cycle per cross section under X100 magnification.

The diameters of nuclei of various germ cell

types were measured by mean of an ocular micrometer. A correction factor was used to obtain the actual numerical density of germ cells (Abercrombie, 1946)

True counts =

$$\text{Crude Counts} \times \frac{\text{Section thickness } (\mu\text{m})}{\text{Section thickness } (\mu\text{m}) + \text{Nuclear diameter } (\mu\text{m})}$$

### Leydig cell area and nuclear diameter

The diameter of one hundred Leydig cells and their nuclei were measured on five sections from each testis with ocular micrometer at X 1000. The values were averaged and expressed as mean nuclear diameter of the Leydig cells.

**Statistical analysis:** All the values of body weights, organs weight, biochemical estimations were averaged; standard error of the mean was calculated. One-way analysis of variance (ANOVA) followed by the Least Significance Difference (LSD) multiple comparison test was used for data analysis. In all cases,  $p < 0.05$  was considered statistically significant. Statistical analyses were performed using SPSS 10.0 software.

## Result and Discussion

**Effect on body and organ weight:** Table 1 represents the effect of Ammonium Molybdate (AM) on body and organ weight of treated rats at three different dose levels. In control rats, a significant ( $P < 0.001$ ; +16.18%) body weight gain was recorded when compared with their initial body weight. A significant but retarded body weight gain was observed in the rats administered with AM at 50 mg/kg b.wt./day ( $P < 0.001$ ; +9.89%) and 100 mg/kg b.wt./day ( $P < 0.01$ ; +8.72%). However, a non significant gain in body weight (+4.41%) was recorded at dose level of 150 mg/kg b.wt./day. After the withdrawal of

the treatment, rats of recovery group depicted a reasonable recovery and there was a significant ( $P < 0.01$ , +9.55%) increase in the body weight when compared with their initial weight. Ammonium molybdate treatment caused a significant dose dependent decline in the relative weight of testis. Weight of reproductive organs significantly improved, after 60 days of the treatment withdrawal when compared with Group-D (150 mg/kg b.wt./day) but it was still significantly lower than control rats. (Table 1)

**Germ cell population dynamics:** The testicular germ cells population i.e. spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and round spermatids counts/ seminiferous tubule cross section are presented in Table 2.

**Spermatogonia:** There was dose dependent decline in the spermatogonia population in testis of rats treated with 50, 100 and 150 mg/kg b.wt./day doses of ammonium molybdate. The decrease was non-significant for low dose (50 mg/kg/ b.wt./day), slightly significant ( $P < 0.05$ ) for medium dose (100 mg/kg/ b.wt./day) and highly significant ( $P < 0.01$ ) for highest dose (150 mg/kg/ b.wt./day) when compared to control values. The population of preleptotene, pachytene spermatocyte and round spermatid in the seminiferous tubules was also declined dose dependently ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ; respectively) in rats exposed with ammonium molybdate at 50, 100 and 150 mg/kg b.wt./ day doses when compared with control.

After 60 days of treatment withdrawal, the spermatogonia count was restored significantly ( $P < 0.001$ ) when compared with Group-IV (150 mg/kg b.wt./day) rats, but it was significantly unchanged when compared with control rats.

**Preleptotene spermatocytes:** After 60 days of the ammonium molybdate treatment, a significant dose dependent decline ( $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ ) in preleptotene spermatocytes counts was observed in ammonium molybdate (50, 100 and 150 mg/kg b. wt./ day; respectively) treated animals when compared with control rats.

The number of preleptotene spermatocytes was increased significantly ( $P<0.001$ ) in rats after 60 days of treatment withdrawal as compared to group IV (150 mg/kg b.wt./day), although it was still low ( $P<0.05$ ) when compared to control rats.

**Pachytene spermatocytes:** However, after 60 days of treatment withdrawal, number of pachytene spermatocyte was increased significantly ( $P<0.001$ ) when compared with Group-IV (150 mg/kg/ b.wt./day) rats, but it was significantly ( $P<0.05$ ) lower than the control rats suggesting partial recovery.

**Round spermatids:** There was significant ( $P<0.05$ ,  $P<0.001$ ,  $P<0.001$ ) decline in the population of round spermatids per seminiferous tubule cross section in ammonium molybdate (50, 100 and 150 mg/kg b.wt./day; respectively) treated animals when compared with control.

After 60 days of cessation of ammonium molybdate treatment the rats of recovery group showed significant increase ( $P<0.001$ ) in the counts of round spermatids as compared to rats of Group-IV (150 mg/kg b.wt./day), although it was still low ( $P<0.01$ ) when compared to control rats.

#### **Histomorphometric analysis (Table 2)**

**Seminiferous tubule diameter:** There was significant ( $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ ) dose dependent decrease in the mean diameter of seminiferous tubules in the rats treated with

three different doses (50, 100 and 150 mg/kg b.wt./day, respectively) of ammonium molybdate as compared to control rats.

The mean diameter of seminiferous tubules was improved significantly ( $P<0.001$ ) after 60 days of treatment withdrawal when compared with Group-IV (150 mg/kg b.wt./day) rats, but it was significantly ( $P<0.05$ ) less than control rats suggesting partial recovery.

#### **Leydig cell nuclear diameter**

Exposure of ammonium molybdate at 100 and 150 mg/kg/ b.wt./day, doses for 60 days in rats, resulted in a significant ( $P<0.01$ ,  $P<0.001$ ; respectively) reduction in Leydig cell nuclear diameter when compared to control rats while reduction in Leydig cell nuclear diameter was no significant at 50 mg/kg b.wt./day dose level.

After 60 days of recovery study, the reduced Leydig cell nuclear diameter was significantly ( $P<0.01$ ) improved as compared with Group-IV (150 mg/kg b.wt./day) rats, but it was still low ( $P<0.05$ ) when compared to control values.

**Cauda epithelial cell height:** Measurement of cauda epididymal epithelial cell height in rats treated with 50, 100 and 150 mg/kg/ b.wt./day doses of ammonium molybdate showed significant ( $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ ; respectively) dose dependent decrease when compared to control.

After 60 days of cessation of ammonium molybdate treatment, the rats of recovery group showed significant recovery ( $P<0.001$ ) in cauda epididymal epithelial cell height as compared to rats of Group-IV (150 mg/kg b.wt./day), although, it was still low ( $P<0.05$ ) when compared to control rats.



### **Histopathological findings in the testis**

The testicular histology of the control rats displayed normal structure of seminiferous tubules surrounded by vascularized dense fibrous connective tissue with prominent Leydig cells. The germinal epithelium exhibited normal shape, size and characteristic arrangement of all successive germ cell types. The tubular lumen was fully occupied by large number of healthy spermatozoa (Fig.1).

The histological picture of the testis treated with low dose of ammonium molybdate (50 mg/kg b.wt./day) showed mild degenerative changes as indicated by slight shrinkage of seminiferous tubules and presence of less sperms in tubular lumen. The Leydig cells also showed mild degenerated changes and reduction in the nuclear diameter (Fig.2 – Top left and right). The histoarchitecture of the testis in rats treated with ammonium molybdate (100 mg/kg b.wt./day) showed marked degenerative changes in seminiferous tubules and Leydig cells. The seminiferous tubules were reduced in size and showed disruption of normal epithelial organization. The lumen showed presence of exfoliated germ cells and fewer spermatozoa. Leydig cells were shrunken and showed atrophic changes (Fig.2 – Bottom left and right). The histological examination of the testis treated with highest dose of ammonium molybdate (150 mg/kg b.wt./day) revealed severe degenerative changes in both the seminiferous tubules and the interstitial tissue. Additionally, the lumen of seminiferous tubule was characterized by the presence of tail remnants of degenerating spermatozoa and exfoliated germ cells. The Leydig cells also showed marked degeneration, decreased in nuclear diameter and decline in number (Fig.3 Top – left and right).

After 60 days of the cessation of treatment

in highest dose group of ammonium molybdate (150 mg/kg b.wt./day), testicular histoarchitecture represented almost normal appearance depicting seminiferous tubules with all the developing stages of spermatogenic cells, moderate number of spermatozoa in the lumen and near normalized Leydig cells (Fig.3 Bottom – left and right).

Reproductive toxicity from metal exposure in males is one of the areas of concern in toxicology today. Our result in this present study showed that exposure to Ammonium molybdate in rat caused stern testicular toxicity resulting in the obstruction of spermatogenesis and steroidogenesis in a dose dependent manner however the toxic effects return towards the normal side after cessation of treatment. The observed reductions in body weight gain reflect a pharmacological effect or a threshold toxic effect of ammonium molybdate. Similar decline in body weight gain was formerly observed in numerous studies in molybdate treated experimental animals (Johnson and Miller., 1963; Pandey *et al.*, 2012b; Lyubimov *et al.*, 2004).

Testicular weight is an important parameter in the reproductive evaluation of males owing to its high and positive correlation to sperm production (França and Russell, 1998). In present study, ammonium molybdate represented a significant dose dependent decline in the relative weight of testis in treated rats. Decline in testis weight could be correlated with degeneration of germinal epithelium, disruption of spermatogenesis, or inadequate supply of testosterone (Pandey and Singh, 2002; Zhai *et al.*, 2014)

Counting of germ cells in seminiferous tubule at specific stage is an appropriate method to detect testicular toxicity. The different germ cell population of

spermatogonia, spermatocyte and spermatids each display their own sensitivity to different toxicants (Takahashi and Matsui, 1993).

The present study shows that ammonium molybdate produced a reduction in the tubular population of spermatogonia, preleptotene and pachytene spermatocytes, as well as in round spermatids in stage VIII of seminiferous cycle in per cross section of seminiferous tubule. These results corroborate the histological findings including reduced tubular diameter and seminiferous epithelium height, indicating a reduction of spermatogenic activity. Titenko-Holland *et al.* (1998) reported genotoxic effects of molybdenum on post meiotic germ cells of spermatogenesis via formation of free radicals and impairment in steroidogenesis. Monsees *et al.* (2002) reported that reproductive toxicants may alter germ cell attachment, disturb apical cytoskeletal transport, or induce microtubule dependent transport defects. This in turn will lead to germ cells loss and disruption of the seminiferous epithelium.

The seminiferous tubular diameter is used as a relevant parameter for the evaluation of spermatogenic activity in experimental and toxicological assays. There is a positive relationship exists between the tubular diameter and the spermatogenic activity of the testis (França and Russell, 1998; Predes *et al.*, 2011). In present study, seminiferous tubule diameter was found to be significantly reduced in animals exposed to ammonium molybdate. Reduced tubule diameter could reflect defective spermatogenesis as evidenced by reduction in the number of various spermatogenic cells.

Leydig cells in the interstitium of testis produce testosterone which acts in a

paracrine manner in the testis to support spermatogenesis. The testicular secretion of androgens depends on the Leydig cell number and also on the activity of Leydig cells (Teerds, 1996). The significant reduction of Leydig cell nuclear diameter was observed in ammonium molybdate treated group. Our results are in accordance with Predes *et al.* (2011) who also found less circular Leydig cell nuclei in cadmium treated animals.

The epididymal epithelial cells perform specific absorptive and secretory activities in order to maintain the unique microenvironment necessary for sperm maturation (Guyonnet *et al.*, 2011). Although there is general agreement in the literature that orchidectomy results in a reduction in epididymal epithelial height and testosterone increases both the re-expansion of existing cells and the number of new cells in regressed epididymis (Hamzeh and Robaire, 2009). Therefore it can be concluded that deficiency of testosterone might be responsible for marked reduction in epididymal cell height.

In the evaluation of male reproductive toxicity, histopathologic examination of the reproductive organs, especially the testis, is recommended as the most sensitive endpoint. Testicular degeneration can be defined as a process that causes deterioration of testicular structure and loss of function (Turner, 2007).

Histopathological examination of rats' testis orally administered with ammonium molybdate (50,100, 150 mg/kg b.wt/day) demonstrated apparent alterations in the testis including marked degeneration in spermatogenic cells, shrunk seminiferous tubules, thinner germinal epithelium, exfoliation of germ cells and depletion of spermatozoa from lumina. Similar

histological changes in testis of rats have been observed in molybdate (Pandey and Singh, 2002) administered rats. Histological alterations in testicular tissue might be resulted from oxidative stress which may cause cell damage and impairment of steroidogenic activity of Leydig cells. Degenerative changes in germ cells found in the present study might be due to hormonal deficiency as reduced steroidogenesis resulted in altered spermatogenesis and spermatogenic failure. A similar correlation between free radicals induced oxidative stress and testicular damage has been reported by various researchers in experimental animals treated with arsenic (Morakinyo *et al.*, 2010), cadmium (Predes *et al.*, 2011), chromium (Aruldas *et al.*, 2005), lead (Dorostghoal *et al.*, 2013), mercury (Kalender *et al.*, 2013) and nickel (Hassan and Barkat, 2008).

Pandey and Singh, (2002) suggested that

molybdenum induced histopathological alterations in testis could be correlated with decreased activity of marker testicular enzymes viz. SDH, which is known to be associated with germ cell maturation along with increased activity of LDH and  $\gamma$ -GT (the enzymes related with germinal epithelium and Sertoli cell, respectively). In present study, we noticed that molybdenum generally induced toxic effects on post meiotic germ cells so restoration in the histoarchitecture of the testis towards the normal side might be possible.

In conclusion, the present study showed that Ammonium Molybdate produced remarkable effect on spermatogenesis as evident in histological degenerative changes observed in the testis. It is assumed that these changes would interfere with the proper function of the testis. Thus it could be concluded that Ammonium Molybdate might contribute to male infertility.

**Table.1** Body and relative organ weights of rats treated with various doses of ammonium molybdate

Treatments		Group-I Control (vehicle)	Group-II Ammonium molybdate (50 mg/kg b.wt./day)	Group-II Ammonium molybdate (50 mg/kg b.wt./day)	Group-II Ammonium molybdate (50 mg/kg b.wt./day)	Recovery- Group (after 60 days of treatment withdrawal)
Body weight (g)	Initial	173.75 ±5.66	179.37 ±2.75	171.87 ±3.19	181.37 ±2.56	189.37 ±3.95
	Final	201.87±4.33 <sup>c</sup> (+16.18%)	197.12±3.27 <sup>c</sup> (+9.89%)	186.59±3.59 <sup>b</sup> (+8.72%)	189.37±3.95 <sup>ns</sup> (+4.41%)	207.46±4.18 <sup>b</sup> (+9.55%)
Testes (mg/100g b.wt.)		1297.92 ±25.01	1226.89 ±23.6 <sup>a</sup>	1175.52 ±26.14 <sup>b</sup>	1099.52 ±19.14 <sup>c</sup>	1208.45 ±18.40 <sup>a***</sup>

**Levels of significance:**

Values in parenthesis indicate % changes Values represent mean ± SEM (n=8)  
 ns- Non significant; a- P<0.05; b- P<0.01; c- P<0.001, final body weight compared with initial body weight.  
 ns- Non significant; a- P<0.05; b- P<0.01; c- P<0.001, ammonium molybdate treated groups compared with control group.  
 +- non significant; \*- P<0.05; \*\*- P<0.01; \*\*\*- P<0.001, recovery group compared with group-IV. (One way ANOVA followed by LSD multiple comparison test)



**Table.2** Spermatogenic cell count/ cross section of seminiferous tubule and morphometric analysis of rats treated with various doses of ammonium molybdate

Treatments	Spermatogonia	Preleptolene spermatocyte	Pachytene spermatocyte	Round spermatid	Seminiferous tubular diameter (μ m)	Leydig Cell nuclear diameter (μ m)	Cauda epithelial cell height (μ m)
<b>Group-I Control</b> (vehicle)	6.75±0.25	22.12 ±0.72	23.12±0.74	68.75±2.69	274.5±7.38	6.75±0.25	24.75±0.86
<b>Group-II Ammonium molybdate</b> (50 mg/kg b.wt./day)	6.37±0.26 <sup>ns</sup>	19.75±0.62 <sup>a</sup>	21.25±0.52 <sup>a</sup>	61.25±2.67 <sup>a</sup>	261.25±3.10 <sup>a</sup>	6.12±0.35 <sup>ns</sup>	22.57±0.68 <sup>a</sup>
<b>Group-III Ammonium molybdate</b> (100 mg/kg b.wt./day)	5.75±0.16 <sup>a</sup>	18.62±0.59 <sup>b</sup>	19.87±0.55 <sup>b</sup>	47.87±2.00 <sup>c</sup>	252.18±3.75 <sup>b</sup>	5.62±0.26 <sup>b</sup>	20.75±0.75 <sup>b</sup>
<b>Group-IV Ammonium molybdate</b> (150 mg/kg b.wt./day)	5.37±0.26 <sup>b</sup>	15.62±0.62 <sup>c</sup>	16.87±0.58 <sup>c</sup>	37.12±1.96 <sup>c</sup>	234.87±2.34 <sup>c</sup>	4.37±0.18 <sup>c</sup>	17.12±0.72 <sup>c</sup>
<b>Recovery-Group</b> (after 60 days of treatment withdrawal)	6.25±0.20 <sup>ns***</sup>	19.56±0.86 <sup>a***</sup>	20.62±0.59 <sup>a***</sup>	58.37±1.96 <sup>b***</sup>	258.62±3.67 <sup>a***</sup>	5.75±0.25 <sup>a**</sup>	21.87±0.85 <sup>a***</sup>

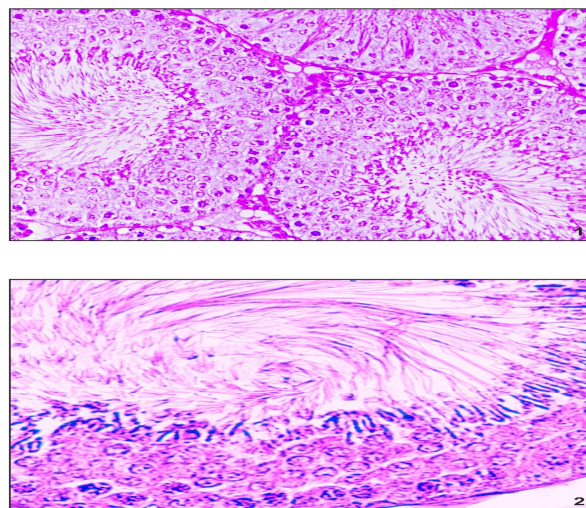
**Levels of significance:**

Values represent mean ± SEM (n=8)

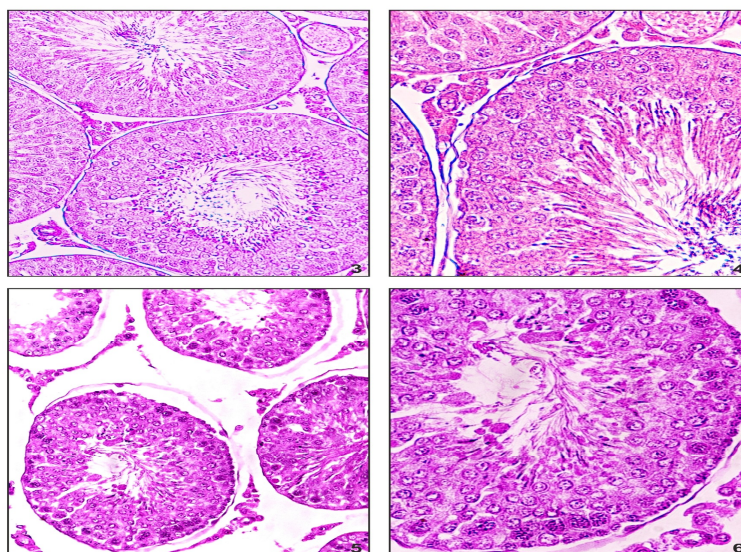
ns- Non significant; a- P<0.05; b- P<0.01; c- P<0.001, ammonium molybdate treated groups compared with control group.

+ - non significant; \*- P<0.05; \*\* - P<0.01; \*\*\* - P<0.001, recovery group compared with group-IV. (One way ANOVA followed by LSD multiple comparison test)

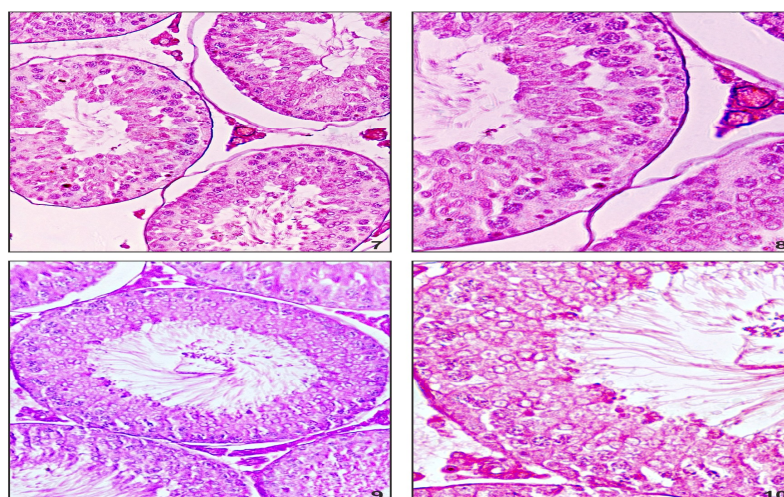
**Figure.1** Photomicrograph of the cross section of testis of control rat showing normal histoarchitecture (H.E. X 200) (Top); Photomicrograph of testicular section at higher magnification exhibiting characteristic tiered arrangement of various types of germ cells in the seminiferous epithelium and normal morphology of the Leydig cells (H.E. X 400) (Bottom)



**Figure.2** Photomicrograph of the cross section of testis of ammonium molybdate (50 mg/kg b.wt./day) treated rat showing mild degenerative changes in seminiferous tubules (H.E. X 200) (Top left); Photomicrograph of the same at higher magnification. Note slight shrinkage of seminiferous tubules, degenerative changes in germinal epithelium and debris in the tubular lumen (H.E. X 400) (Top right); Photomicrograph of the cross section of testis of ammonium molybdate (100 mg/kg b.wt./day) treated rat showing shrinkage of seminiferous tubules with degeneration, increase in intertubular space. (H.E. X 200) (Bottom left); Photomicrograph of the same at higher magnification. Note degeneration of tubular elements and presence of exfoliated germ cells and reduced number of spermatozoa in the lumen (H.E. X 400) (Bottom right)



**Fig.3** Photomicrograph of the cross section of testis of ammonium molybdate (150 mg/kg b.wt./day) treated rat showing marked shrinkage of seminiferous tubules, degenerative changes in germ cells with disruption of spermatogenesis (H.E. X 200) (Top left); Photomicrograph of the same at higher magnification showing decrease in the diameter of seminiferous tubules accompanied with depletion in the height of germinal epithelium, degenerated Sertoli cells, presence of sperm debris and exfoliated germ cells in lumen and shrunken Leydig cells (H.E. X 400) (Top right); Photomicrograph of cross section of testis of rat after 60 days of withdrawal of ammonium molybdate treatment (150 mg/kg b.wt./day for 60 days) exhibiting almost normal histoarchitecture of seminiferous tubules and lumen almost filled with spermatozoa (H.E. X 200) (Bottom left). Photomicrograph of the same at higher magnification showing almost normal appearance indicating seminiferous tubules with all the developing stages of spermatogenic cells (H.E. X 400) (Bottom right)



## Acknowledgement

The authors are grateful to Prof. P.K.Goyal, Head of the Department and Center for Advances Studies, Department of Zoology, University of Rajasthan for providing necessary facilities. Ms. Geeta Pandey thanks University Grants Commission (UGC), New Delhi for providing Meritorious Research Fellowship.

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