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Assessment of Molybdenum Induced Alteration in Oxidative Indices, Biochemical Parameters and Sperm Quality in Testis of Wistar Male Rats

Geeta Pandey and Gyan Chand Jain
Department of Zoology, Laboratory No. 15, University of Rajasthan, Jaipur, India

Corresponding Author: Gyan Chand Jain, Department of Zoology, Laboratory No. 15, University of Rajasthan, Jaipur, India

ABSTRACT

The present study was carried out to investigate the effects of ammonium molybdate on lipid peroxidation, antioxidant, biochemical and semen parameters in male Wistar rats. Group I served as control while group II, III and IV received 50, 100 and 150 mg kg^{-1} b.wt. day^{-1} ammonium molybdate orally respectively for 60 days. Group V rats received 150 mg kg^{-1} b.wt. day^{-1} for 60 days and thereafter left for recovery study for 60 days. The present study examined the effects of ammonium molybdate on serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) along with sperm parameters. Estimations were also made for marker biochemical and antioxidant parameters in testis. Ammonium molybdate treatment resulted in a dose dependent reduction in serum testosterone, FSH, LH, sperm count, motility and viability. The concentration of total protein and sialic acid as well as activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were decreased in a dose dependent manner while there was a significant increase in the glycogen and cholesterol level in testis. The level of Thiobarbituric Acid Reactive substance (TBARs) was increased while the activities of superoxide dismutase and concentration of glutathione and ascorboic acid was decreased significantly in testis of rats treated with ammonium molybdate. All the parameters showed significant improvement after 60 days of treatment withdrawal in highest dose group. The findings suggested that ammonium molybdate exerts a deleterious effect on testicular function by altering biochemical and antioxidant status of the testes.

Key words: Molybdate, testosterone, sperm, biochemical, rats

INTRODUCTION

Molybdenum (Mo) is fourth member of the second transition series with an atomic number of 42 and atomic weight of 95.95 (Burguera and Burguera, 2007). It is of essential importance for all biological systems as molybdenum enzymes are ubiquitous in the biosphere. It has also been reported that molybdenum significantly affects protein synthesis, as well as metabolism of phosphorus, sulphur, potassium, copper, zinc and iodine (Mendel, 2013).

For the general population, diet is the most important source of molybdenum exposure. Among foods, Mo is found at higher concentrations in leafy vegetables and legumes. Beside this, persons get exposed to molybdenum in the areas involved in the mining of Mo ore and certain industrial operations (CDC., 2005).

Molybdenum supplementation may be of therapeutic use in patients with sulfite sensitivity (Wright and Littleton, 1989), Wilson's disease (Brewer, 2005), dental caries

(Curzon and Cutress, 1983), malabsorption states (Underwood, 1977) and certain type of cancers (Waern and Harding, 2004; Brewer, 2005). Molybdenum has also been recommended as antidiabetic agent. Molybdate treatment also showed beneficial effects on antioxidant system and postischaemic cardiac function of diabetic rats (Pandey *et al*., 2012a). Additionally, it is also present in multi-vitamin and mineral supplements currently at level up to 25 μg day^{-1} (Pennington and Jones, 1987).

Several animal studies have reported that high levels of Mo interfere especially with copper metabolism in animals which in turn results in alteration of normal hematological profile (Kusum *et al*., 2010; Pandey *et al*., 2012b). Histopathological examination of testis revealed high proportion of degenerated seminiferous tubules, with marked attenuation of the lining epithelium and diminished spermatogenesis in molybdenum treated sheep (Haywood *et al*., 2004), bulls (Thomas and Moss, 1951), rats (Pandey and Jain, 2015) and rabbits (Bersenyi *et al*., 2008).

Molybdenum-induced changes at semen, hormonal and biochemical level in testis along with recovery study after treatment withdrawal have not so far been studied. Therefore, the present study was designed to investigate the effect of ammonium molybdate on reproductive hormones, sperm quality and marker biochemical parameters in testis of male rats.

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 I: Control rats received 0.5 mL day^{-1} of the vehicle, i.e., distilled water

Group II: Rats were treated with ammonium molybdate ($(NH_4)_6$ $Mo_7O_{24}.4H_2O$) at 50 mg kg^{-1} b.wt. day^{-1}

Group III: Rats were treated with ammonium molybdate ($(NH_4)_6$ $Mo_7O_{24}.4H_2O$) at 100 mg kg^{-1} b.wt. day^{-1}

Group IV: Rats were treated with ammonium molybdate ($(NH_4)_6$ $Mo_7O_{24}.4H_2O$) at 150 mg kg^{-1} b.wt. day^{-1}

Group V: Recovery of 60 days after treatment withdrawal in highest dose treatment group (i.e., Group IV)

Autopsy schedule: The rats were sacrificed within 24 h of the last administration of the compound The testes were excised, dissected and freed of fat/blood vessels and were kept frozen at -20-70°C for biochemical estimations.

Serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH): Serum samples were examined for serum testosterone (Mourao *et al*., 2003), LH and FSH (Rojanasakul *et al*., 1994) through chemiluminescence assay.

Sperm parameters

Sperm motility and density: To determine the sperm motility and density (sperm counts), 100 mg of cauda epididymis was minced with a sharp razor blade suspended in 2.0 mL normal saline (0.9% NaCl, 37°C). The suspension was passed through a nylon mesh to separate the tissue from the sperm. One drop of the evenly mixed sample was applied to a Neubauer's counting chamber under coverslip. Quantitative motility expressed as percentage was determined by counting both motile and immotile spermatozoa in different areas under light microscope at a magnification of 100X. Sperm counts were made by the routine procedure and expressed as million mL^{-1} of suspension (Prasad *et al*., 1972).

Sperm viability: Sperm vitality was assessed by nigrosin-eosin staining method. One drop of the 1% aqueous solution of eosin-y and 10% aqueous solution of nigrosin was placed in a microcentrifuge tube. A drop of well mixed sperm sample was added to it and mixed thoroughly. The mixture was dropped on a glass slide and observed under 400X magnification. The percentage of alive (without stain) and dead (red) cells were determined by at least counting 200 cells (Bjorndahl *et al*., 2003).

Tissue biochemistry: Frozen tissue of testis was analysed for total protein (Lowry *et al*., 1951), glycogen (Montgomery, 1957), sialic acid (Warren, 1959), total cholesterol (Zlatkis *et al*., 1953), acid phosphatase and alkaline phosphatase (Kind and King, 1954).

Lipid peroxidation and antioxidant defense system markers: Lipid peroxidation (TBARs, Ohkawa *et al*., 1979), superoxide dismutase (Marklund and Marklund, 1974), glutathione (Moron *et al*., 1979) and ascorbic acid (Roe and Kuether, 1943) were analysed in testis.

For all quantitative estimations, tissues obtained from different animals of a treated and control group were pooled to prepare the homogenate. At least seven samples from each group were taken and averaged.

RESULTS

Serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH): The level of serum testosterone, FSH and LH showed a significant dose dependent decline ($p<0.05$, $p<0.01$, $p<0.001$, respectively) in rats exposed with ammonium molybdate (50, 100 and 150 mg kg^{-1} b.wt. day^{-1}) when compared with the rats of control group (Fig. 1-3).

The rats of recovery group showed significant improvement ($p<0.01$) in serum testosterone, FSH and LH level when compared with high dose group (150 mg kg^{-1} b.wt. day^{-1}) but it was still lower as compared to control rats.

Sperm count, motility and viability: A significant dose dependent decline was observed in the sperm count, motility and viability in cauda epididymis of ammonium molybdate (50, 100 and 150 mg kg^{-1} b.wt. day^{-1}, respectively) treated rats when compared with control (Table 1).

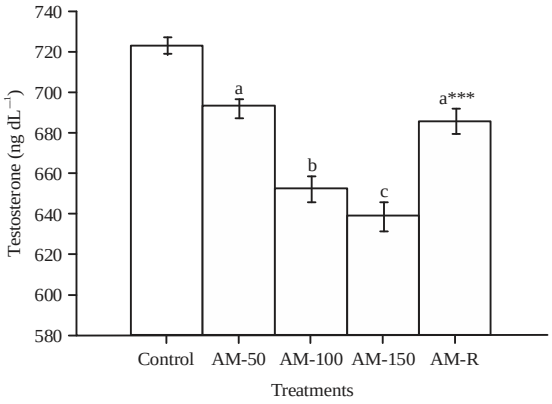

Fig. 1: Serum testosterone level of rats treated with different doses of ammonium molybdate

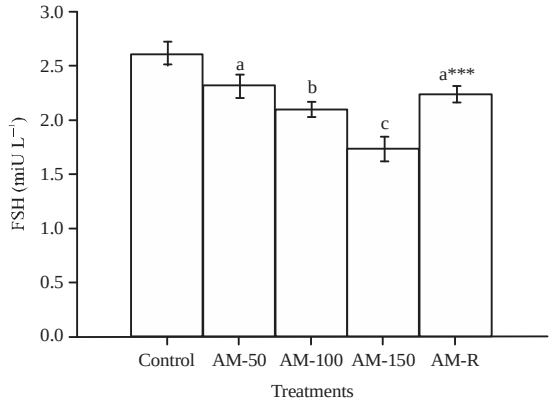

Fig. 2: Serum FSH level in ammonium molybdate treated rats at different dose levels

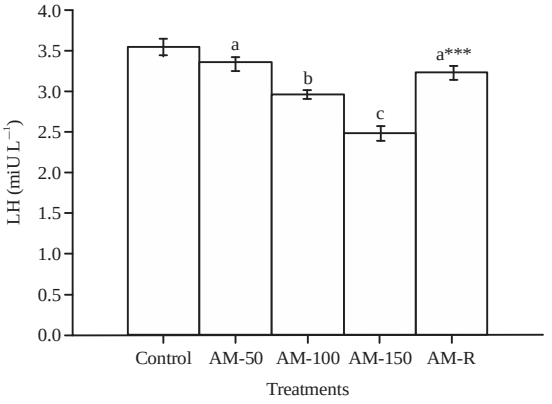

Fig. 3: Serum LH level in ammonium molybdate treated rats at different dose levels

The rats of recovery group showed significant improvement ($p<0.001$) in sperm parameters as compared to rats of group-IV (150 mg kg^{-1} b.wt. day^{-1}) after 60 days of treatment cessation. However, all these parameters were significantly ($p<0.05$) lower than control rats suggesting partial recovery.

Table 1: Cauda epididymal sperm analysis and reproductive performance of rats treated with various doses of ammonium molybdate

Treatments	Sperm count (Million mL^{-1})	Sperm motility (%)	Sperm viability (%)	Fertility (%)	Litter size
Group I					
Control (vehicle)	46.50±1.95	75.08±2.45	82.6±2.99	93.75 (15/16)	10.12±0.26
Group II					
Ammonium molybdate (50 mg kg^{-1} b.wt. day^{-1})	41.65±1.06[a]	68.58±2.11[ns]	74.6±2.28[a]	81.25 (13/16)	9.12±0.42[a]
Group III					
Ammonium molybdate (100 mg kg^{-1} b.wt. day^{-1})	37.56±1.88[b]	60.97±3.71[b]	69.37±2.67[b]	62.50 (10/16)	8.12±0.57[b]
Group IV					
Ammonium molybdate (150 mg kg^{-1} b.wt. day^{-1})	25.72±1.42[c]	38.56±2.76[c]	45.75±2.35[c]	43.75 (7/16)	5.12±0.27[c]
Recovery-group (after 60 days of treatment withdrawal)	40.23±1.90[a]***	65.16±2.32[a]***	71.37±2.52[b]***	75.00 (12/16)	8.62±0.39[a]***

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)

Fertility (%): The fertility (%) was reduced to 81.25, 62.50 and 43.75%, respectively in ammonium molybdate administered rats at dose regimen of 50, 100 and 150 mg kg^{-1} b.wt. day^{-1} as compared to control rats (93.75%) (Table 1).

After 60 days of the termination of ammonium molybdate exposure, the fertility rate was increased up to 75%.

Litter size: The mean litter size was also turned down dose dependently in ammonium molybdate (50, 100 and 150 mg kg^{-1} b.wt. day^{-1}) treated rats (9.12±0.42, 8.12±0.57 and 5.12±0.27, respectively) as compared to control rats (10.12±0.26) (Table 1).

A significant ($p<0.001$) improvement in the mean litter size was noticed in rats after 60 days of treatment withdrawal when compared with group-IV (150 mg kg^{-1} b.wt. day^{-1}) rats, although it was significantly ($p<0.05$) lower in comparison to control rats.

Tissue biochemistry: A dose dependent decrease in the concentration of total protein, sialic acid, ACP and ALP in testis while there was a significant increase in the glycogen and cholesterol level in testis of rats treated with ammonium molybdate (Table 2).

After 60 days of treatment withdrawal, the rats of recovery group showed significant improvement in all biochemical parameters in testis as compared to rats of group-IV. However, it was still low when compared to control rats.

Lipid peroxidation and antioxidant defense system: Antioxidant defense parameters like lipid peroxidation, superoxide dismutase, glutathione and ascorbic acid level were observed after the completion of treatments. The concentration of TBARs was measured for the quantitative measurement of lipid peroxidation. The rate of lipid peroxidation (TBARs) showed significant ($p<0.05$, $p<0.01$, $p<0.001$) dose dependent increase while a significant ($p<0.05$, $p<0.01$, $p<0.001$) dose dependent decline was found in the activity of superoxide dismutase (SOD) as well as ascorbic acid and glutathione content in testis of rats treated with ammonium molybdate (50, 100 and 150 mg kg^{-1} b.wt. day^{-1}, respectively), when compared with control group (Table 3).

The increased rate of lipid peroxidation (TBARs level) and reduced concentration of ascorbic acid, glutathione and superoxide dismutase (SOD) activity was significantly recovered in testis after 60 days of treatment withdrawal when compared with Group-IV (150 mg kg^{-1} b.wt. day^{-1}) rats. However, it was significantly ($p<0.05$) higher than control rats suggesting partial recovery.

Table 2: Tissue biochemistry in testis and epididymis of rats treated with various doses of ammonium molybdate

	Total protein (mg g^{-1})		Glycogen (mg g^{-1})		Sialic acid (mg g^{-1})		Total cholesterol (mg g^{-1})		Acid phosphatase (KA unit)		Alkaline phosphatase (KA unit)		Fructose (mg g^{-1})
Treatments	T	E	T	E	T	E	T	E	T	E	T	E	Seminal vesicle
Group I													
Control (vehicle)	216.37±5.81	221.5±6.58	3.69±0.09	3.50±0.13	5.06±0.14	5.37±0.21	6.59±0.18	6.04±0.17	12.96±0.49	11.57±0.42	54.27±2.04	52.23±2.13	5.79±0.22
Group II													
Ammonium molybdate (50 mg kg^{-1} b.wt. day^{-1})	201.12 ±4.29^{a}	204.25±6.43^{ns}	3.87±0.14^{ns}	3.78±0.11^{ns}	4.57±0.18^{a}	4.74±0.16^{a}	7.18±0.15^{a}	6.73±0.19^{a}	12.02±0.26^{a}	10.45±0.24^{a}	48.33±1.20^{a}	46.95±1.53^{a}	5.17±0.18^{a}
Group III													
Ammonium molybdate (100 mg kg^{-1} b.wt. day^{-1})	186.62±6.28^{b}	189.60±5.30^{b}	4.10±0.15^{a}	3.94±0.16^{a}	4.28±0.19^{b}	4.41±0.17^{b}	7.63±0.20^{b}	7.01±0.18^{b}	11.29±0.26^{b}	9.77±0.42^{b}	45.18±1.41^{b}	41.00±2.23^{b}	4.79±0.12^{b}
Group IV													
Ammonium molybdate (150 mg kg^{-1} b.wt. day^{-1})	165.12±5.56^{c}	173.62±5.14^{c}	4.50±0.18^{c}	4.39±0.16^{c}	3.83±0.11^{b}	4.09±0.16^{c}	8.72±0.24^{c}	8.21±0.18^{b}	8.40±0.24^{c}	7.28±0.49^{c}	38.70±1.99^{c}	34.53±1.64^{c}	3.98±0.21^{c}
Recovery-group													
After 60 days of treatment withdrawal	193.25±5.83***	198.25±5.72***	3.98±0.10^{ns***}	3.88±0.17^{ns***}	4.44±0.16***	4.58±0.18***	7.16±0.16^{a***}	6.85±0.17***	11.81±0.29^{a***}	10.37±0.30^{a***}	46.92±1.60^{a***}	45.20±1.89^{a***}	4.91±0.18^{a***}

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), T: Testis, E: Epididymis

Table 3: Lipid peroxidation and antioxidant defense system markers in testis and epididymis of rats treated with various doses of ammonium molybdate

	Lipid peroxidation (TBARs) (n mol MDA mg^{-1} tissue)		SOD (unit mg^{-1} protein)		Glutathione (n mol/g tissue)		Ascorbic acid (mg g^{-1} tissue)	
Treatments	Testis	Epididymis	Testis	Epididymis	Testis	Epididymis	Testis	Epididymis
Group I								
Control (vehicle)	1.59±0.13	1.71±0.09	8.54±0.17	8.19±0.21	2.78±0.09	2.98±0.10	1.36±0.06	1.42±0.05
Group II								
Ammonium molybdate (50 mg kg^{-1} b.wt day^{-1})	1.99±0.10[a]	1.98±0.11[a]	7.89±0.22[a]	7.48±0.21[a]	2.49±0.08[a]	2.67±0.07[a]	1.13±0.04[a]	1.21±0.06[a]
Group III								
Ammonium molybdate (100 mg kg^{-1} b.wt. day^{-1})	2.19±0.11[b]	2.15±0.11[b]	7.61±0.20[b]	7.13±0.24[b]	2.32±0.07[b]	2.51±0.10[b]	1.04±0.07[b]	1.09±0.06[b]
Group IV								
Ammonium molybdate (150 mg kg^{-1} b.wt. day^{-1})	2.67±0.12[c]	2.52±0.12[c]	6.72±0.15[c]	6.49±0.17[c]	1.84±0.06[c]	2.05±0.11[c]	0.75±0.06[c]	0.87±0.04[c]
Recovery-group								
After 60 days of treatment withdrawal	2.03±0.08[a]***	2.05±0.06[a]***	7.78±0.20[a]***	7.44±0.23[a]**	2.41±0.11[a]***	2.58±0.09***	1.12±0.08[a]**	1.19±0.07[a]**

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)

DISCUSSION

Reproductive hormones play an important and complicated role in the regulation of spermatogenesis and sperm development. Testosterone, an important androgen, plays a pivotal role in maintenance of structural integrity of testis and accessory sex organs, spermatogenesis as well as in expression of secondary sexual characters. It is formed and secreted by Leydig cells in testes in response to stimulation by LH (Steinberger, 1971).

A significant dose dependent decrease has been found in the level of serum testosterone in ammonium molybdate exposed rats. In a case study, Meeker *et al*. (2010) observed similar decline in level serum testosterone after exposure of molybdenum. Decreased testosterone level in treated rats seems to be due to decreased LH secretion by pituitary or underutilization of cholesterol in testosterone synthesis which is in consonance with the results obtained in the present study. Increased oxidative stress might also influence the normal functioning of the leydig cells.

The FSH plays a crucial role in the induction and maintenance of spermatogenesis. It also stimulates the proliferation of sertoli cells, induces the synthesis of ABP which is implied in the transport of testosterone and dehydrotestosterone from the testis towards the epididymis, maturation of germ cells within the testes and secretion of inhibin which has a negative feedback directly to the anterior pituitary (Plant and Marshell, 2001). In males, LH is the key regulator of leydig cell function and it is indispensable for functional differentiation and proliferation of leydig cells (Benton *et al*., 1995). In the present investigation, significant decline in the levels of serum FSH and LH was observed in ammonium molybdate treated rats. Similar decline in the level of FSH and LH has been observed in previous studies related to metal exposure in rats (Chandra *et al*., 2007; Al-Azemi *et al*., 2010).

The diminished level of FSH and LH could be due to copper deficiency caused by molybdenum exposure (Phillippo *et al*., 1987). It was further elucidated by Haywood *et al*. (2004) that reduced copper content make it unavailable for a copper-dependent enzyme peptidylglycine α-amidating mono-oxygenase (PAM) that appears to be crucial for the bioactivation of many peptide hormones, including FSH and LH.

The decreased level of serum testosterone, FSH and LH showed significant recovery after 60 days of treatment withdrawal in ammonium molybdate treated rats by virtue of restoration of androgen biosynthesis in the Leydig cells.

Sperm analysis endpoints, such as sperm count, motility and viability are prime parameters that provide a good general assessment of male fertility and reproductive health status. Alterations

in these parameters are suggested as optional procedures for confirmation of histopathologic findings or to better characterize adverse effects (Kaneto *et al*., 1999). In the present investigation, an adverse impact on sperm parameters were noticed which provides sufficient evidence in favor of reproductive toxicity. The observed significant decline in sperm parameters are in agreement with previous reports of molybdate (Lyubimov *et al*., 2004; Meeker *et al*., 2008; Zhai *et al*., 2013) exposure in experimental animals.

The significant diminution in epididymal sperm count and sperm motility, with different doses of ammonium molybdate, may be due to sperm toxic effects of molybdenum (Pandey and Singh, 2002). The reduced sperm characteristics caused by molybdate administration may be due to the low testosterone concentration observed in this study as high level of testosterone is critically required for normal spermatogenesis, development and maintenance of normal sperm morphology and physiology of seminiferous tubules (Sharpe and Skakkebaek, 1993).

The deterioration in sperm parameters might be correlated with enhanced lipid peroxidation. Excess ROS and low antioxidant levels in semen enhanced susceptibility of sperm DNA to denaturation and might cause mitochondrial DNA mutations which impairs the fertilizing capacity of spermatozoa (Badade *et al*., 2011). Mammalian spermatozoa are rich in polyunsaturated fatty acids which makes them susceptible to oxidative damage. So, decreased level of antioxidants or increased ROS level disrupts the physiological functions of the spermatozoa and impairs motility, viability and structural integrity of spermatozoa (Rivlin *et al*., 2004).

Heavy metals such as lead, cadmium and chromium have shown a similar adverse effect on the spermatogenic process via mechanisms that involved the induction of lipid peroxidation, depletion of ROS scavengers and disruption of testicular antioxidant enzyme activity (Priya and Reddy, 2012; Pires *et al*., 2013; Akunna *et al*., 2012).

Sperm parameters were significantly improved in ammonium molybdate treated recovery group after 60 days of treatment withdrawal possibly via restoration in antioxidant status or steroidiogenesis.

Fertility is an important consideration in the evaluation of male reproductive toxicity, as it provides an evaluation of many aspects of sperm production/function as well as the reproductive tract and endocrine system. The standard method for determining adverse effects of chemical compounds on fertility in animal experiments is based on mating trials to determine number of pregnant females and litter size (Mangelsdorf *et al*., 2003). In the present study, the fertility (%) and litter size declined in a dose dependent manner. The decline in fertility and litter size might be the result of altered sperm parameters or biochemical milieu of reproductive organs. Previous studies with molybdate (Pandey and Singh, 2002) also reported similar effect on fertility and litter size. Turner and Lysiak (2008) found that detrimental effect of ROS and oxidative damage to biomolecules may contribute reduced male fertility and litter size by deteriorating sperm quality. Both fertility (%) and litter size were significantly improved in rats of recovery group after cessation of treatment for 60 days suggesting partial reversibility of the adverse effects on fertility.

The biochemical parameters evaluated in this study are useful 'marker' indices of male testicular function. Testicular proteins which are androgen dependent, are one of the constituents that ensure the maturation of spermatozoa. Protein biosynthesis is the key for testicular development and spermatogenesis. Sertoli cells coordinate the spermatogenic process by synthesizing a variety of proteins required during the different phases of germ cell maturation (Weinbauer *et al*., 2010). Treatment of ammonium molybdate in rats resulted in a significant dose dependent decline in testicular protein level. Similar decline in testicular protein content has been reported in molybdenum (Mills *et al*., 1958) treated rats. The reduction in protein content observed in the present study may be attributed to disturbances in protein synthesis or metabolism or

reduction in the number of germ cells in testis. The restoration in the protein content of testis towards normal value after 60 days of treatment withdrawal suggests modulation of protein metabolic disturbances.

The glycogen content in the cell indicates energy storage. Testicular glycogen is a crucial requirement for gonadal maturation and proper functioning (Datta *et al*., 1988). The increased level of glycogen in the testis observed in the present study reflects accumulation of nonutilizable sugars in the sertoli cells due to an arrested spermatogenesis or lowering of the rate of glycolysis by virtue of inhibition of enzyme activity. The increased level of glycogen in testis showed significant recovery after 60 days of treatment withdrawal by virtue of increased utilization by spermatozoa.

Sialic acid (N-acetylneuraminic acid) is a derivative of N-acetylmannose and pyruvic acid. It is an important constituent of glycoprotein and glycolipids (Resenberg, 1999). According to Mann (1964) sialic acid concentration in the testes declined with the decrease in the rate of spermatogenesis. Therefore, it might be suggested that impaired spermatogenesis further contributes to the declined level of sialic acid in the testes of exposed rats. The results are in accordance with previous researchers who also reported similar decline in sialic acid in testis due to the antiandrogenic or antispermatogenic impact of the metals (Chinoy *et al*., 2005; Jain *et al*., 2007). The level of sialic acid was significantly improved in ammonium molybdate treated rats after 60 days of treatment withdrawal probably due to restoration of androgen level.

Cholesterol is required for the normal testicular activity. Testicular cells have a dual requirement for cholesterol: they need cholesterol for membrane biogenesis and cell signaling as well as precursor required for androgen synthesis (Hu *et al*., 2010). Increased cholesterol content in testis of treated rats might be due to inhibition of testosterone biosynthesis as observed in the present study. The increased level of cholesterol content in testis showed significant decline after 60 days of treatment withdrawal probably due to its utilization in androgen biosynthesis by the leydig cells or restored sperm count in epididymis.

Acid phosphatase (ACP) is a lysosomal enzyme which is used as marker parameter of specific androgen dependent steps in spermatogenesis. Acid phosphatase is widely distributed in the testes and is important in the physiology of sperm (Peruquetti *et al*., 2010). Reduced activity of ACP in testis might reflect testicular degeneration which may be a consequence of decreased testicular steroidogenesis in the treated-rats and this may be correlated with the reduced secretion of gonadotrophins (Aladakatti *et al*., 2010). Decreased activity of testicular acid phosphatase appeared to be reversible after 60 days of cessation of treatment in highest dose group by virtue of androgen restoration.

Alkaline phosphatase (ALP) is an excellent histochemical as well as biochemical marker for the germ cells of several mammalian species including rats (Turner and McDonnell, 2003). In the present study, a significant decline was observed in ALP activity in testis of rats treated with ammonium molybdate was observed. Rahman *et al*. (2000) suggested that the decrease in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis in treated animals. Similar decline in the activity of testicular alkaline phosphatase was observed in animals exposed with chromium (Dey and Roy, 2009), mercury (Saxena and Kumar, 2004) and stannous chloride (El-Demerdash *et al*., 2005). Reduced activity of ALP in testis was significantly but partially improved in rats after 60 days of treatment withdrawal suggesting partial modulation of the degenerative effects.

Estimation of end products of lipid peroxidation such as malondialdehyde (MDA) is an index of the extent of oxidative damage to cellular structures (Sharma and Agarwal, 1996). Rats treated with ammonium molybdate exhibited an increase in lipid peroxidation in the testis which could be

due to concomitant increase in the generation of free radicals or reduced content/activity of antioxidants in testis. Reduced level of intratesticular testosterone might also be responsible for suppressed expression of antioxidant enzyme with a concomitant increase in peroxidative damage, disruption of spermatogenesis and an increase in germ cell apoptosis (Peltola *et al*., 1996; Chaki *et al*., 2006).

Superoxide dismutase (SOD) is considered as the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (Nehru and Anand, 2005). Administration of ammonium molybdate in rats caused significant dose dependent decline in SOD activity in testis. Super oxide dismutase requires copper and zinc for its functional activity and stability. Molybdate can induce abnormal metabolism or displacement of metals like Zn and Cu which might be one of the possible reasons of reduced SOD activity as observed in the present study (Grace *et al*., 2005).

Glutathione deficiency can lead to instability of the mid-piece of sperm, resulting in defective sperm motility. It protects plasma membrane from lipid peroxidation, scavenges superoxide and prevents O_2 formation (Lushchak, 2012). The reduced level of GSH after administration of ammonium molybdate may be due to increased utilization in trapping free radicals generated due to toxic effect of molybdate in testis. It is postulated that ROS has a greater affinity for the thiol groups of biomolecules, thus depleting intracellular thiols inducing reduced GSH content in testicular tissue which made spermatogenic cells more vulnerable to free radicals resulting in lessen sperm count (Aitken, 2013).

Vitamin C (ascorbic acid) is essential for the normal integrity and function of the testis. Low ascorbate level has been associated with disrupted spermatogenesis and steroidogenesis (Turner and Lysiak, 2008). In the present study, there was a significant dose dependent decline in the concentration of ascorbic acid in testis of ammonium molybdate exposed rats. It has been reported that high intake of molybdenum resulted in diminished reserves of ascorbic acid as molybdenum interferes with some aspects of ascorbic acid metabolism in rats (Sasmal *et al*.,1968).

Our results are in consonance with the findings of other studies who reported alteration in antioxidant defense parameters in testis of rats treated with metals like chromium (Elgharabawy and Emara, 2014), mercury (El-Desoky *et al*., 2013), molybdate (Zhai *et al*., 2013) and nickel (Hassan and Barkat, 2008). Levels of TBARs, SOD activity in testis were significantly but partially improved in rats after 60 days of treatment withdrawal suggesting partial recovery of the degenerative effects by virtue of partial alleviation of free radical generation.

CONCLUSION

On the basis of the results, we concluded that ammonium molybdate exerts an adverse impact on steroidogenesis and sperm quality by varying the biochemical milieu of testis. A significant rise in the lipid peroxidation and simultaneously reduction in antioxidant levels in the testes indicates excessive generation of free radicals resulting in oxidative stress which might be responsible for decline in male reproductive dysfunctions. However, significant recovery is possible after treatment withdrawal.

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