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Effects of molybdenum on fertility of male rats

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Abstract

Sodium molybdate was administered orally to adult male rat at dose level of 10, 30, and 50 mg kg body weight (5 days per week) for 60 days. At higher dose levels significant decrease in absolute and organ-to-body weight ratios of testes, epididymides, seminal vesicles and ventral prostate was observed. The sperm abnormality, associated with decrease in sperm motility and sperm count was also observed. Significant alterations in the activities of marker testicular enzymes, viz. sorbitol dehydrogenase (decreases), lactate dehydrogenase (increases) and γ-glutamyl transpeptidase (increases) associated with histopathological changes in testes was also observed. Accumulation of molybdenum in testes, epididymides and seminal vesicles was also observed. The study reveals that the oral ingestion of molybdenum may affect the histoarchitecture of testes and sperm morphology. The testicular and spermatotoxic changes may be responsible for observed male mediated developmental toxic effects.

Introduction

Molybdenum is used in manufacture of electric and electronic parts, a wide variety of glass, ceramic, lubricant, dyes, in production of catalyst, pigment and in alloying the steels. Persons get exposed to molybdenum in the weapons industry, aeronautical engineering, chemical industry, automobile industry, mining and refining of this metal (Mills 1987).
Molybdenum is an essential, trace and micronutrient element and play an important role in animal and plant physiology (Schroeder *et al.* 1962; Mills & Davis 1987; Pennington & Jones 1987). Molybdenum is a constituent of at least three mammalian metaloflavoprotein xanthine oxidase, aldehyde oxidase and sulphite oxidase and that of nitrate reductase of plant protein (Schroeder *et al.* 1962; Anke *et al.* 1985). The importance of molybdenum in animal is well recognised. Its antagonistic effects on copper metabolism in ruminants have always attracted much attention (Mason 1986; Mills & Davis 1987). Molybdenum is known to act as an anticarcinogen (Luo *et al.* 1983). Low molybdenum levels in soils, plants, drinking water, food and human tissues may be responsible for high mortality from oesophageal cancer. In several animal studies molybdenum has been reported to inhibit gastrointestinal cancers (Luo *et al.* 1983). The recent findings have indicated that molybdenum has direct effects on biological processes controlling growth and reproductive performance (Dixon 1986).
In the present investigation, the studies have been undertaken to evaluate the effect of orally administered molybdenum on histoarchitecture of different compartments of testes, sperm count, motility and abnormalities in different regions of spermatozoa. The study also describes the bioaccumulation pattern of molybedenum in reproductive organs, effects on enzyme considered to be marker of testicular function and male mediated developmental toxic effects.

Material and methods

Sodium molybdate of AR grade was procured from E-merck. All other chemicals used in study were of the highest purity available.

Table 1. Effect of sodium molybdate exposure on organ weights of rats.

Group		Testis	Epididymis	Accessory sex organs	
				Seminal vesicle	Prostate gland
I	A	2.50 ± 0.08	0.81 ± 0.01	0.18 ± 0.013	0.11 ± 0.010
	B	1.20 ± 0.03	0.38 ± 0.01	0.08 ± 0.006	0.05 ± 0.006
II	A	2.50 ± 0.03	0.78 ± 0.02	0.17 ± 0.016	0.11 ± 0.006
	B	1.20 ± 0.04	0.37 ± 0.01	0.08 ± 0.008	0.05 ± 0.004
III	A	2.40 ± 0.05	$0.50 \pm 0.02^*$	$0.09 \pm 0.012^*$	0.09 ± 0.004
	B	1.15 ± 0.03	$0.30 \pm 0.02^*$	0.05 ± 0.008	$0.04 \pm 0.002^*$
IV	A	2.40 ± 0.03	0.49 ± 0.02	$0.08 \pm 0.010^*$	$0.05 \pm 0.010^*$
	B	$1.15 \pm 0.03^*$	$0.32 \pm 0.02^*$	$0.05 \pm 0.008^*$	$0.03 \pm 0.005^*$

Mean ± SE of requisite number of rat in each group.
$^*P < 0.05$ considered to be statistically significant.
A = Absolute body weight/whole animal weight (g)
B = relative (organ to whole animal weight) weight
Group I – Control
Group II – Treated with 10 mg Sodium molybdate kg body weight.
Group III – Treated with 30 mg Sodium molybdate kg body weight.
Group IV – Treated with 50 mg Sodium molybdate kg body weight.

Table 2. Effect of sodium molybdate exposure on motility and total epididymal sperm count of rats treated for 60 days.

Group	Sperm motility (%)	Total sperm count (Per epidydims) $\times 10^7$
I	86.0 ± 2.3	8.0 ± 0.17
II	85.0 ± 1.2	8.2 ± 0.08
III	$65.0 \pm 1.2^*$	$6.0 \pm 0.07^*$
IV	$49.1 \pm 1.3^*$	5.0 ± 0.05

Means ± SE of requisite number of rats in each group.
$^*P < 0.05$ considered to be statistically significant.
Group I – Control
Group II – Treated with 10 mg Sodium molybdate kg body weight.
Group III – Treated with 30 mg Sodium molybdate kg body weight.
Group IV – Treated with 50 mg Sodium molybdate kg body weight.

Treatment of animals

Adult male Druckery rats (120 ± 10 g) bred at Industrial Toxicology Research Centre, Lucknow, Animal house colony, were used in the present study. The animals were fed on pellet diet (Lipton India Limited) and water ad libitum, maintaned under standard laboratory conditions. The rats were acclimatised for fortnight before oral administration of test chemicals.

The rats were equally divided into four groups consisting of ten animals in each group. The animals of group I were orally administered 0.2 ml of distilled water while rats of group II, III, IV were orally administered 10, 30 and 50 mg sodium molybdate kg body weight, respectively, five days a week for a 60 days. Desired amount of sodium molybdate was dissolved in distilled water and 0.2 ml was orally fed to rats with the help of cannula. The body weights of rats were recorded at the initiation and termination of experiment. The rats were sacrificed by cervical dislocation on 61st day of the experiment. Testes, epididymes, seminal vesicles and prostate glands were quickly removed and weighed.

The tissues (one from each pair) like testis, epididymis and seminal vesicle of rats from each group were used for determination of molybdenum contents. While the remaining same tissues from same group were used for histopathological and biochemical studies.

Spermatozoa count

Epididymal sperms were obtained by mincing cauda epididymis in normal saline and filtering through nylon mesh. The sperm were counted using Neubauer Chamber (Freund & Carol 1964).

Sperm motility assay

The motility of sperm was assayed microscopically within 5 min following their isolation from cauda epididymis at 37 °C and data were expressed as percent mołility (Adelman & Cahil 1936).

Morphological abnormalities

The morphological abnormalities in sperm were enumerated by the methodology as reported by Hemavathi & Rahiman (1993) using light microscope.

Testicular enzyme assay

A portion of testis was homogenised (1:9) in 0.2 M Tris/HCl buffer pH 7.0 having 0.1% cetyltrimethylammoniumbromide (CTNB) using Potter Elvejham homogeniser for the estimation of sorbitol dehydrogenase (SDH) following the method of Gerlach (1983). In the same homogenate lactate dehydrogase (LDH) was also estimated (Vassault 1983).
Another portion of testis was homogenised (1:9) in 0.05 M Tris/HCl buffer pH 7.4 for the assay of γ-glutamyl transpeptidase following the methodology of Roomi & Goldberg (1981). Protein contents of the sample were estimated by the method of Lowry *et al.* (1951).

Histological techniques

Testes and other accessory sex organs were fixed in 10% neutral buffered formalin, dehdrated and embedded in paraffin. Sections from each block (5 μm) were prepared and stained with haemotoxylin-eosin following standard procedures (Putt 1972).

Molybdenum analysis

The testis, epididymis and seminal vesicle were soaked on the filter paper and weighed immediately. The tissues were digested twice with the nitric acid and finally with acid mixture of nitric, perchloric and sulphuric acids ($2 + 1 + 0.5$ ml) (Morrice *et al.* 1989). The digested samples were dissolved in 1% HNO_3 and made upto 5 ml. In the similar fashion acid blanks were prepared. The presence of metal was also checked in the diet and drinking water of animals (normal as well as exposed) and its quantity was 0.011 ng gm and 0.001 ng ml, respectively. The processed and digested samples were analysed on Graphite furnace atomic absorption spectrometer (Varian GTA-97, SpectrAA-250 Plus). Recommended operating conditions for the AAS:

Wave length	:	390.3 nm
Lamp current	:	7 mA
Injection volume	:	10 μl
Fuel	:	acetylene
Support for fuel	:	nitrous oxide
Flame stochiometry	:	stronly reducing

Male mediated developmental toxicity studies

Twenty male rats of proven fertility, were administered 30 mg sodium molybdate kg body weight 5 days a week for a period of 60 days. Desired amount of sodium molybdate was suspended in distilled water and 0.2 ml was orally fed to rats with the help of cannula. Similarly twenty male rats were given equivalent amount of distilled water in an identical manner which served as vehicle control. The treated male and non treated female of proven fertility were housed overnight on a 1:2 basis in the home cage of the male. The maximum duration of pairing was 1 or 2 weeks. Positive evidence of copulation was confirmed by the presence of sperm in vaginal smear taken each morning during cohabitation (Dunnick *et al.* 1984). The day on which evidence of copulation identified was termed day zero of gestation. The number of pregnant rats with each sodium molybdate exposed or the control group was recorded for determination of fertility index. On the 20^{th} day of gestation laparotomies were performed and number of corpora lutea were counted and foetuses were removed by uterine opening. The number of live and resorbed foetuses (embryo) and total number of implantation were recorded. Fertility index, pre and post implantation loss were calculated. Foetal weight and crown rump lengths were recorded.

Statistical analysis

The data were statistically analysed using Student's t-test (Fisher 1950). $P < 0.05$ was considered significant.

Results

General toxicity

The animals did not show any mortality. Only sluggishness is observed at highest dose level.

Table 3. Effect of sodium molybdate exposure on different types of morphological abnormalities in rats spermatozoa.

Group	Percent abnormalities								Percent total abnormalities
	Head	Neck		Tail					
	Banana	Detached	Curved	Curved	Bent	Round	Loop	Signet	
I	1.0 ± 0.36	1.0 ± 0.16	1.4 ± 0.16	1.5 ± 0.22	1.5 ± 0.42	1.4 ± 0.13	1.0 ± 0.11	0.8 ± 0.30	10.3 ± 0.61
II	1.1 ± 0.16	1.3 ± 0.21	1.6 ± 0.33	1.4 ± 0.21	1.3 ± 0.21	1.6 ± 0.33	1.0 ± 0.32	1.2 ± 0.25	11.1 ± 0.40
III	$2.2 \pm 0.21^*$	$1.4 \pm 0.10^*$	$2.0 \pm 0.21^*$	$2.0 \pm 0.30^*$	$2.0 \pm 0.26^*$	2.2 ± 0.27	$1.4 \pm 0.12^*$	$1.5 \pm 0.23^*$	$16.1 \pm 0.99^*$
IV	$3.1 \pm 0.10^*$	$2.5 \pm 0.20^*$	$3.7 \pm 0.22^*$	$5.3 \pm 0.42^*$	$4.1 \pm 0.48^*$	$2.5 \pm 0.25^*$	$1.6 \pm 0.30^*$	2.0 ± 0.12	$23.1 \pm 1.40^*$

$^*P < 0.5$ considered to be statistically significant
Mean ± SE of requisite number of rats in each group.
Group I – Control
Group II – Treated with 10 mg Sodium molybdate kg body weight.
Group III – Treated with 30 mg Sodium molybdate kg body weight.
Group IV – Treated with 50 mg Sodium molybdate kg body weight.

Body gain profile

The body gain profile is insignificant at any dose level.

Organs weight

The organ weight data is presented in Table 1, there was no significant change in absolute weight of testes and accessory organs at the dose levels tested, however relative weights of testes were decreased at 30 and 50 mg dose level.

Effect on sperm motility, sperm count and morphological sperm abnormality

The results indicate significant dose dependent decrease in sperm motility, total epididymal sperm count (Table 2) and increase in morphological abnormalities (Table 3) in different regions of spermatozoa of rats exposed to 30 and 50 mg sodium molybdate kg body weight. However, such effects were not at the lowest dose of sodium molybdate.

Effect on testicular enzyme activities

The effect of sodium molybdate treatment on the specific activities of marker testicular enzymes associated with specific cell types is presented in Table 4. The results indicate significant decrease in the activity of SDH and an increase in the activities of LDH and γ-GT with different doses of sodium molybdate in a dose dependent manner.

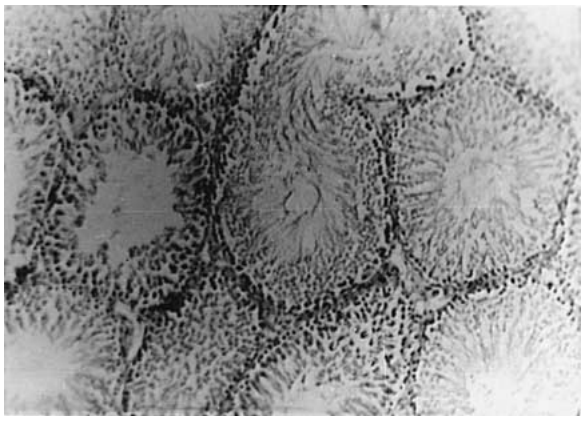

Fig. 1. Control testis shows compact seminiferous tubules, the tubules have well-developed germinal epithelial cells (HE&160).

Histopathological observation

The histological examination of testis obtained from rats treated with lowest dose of sodium molybdate (10 mg kg body weight showed almost normal structural appearance. However high doses of sodium molybdate caused degeneration of seminiferous tubules in testes. The seminiferous tubules shrinked in size resulting in increased intertubular space associated with degeneration of interstitial cells (Figure 1A & B). No more changes were observed in other accessory organs.

Table 4. Effect of sodium molybdate exposure on marker testicular enzymes of rats treated for 60 days.

Enzymes	Group-I	Group-II	Group-III	Group-IV
Sorbitol dehydrogenase	3.92 ± 0.51	3.85 ± 0.62	2.60 ± 0.45°	1.58 ± 0.38*
γ-glutamyl transpeptidase	12.72 ± 1.38	13.72 ± 0.89	24.81 ± 2.57*	35.23 ± 1.33*
Lactate dehydrogenase	250.60 ± 18.50	265.01 ± 15.01	398.01 ± 28.50	516.02 ± 15.52*

Mean ± SE.
* $P < 0.05$ considered to be statistically significant
Enzyme activities are expressed as specific activities (n moles of substrate oxidised or continue product formed/min/mg protein).
Group I – Control
Group II – Treated with 10 mg Sodium molybdate kg body weight.
Group III – Treated with 30 mg Sodium molybdate kg body weight.
Group IV – Treated with 50 mg Sodium molybdate kg body weight.

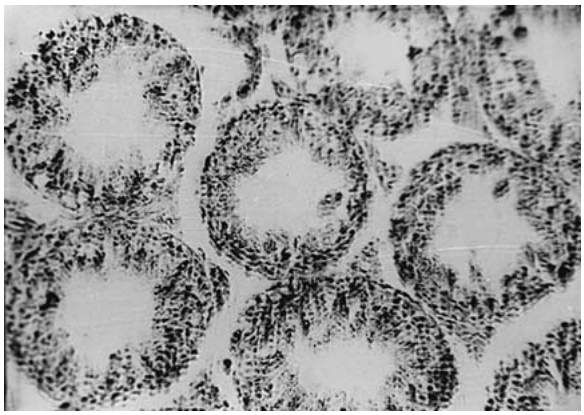

Fig. 2. In treated rats at 50 mg/kg b.<t. intertubular space and loss in spermatids (HE&160).

Table 5. Distribution of molybdenum in male reproductive tissue of rats.

Tissues	Group I (ng g)	Group IV (ng g)
Testis	229.86 ± 2.15	245.57 ± 2.11
Epididymis	315.00 ± 1.99	417.78 ± 2.00*
Seminal vesicle	283.16 ± 2.51	427.43 ± 1.75*
Prostate gland	031.93 ± 1.91	241.65 ± 1.90*

Mean ± SE
* $P < 0.05$ considered to be statistically significant.
Group I – Control
Group IV – Treated with 50 mg Sodium molybdate kg body weight.
No metal accumulation was recorded in group II and III.

Accumulation of molybdenum

Molybdenum was present in appreciable quantity in the male reproductive organs even in control animals and its is clear that after the oral administration of molybdenum salt at dose of 50 mg kg body weight resulted in significant amount of metal accumulation in testis, epididymis, seminal vesicle and prostate gland (Table 5). No metal accumulation was recorded at lower dose levels.

The degree of accumulation of molydenum in the male reproductive organs of exposed animals was observed as follows:

Seminal vesicle > Epididymis > Testis > Prostate gland.

Male mediated developmental toxicity

No treatment related mortality or overt clinical signs of toxicity were observed in the rats during this period. The fertility index of exposed male rats were calculated on their ability to imprignate unexposed female rats. At 30 mg kg body weight dose level fertility index was 60% in comparison to control rats 80%. The number of corpora lutea was calculated in treated and control group, and it was found number of implantation was significantly reduced. The foetal weight was observed 3.70 g in control and 3.65 g in treated group. Grown rump length was found reduced (Table 6).

Table 6. Male mediated embryotoxicity studies with sodium molybdate.

	Group I	Group IV
Number of dams	10.00	10.00
Copora lutea	12.30 ± 0.21	12.00 ± 0.25
Implantation	12.50 ± 0.26	10.30 ± 0.42*
Pre-implantation loss (%)	06.47 ± 1.57	14.92 ± 3.09*
Live foetuses	11.90 ± 0.20	08.80 ± 0.41*
N. of resorptions	00.60 ± 0.16	01.50 ± 0.50*
Post-implantation loss (%)	04.69 ± 0.97	13.80 ± 4.27*
Foetal crown-rump length (mm)	39.30 ± 0.07	30.50 ± 0.04*
Foetal weight (g)	03.70 ± 0.05	02.81 ± 0.03*

* $P < 0.05$ considered to be statistically significant.
Pre-implantation loss (%) = (Corpora lutea-Implantation/Corpora lutea × 100.
Post-implantation loss (%) = (Implantation-Live foetuses/Implantation) × 100.
No such results were observed in remaining groups of animal.

Discussion

No mortality, in exposed rats, indicates such molybdate does not show acute toxicity at their dose levels. The decrease in body organ weight gain profile (testes, epididymides, seminal vesicles and prostate gland) of rats may be due to cellular loss during the histopathological changes. Weight loss in reproductive organs and accessory reproductive organs are well in accordance to nickel (Pandey *et al.* 1999), sodium selenite (Nebbia *et al.* 1987), carbofuran (Plant *et al.* 1995), quinolphos (Ray *et al.* 1991) and lead (Ronis & Badger 1996).

The studies have shown that the activity of certain enzymes are associated with specific cell type of testis of germ cell maturation (Blackshaw 1970; Hodgen & Sherins 1973; Sherins & Hodgen 1976). The 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 γ-GT (the enzymes related with germinal epithelium and Sertoli cell, respectively) indicate damage to these particular cell types of testes by different dose of sodium molybdate in a dose dependent manner. The biochemical alterations in the activities of marker testicular enzymes, associated with specific cell types of testes, indicating testicular damage by sodium molybdate (Pandey & Singh 1999) is well supported with histopathological observations indicating degeneration of seminiferous tubules, disturbed spermatogenesis, increase in intertubular spaces and either few or absence of spermatozoa (Pandey *et al.* 1999). The lumen of tubule is completely devoid of spermatozoa which is also supported by Dixit (1976).

The significant reduction in total epididymal count, the decreased activity of marker testicular enzyme viz SDH is known to be associated with germ cell maturation along with increased activity of LDH and γ-GT (the enzymes related with germinal epithelium and Sertoli cell, respectively) indicate damage to these particular cell types of testes by different dose of sodium molybdate in a dose dependent manner. The biochemical alterations in the activities of marker enzymes, associated with specific cell types of testes, indicating testicular damage by sodium molybdate is well supported with histopathological observations indicating degeneration of seminiferous tubules (Saxena *et al.* 1990), disturbed spermatogenesis and degenerative changes of Sertoli cells. It has been suggested from these that Sertoli cell damage may be responsible for germ cell degeneration (Pant *et al.* 1995; Srivastava *et al.* 1990, 1992).

The significant reduction in total epididymal sperm count and sperm motility, with different doses of sodium molybdate, may be due to sperm toxic effects of molybdenum. The increased percentage of morphological abnormalities, observed in different regions of spermatozoa (Sobti & Gill 1989) following sodium molybdate exposure, may be due to toxic potential of this heavy metal (Pandey & Srivastava 2000).

Exposure of 30 mg sodium molybdate to male rats resulted in decrease in fertility index (Meistrich 1989). Exposed male rats were able to impregnate unexposed female but comparatively in lower number. Many metals such as lead and cadmium have shown deleterious effects on fertility and histopathology of testis. The observed decrease in fertility in males have been attributed to a direct cytotoxic ac-

tion on testes resulting increase in sperm abnormalities (Working *et al.* 1985a). The results from rats dosed with 50 mg sodium molybdate kg demonstrated significantly decreased organ weights (Davis 1967) while histopathological examination revealed severe effects on spermatogenic cells in the testes and degeneration of seminiferous tubules (Hoey *et al.* 1966) and lumen devoid of spermatozoa. Analysis of sperm from the rats in the 50 mg kg group further revealed the decrease in epididymal sperm count poor motility as well as increase in abnormally shaped sperms. These testicular and spermatotoxic changes could have been expected to lead to poor reproductive performance (Oehninger *et al.* 1989; Morales *et al.* 1988). The low implantation observed may be due to damage to spermatogenic cells or aberrations in sperm. Further the decrease in number of implantation suggests that exposure to molybdate induces dominant toxic effects in rats which represents embryonic death (Mathur *et al.* 1978). The foetal loss may occur before and after implant. A decrease in the number of five foetuses observed in post implantation death and decrease in total implantation induced pre implantation death of fertilized ova (Working *et al.* 1985b).

The data suggest that preimplantation loss, may be due to part failure of fertilization due to poor sperm quality and lowered sperm number. The reported testicular and spermatotoxic effects and observed decrease in fertility index and developmental toxicity effect suggest that exposure to sodium molybdate may effect fertility and development of embryo/foetus.

Thus the results of the present study indicate testicular damage and sperm toxic effects of sodium molybdate concomitant with supportive biochemical and histo-pathological alterations in a dose dependent manner. Sodium molybdate has also the potential to cause infertility in male rats through its spermatotoxic influence on its foetus or embryo.

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