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The effect of sub chronic exposure to ammonium molybdate on hematological and hepatic parameters in albino rats

Geeta Pandey, Gyan Chand Jain, Nidhi Mathur

Department of Zoology, University of Rajasthan, Jaipur,India.

Abstract

Objectives: The present study has been undertaken to assess the influence of molybdenum exposure on the hematological, hepatic parameters and oxidative stress biomarkers in male Wistar rats.

Methods: The rats were orally administered with ammonium molybdate at two different doses 50 and 100 mg/kg b. wt. /day for 60 days. TEC, TLC, hemoglobin concentration and hematocrit value were measured in blood sample. AST, ALT, ALP, total bilirubin, total protein, glycogen, cholesterol, triglycerides and the levels of lipid peroxidation and antioxidants were assessed in the liver.

Results: TEC, hemoglobin concentration and hematocrit value showed significantly decrease ($P \leq 0.05$) in group B and ($P \leq 0.01$) in group C while TLC reflect significant change ($P \leq 0.05$) in only group C. Biochemical findings showed a significant decrease in protein and glycogen content in group B ($P \leq 0.05$) and in group C ($P \leq 0.01$) while a significant increase in the levels of total cholesterol and triglycerides in liver in both group B ($P \leq 0.05$) and group C ($P \leq 0.01$). Activities of AST, ALT, ALP and the level of total bilirubin showed significant increase (group B; $P \leq 0.05$ and group C; $P \leq 0.01$).GSH, ascorbic acid levels and the activities of SOD were found to be significantly decreased (group B; $P \leq 0.05$) and Group C; $P \leq 0.01$) however, TBARS was significantly increased (group B; $P \leq 0.05$ and group C; $P \leq 0.01$) .Hepatic histoarchitecture indicated dilated sinusoids, degenerative and necrotic changes in hepatocytes and infilteration of leukocytes at high dose level.

Conclusion: The results indicate hepatotoxicity due to altered enzymatic activity and antioxidant status caused by ammonium molybdate in rats.

Key Words: Ammonium Molybdate, Serum Transaminases, Lipid Peroxidation,Hematocrit, Hepatic Histoarchitecture.

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INTRODUCTION

Molybdenum (Mo) plays an important role in animal and plant nutrition as well as in industrial society. Molybdenum has been identified as an essential trace element for all plants and animals because it is bound to and required for the function of several molybdoenzymes [1]. Molybdate salts and other compounds are used in metallurgical processes, electronic parts, glass, ceramics, lubricants, in the production of catalyst and pigments, as a component of contact lens solution and a color additive in cosmetics [2].

Molybdenum supplementation may be of therapeutic benefits in patient with molybdenum cofactor deficiency, a disease in which deficiency of molybdenum cofactor causes severe neurological abnormalities and mental retardation, copper poisioning, improper carbohydrate metabolism. Due to its copper chelating property ammonium tetrathiomolybdate (TTM) has also been used in the cancer therapy and for treating Wilson's disease. Being a transition metal it is also known to act as insulin mimics and may be cardio- protective[3, 4].

The wide distribution of this contaminant in the environment is the result of the mining and refining of molybdenum ore industrial operations, uranium processing and combustion processes. Legumes, grains, and organ meat are major contributors of molybdenum [5, 6]. An outbreak of genu valgum (knock knees) in India was attributed to an increase in Mo levels in sorghum; the main staple food of the region [7]. Molybdenum exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of reactive oxygen species (ROS).Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, altered anti- oxidant system, DNA damage, altered gene expression and apoptosis[8].

There are few reports which reveals that exposure to molybdenum might cause adverse effects on the various organ systems in experimental animals. Experimental animals exposed to molybdate exhibit decreased hemoglobin concentration, depression of growth, mild renal failure, diuresis and proteinuria, body weight loss, altered histological changes in kidney, liver and reproductive organs [9, 10]. Bompart *et al*. [11] investigated that high doses of Molybdenum (80 Mo mg/kg b. wt) for 8 weeks resulted in a delay in body weight gain associated with mild renal failure marked by decrease in glomerular filtration.

Liver is considered as one of the most vital organs that functions as a centre of metabolism of nutrients and excretion of xenobiotics from the body. Liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. The liver is vulnerable to a wide variety of toxic chemicals and microbes. The liver is placed at increased risk for toxic damage by enhanced generation of free radical and depleting antioxidant system [12]. A study by Rana et al.[13] reveals that the chronic administration of metal salts (Pb, Hg, Cd, Cr, Mn, Mo and Co) cause marked deviation from normal liver glycolysis ,citrate cycle function and glycogen metabolism. However, in a recent study Eidi et al. [14] have reported protective effects of molybdate.

There are limited and inconclusive data regarding the

Address for correspondence*

Gyan Chand Jain,

Department of Zoology,University of Rajasthan, Jaipur.

Email: jain_gc@yahoo.co.in

effect of high molybdenum intakes on hematology, liver function and histology. Therefore, in the present investigation, an attempt has been undertaken to evaluate the effect of orally sub chronic administration of molybdenum on hematological and biochemical parameters along with histological changes in liver.

MATERIAL AND METHODS

Chemicals

Ammonium molybdate (AM) was purchased from Merck, India Ltd., Mumbai, India.

Experimental design

Twenty four male albino rats of the Wistar strain, weighing 150-190 g each, were left under normal healthy conditions at the animal house. Animals were fed on basal diet (Aashirwad Food Industries, Chandigarh) and water was supplied *ad libitum*. They were housed in polypropylene cages. Drug and/or vehicle were given orally with the help of feeding needle. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations. The study was approved by institutional Ethical committee, Department of Zoology, University of Rajasthan, Jaipur, India.

Animals were divided into three groups as follows:

Group A: Rats received 0.5 ml/day of the vehicle, i.e., distilled water. (Control group)
Group B: Rats received 50 mg Ammonium molybdate (AM)/kg b.wt./day for 60 days.
Group C: Rats received 100 mg Ammonium molybdate (AM)/kg b.wt./day for 60 days.

At the end of the experimental period, animals were fasted overnight. The animals were weighed and sacrificed under mild ether anesthesia. Total liver weight of rats from each group was also recorded before the tissue was processed for analyses. The liver was washed in ice cold saline solution, blotted and a small portion was cut and weighted for homogenization. The homogenate was kept in the refrigerator (-20° C) for the determination of biochemical activities.

The blood sample was collected by cardiac puncture in rats of all groups. Each blood sample was divided in to two portions; the first was collected in EDTA vials for hematological study while the second portion was collected in plain tubes, allowed to clot and then was centrifuged at 3000 rpm for ten minutes to obtain serum for biochemical assays.

Hematological study

Total erythrocyte and total leukocytes were counted by the method of Lynch et al. [15] . Hematocrit value [16] and Hemoglobin concentration [17] were also determined in each sample.

Serum biochemistry

Serum sample were analyzed for Transaminases (AST and ALT; [18]), alkaline phosphatase [19] and total bilirubin[20].

Tissue biochemistry

Frozen liver sample was analyzed for quantitative biochemical estimations of total protein [21], glycogen [22], total cholesterol [23] and triglycerides [24].

Lipid peroxidation and antioxidant defense system

Frozen liver sample was used for estimation of lipid peroxidation (TBARS)[25], glutathione (GSH) [26], ascorbic acid [27] and superoxide dismutase (SOD) [28].

Histopathological study

For histopathological observation Bouin's fixed tissues were cut into small pieces and processed through ethanol-xylene series. The tissues then were embedded in paraffin wax. The 5 µ thick sections were prepared with the help of microtome and stained in haematoxylin – eosin and observed under light microscope for histopathological changes. Photomicrographs of the histopathological slides were taken using a Nikon digital camera attached to a light microscope.

Statistical analysis

All the values of body weights, organs weight, biochemical estimations were averaged; standard error of the mean was calculated and compared by applying Student's test.

RESULTS

Body and liver weight

The control rats represent significant ($p \leq 0.001$; 16.18%) gain in the mean body weight. However the body weight gain was less in AM treated group than the control group (group B $p \leq 0.01$; 9.88% and group C $p \leq 0.05$; 8.72%).The relative weight of liver exhibited a significant increase in group B ($p \leq 0.05$) and group C ($p \leq 0.001$) as compared to control .(Table 1)

Hematological parameters

Table 2 summarizes the total erythrocyte, total leukocyte count, hemoglobin and hematocrit data obtained for control and AM treated rats. Total erythrocyte count, hemoglobin and hematocrit value depicted significant dose dependent decrease in group B ($P \leq 0.05$) and in group C ($P \leq 0.01$) while total leukocyte count reflects significant change ($P \leq 0.05$) in only group C.

Biochemicals results

Tissue and serum biochemical results are shown in table 3. The protein and glycogen content in liver was significantly reduced in group B ($P \leq 0.05$) and in group C ($P \leq 0.01$) when compare to controls. The cholesterol and triglyceride concentration in liver showed significantly increment in both group B ($P \leq 0.05$) and group C ($P \leq 0.01$) when compared to group A (Table 3).

Rats fed with ammonium molybdate showed dose dependent significant increase in serum levels of AST, ALT, ALP and total bilirubin in both group B ($P \leq 0.05$) and Group C ($P \leq 0.01$) as compared to control rats.(Table 3).

The effects of ammonium molybdate exposure on selected oxidative stress biomarkers are presented in Table 4. In treated rats TBARS level showed dose dependent significant increase in lipid peroxidation in group B ($P \leq 0.05$) and group C($p \leq 0.01$) in contrast to this the levels of glutathione(GSH), ascorbic acid and activities of superoxide dismutase (SOD) were significantly decreased in liver of both group B ($P \leq 0.05$) and group C($p \leq 0.01$) animals.(Table 4)

Table 1: Effect of *Ammonium molybdate* on body weight in treated Rats

Treatments		Group A (Control)	GroupB (50mg/kgb.wt./day)	Group C (100mg/kg b.wt./day)
Body weight (gm)	Initial	173.75 ± 3.65	179.37 ± 2.75	171.87 ± 3.6
	Final	201.87[c] ± 4.33 (16.18%)	197.12[b] ± 3.55 (9.88%)	186.87[a] ± 4.01 (8.72%)
Liver weight (mg/ 100g b.wt.)		3229.53 ± 72.25	3468[a] ± 64.15	3808.46[b] ± 124.5

Values are mean ± SEM (n≤8) Levels of significance: [ns] non significant; [a] p<0.05; [b] p<0.01; [c] p<0.001, AM (Ammonium molybdate) treated rats compared with Group A control rats

Table 2: Effect of *Ammonium molybdate* on Various Hematological Parameters intreated Rats.

Treatments	Total RBCs count (millions/mm^3)	Total WBC count (number /mm^3)	Hematocrit (%)	Hemoglobin (%)
Group A (Control)	5.09 ± 0.13	6355.10 ± 203.71	40.99 ± 0.65	13.38 ± 0.22
Group B (50mg/kg bd.wt./day)	4.60[a] ± 0.16	6284.75[ns] ± 261.85	38.33[a] ± 0.81	12.16[a] ± 0.36
Group C (100mg/kg bd.wt./day)	4.45[b] ± 0.18	6069.37[a] ± 263.88	36.26[b] ± 1.02	11.05[b] ± 0.62

Values are mean ±SEM (n≤8) Levels of significance: [ns] non significant; [a] p<0.05; [b] p<0.01; [c] p<0.001, AM(Ammonium molybdate) treated rats compared with Group A control rats

Table3: Effect of *Ammonium molybdate* on Various Tissue and serum Biochemical Parameters in treated Rats.

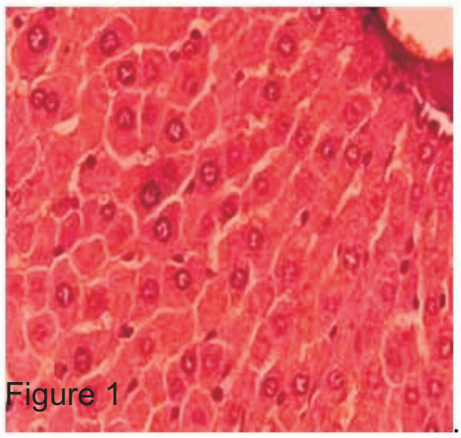
Treatments	Liver weight (mg/100g b.wt.)	Tissue biochemistry				Serum biochemistry			
		Protein (mg/gm)	Glycogen (mg/gm)	Cholesterol (mg/gm)	Triglyceride (mg/gm)	AST (U/ml)	ALT (U/ml)	ALP (KA Unit)	Total bilirubin (mg/dl)
Group A (Control)	3229.53 ± 72.25	173.76 ± 6.82	5.30 ± 0.12	8.71 ± 0.17	8.01 ± 0.28	61.69 ± 2.41	43.77 ± 3.96	12.96 ± 0.87	0.73 ± 0.10
Group B (50mg/kg bd.wt./day)	3468[a] ± 64.15	154.28[a] ± 5.86	5.01[a] ± 0.08	9.51[a] ± 0.25	9.50[a] ± 0.54	79.6[a] ± 4.52	71.83[a] ± 5.46	15.72[a] ± 0.92	1.18[a] ± 0.14
Group C (100mg/kg bd.wt./day)	3808.46[b] ± 124.5	146.78[b] ± 5.12	4.64[b] ± 0.17	12.88[b] ± 1.04	12.45[b] ± 1.12	112.36[b] ± 5.91	100.26[b] ± 5.96	19.52[b] ± 1.37	1.44[b] ± 0.16

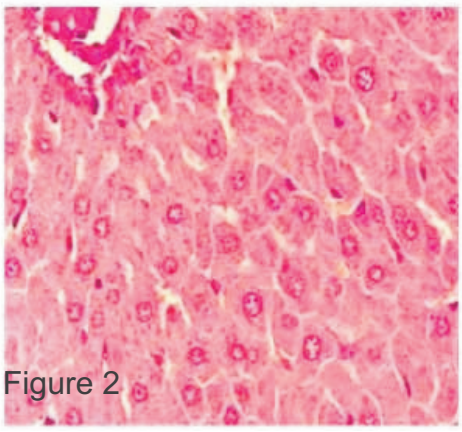
Values are mean ±SEM (n≤8) Levels of significance: [ns] non significant; [a] p<0.05; [b] p<0.01; [c] p<0.001, AM (Ammonium molybdate) treated rats compared with Group A (control rats)

Table 4: Effect of *Ammonium molybdate* on Lipid peroxidation and antioxidant Parameters in liver of treated Rats

Treatments	Lipid Peroxides (n mole/mg tissue)	Superoxide Dismutase (SOD) (U/mg/protein)	Reduced Glutathione (GSH) (µ mole/gtissue)	Ascorbic Acid (mg/g tissue)
Group A (Control)	2.8 ± 0.16	15.56 ± 0.86	3.80 ± 0.22	1.88 ± 0.24
Group B (50mg/kg bd.wt./day)	3.46^{a} ± 0.18	12.66^{a} ± 0.51	3.06^{a} ± 0.19	1.24^{a} ± 0.15
Group C (100mg/kg bd.wt./day)	6.85^{b} ± 1.02	10.84^{b} ± 0.94	2.27^{b} ± 0.31	0.85^{b} ± 0.11

Values are mean ±SEM (n≤8) Levels of significance: ^{ns} non significant; ^{a} $p<0.05$; ^{b} $p<0.01$; ^{c} $p<0.001$, AM (Ammonium molybdate) treated rats compared with Group A (control rats)





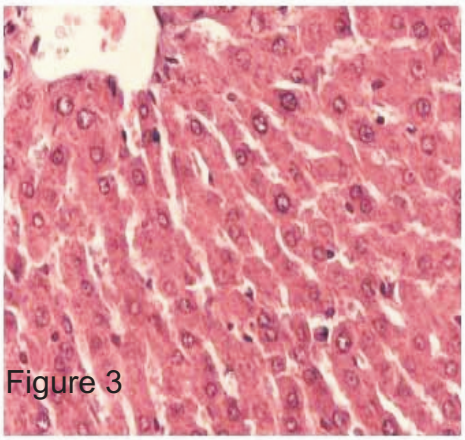


Figure 1. Photomicrograph of the liver of control rats showing normal hepatocytes radiating from the central vein. (H & E, x400).Figure 2. Photomicrograph of the liver of Ammonium molybdate 50mg/kg b.wt (AM-50) treated rats showing mild degenerative and necrotic changes.(H& E, X 400).Figure 3. Photomicrograph of the liver of Ammonium molybdate 100mg/kg b.wt (AM-100) treated rats showing degenerative and necrotic changes, dilation of sinusoids and infilteration of leukocytes. (H and E X 400)

Histopathological results

The histopathological examination of liver of control rats revealed normal histoarchitecture of showing normal hepatocytes radiating from the central vein with prominent nuclei & characteristic cord like arrangement separated by sinusoids (Figure, 1). Exposure of AM at the dose level of 50 mg/kg b. wt showed a less normal lobular pattern with mild degree of degenerative changes and leukocyte infiltration (Figure 2). Section of liver tissue of AM (100 mg/kg b.wt) treated rats revealed degenerative and necrotic changes in liver, swelling of hepatocytes, dilation of sinusoids and infiltration of leukocytes (Figure 3).

DISCUSSION

The liver plays a critical role in metabolizing foreign substances, including nutrients, therapeutic drugs, and environmental toxicants, some of which can have an adverse effect on it [29] . Although molybdenum is a necessary trace element in the body but has also the potential to cause toxicity**.** It promotes an early oxidative stress and contributes to the development of various pathological conditions in different tissues including liver [30] . Our paper explores molybdenum induced oxidative stress, alterations in hematology, hepatic marker biochemical parameters and hepatic histoarchitecture.

In our experimental study AM treated rats showed low body weight gain as compared to normal rats which may be due to gastrointestinal disturbances [31] . There was an increase in the relative weight of liver in AM treated rats. Increase in liver weight could be due to increased blood contents as a result of the dilation of the sinusoids and increased accumulation of fat in the hepatocytes. Similar increase in liver weight has been observed in rats after exposure with chromium [32], *cadmium* [33] *metals.*

Hemoglobin content, total erythrocyte count and hematocrit values revealed significant decrease in both group B and C while total leucocyte counts showed significant decline in only high dose group .The fall in hemoglobin content can be attributed to secondary Cu deficiency effect resulted due to AM treatment [34] . The decrease in total erythrocyte count might be due to failure of hematopoiesis and defective iron metabolism [35] . The present results are similar to Lyubimov et al.[36] who reported similar decline in erythrocyte count, hemoglobin concentration and hematocrit in tetrathiomolybdate (12mg/kg/day)treated male rats. Further the decline in total erythrocyte count might be due to hemolysis caused as a result of membrane damage by virtue of excessive generation of free radicals in AM treated rats [37] .

Exposure of AM caused decline in hepatic protein level in treated rats, which may be due to alteration in protein

metabolism. Interference with protein metabolism and enzyme impairment has been described in animals fed a diet with a high level of molybdenum [38] . Decrease in hepatic glycogen level in AM treated rats might be due to increased glycogenolysis in hepatocytes. Fall in glycogen level indicates depression of glycokinase which slows down the conversion of glucose to glycogen [39] . Administration of AM to rats also led to significant elevation in liver total cholesterol and triglycerides which might be due to disturbances of lipid metabolism as also observed in chromium [32] treated rats.

The aminotransferase enzymes (ALT and AST), ALP and bilirubin have been shown to be crucial biomarkers for the assessment of hepatocellular injury [40] . Serum biochemical findings in this study showed that the oral administration of ammonium molybdate for 60 days resulted in a significant increase in serum AST, ALT, ALP and total bilirubin. Elevated level of AST and ALT indicates the cellular leakage and loss of functional integrity of hepatic membrane architecture which results in release of these enzymes from the cell cytosol into blood [41-42] . In an earlier report, Rana and Chauhan [43] also observed a similar increase in serum transaminases in rats administered with sub lethal dose of molybdenum (50 mg/100 g body weight).

Alkaline Phosphatase (ALP) enzyme is a sensitive biomarker of liver function test since it is a membrane bound enzyme related to the transport of various metabolites [44] . The increase in the activities of blood ALP observed in AM treated rats indicates the possibility of the increased permeability of plasma membrane or cellular necrosis in liver. These results are in accordance with the findings of Van Reen and William [45] . The increase in serum total bilirubin level in the AM treated rats indicates hepatotoxicity. The induction rate in serum bilirubin may be associated with free radical production [46] . The increase in serum total bilirubin may result from decreased liver uptake, conjugation or increased bilirubin production from haemolysis [47] . These observations are similar to the data reported in cobalt [39] and nickel [48] treated rats.

Lipid peroxidation is one of the main manifestations of oxidative damage and can cause protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products, MDA and 4-hydroxynonenal [49] . Elevated lipid peroxidation (TBARS) concentration in liver has been observed in AM treated rats. The elevated levels of lipid peroxides in the liver reveal the degree of lipid peroxidation in hepatic tissues and are considered as the indicator of hepatocyte damage [50] . It has been suggested that molybdenum has a variety of oxidative states, and could cause oxidative damage through the formation of free radicals [51] .

Alteration of the antioxidant system in liver of AM treated group was confirmed by the significant decline of GSH, SOD and ascorbic acid. Glutathione (GSH), a crucial component of the antioxidant defense mechanism, functions as a direct reactive free-radical scavenger. Reduced activities of GSH in liver tissues may be due to a direct attack of reactive oxygen species. In fact, it has been reported that the sulfhydryl group of cysteine moiety of glutathione has a high affinity for metals forming thermodynamically stable mercaptides complexes which are inert and excreted *via* the bile [52] . SOD activity fairly well reflected status of copper nutrition of rats. Decreased activity of SOD may be correlated with the copper deficiency caused by molybdenum. As the concentration of Mo increases, other kind of Mo-Cu complexes may be formed which resulted in shielded and unavailable Cu to be incorporated into copper enzymes[53].Ascorbic acid is an excellent hydrophilic antioxidant; it readily scavenges ROS and peroxyl radical. Decreased level of ascorbic acid might be correlated with the increased utilization of ascorbic acid in deactivation of the increased level of reactive oxygen species or with the decreased GSH level. Since, the GSH is required for the recycling of ascorbic acid [54] .

The biochemical results also confirmed the histopathological findings .Sections of liver tissue from rats treated with ammonium molybdate for 60 days showed hepatocytes with shrunken and pycknotic nuclei, necrosis and increased in infinteration of leukocytes which might be correlated with the increased level of AST, ALT and ALP (Figure 2, 3). Similar histopathological alterations have been reported in rats receiving 489 mg Mo/kg/day (as ammonium molybdate) in their diet for 20 days [55] .

CONCLUSION

Our results indicated that sub chronic exposure of ammonium molybdate caused adverse impact on hematology, hepatic functions and histology in treated rats. Excessive environmental exposure of molybdate by various sources and its medicinal use may cause adverse effect on the hematology and hepatic functions in human beings. Therefore it is recommended that further long term studies at different dose levels be carried out in different animal models.

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