Effect of molybdate and sulfate alone and in combination overload intake on copper outcome in mature male rats

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Summary

This study was designed to investigate the effect of molybdate and sulfate overload alone or in combination overload intake on copper outcome in mature male rats. Twenty eight adult male rats were housed and randomly divided into four equal groups and treated as follows for 60 days: control, groups (T1) rats received sodium molybdate 50 mg/kg B.W and (T2) rats of this group were given orally 500 mg/kg B.W of sodium sulfate. Animals in the (T3) group were given sodium molybdate and sodium sulfate in combination at half dose in comparison with T1 and T2 doses. The severity of toxic symptoms were more noticed at the end of experiment especially in T1 followed by T2, while T3 showed the least toxic symptoms, also the body weight change recorded weight loss during the experiment period at the same following order T1, T2 followed by T3 in comparison with control group that showed normal increase in weight. Biochemical results showed a significant decline in serum glutathione and an increase in serum creatinine, bilirubin and blood urea nitrogen at the same pattern in all treated groups positively proportional with the course of experiment. This was attributed to the recorded deficiency in serum copper that in consequence possibly induced anemia and oxidative changes in T1 and T2 groups, but the addition of sulfate to molybdate at half doses recorded less severity of copper deficiency signs and less toxic consequences indicating a sort of antagonistic effect.

Keywords: Copper, Molybdate, Sulfate.

Introduction

Molybdenum (Mo) is an essential trace element for virtually natural life. Its role is a cofactor for a number of enzymes e.g. (sulfite oxidase, xanthin oxidase, aldehyde oxidase *etc*.) that catalyze important chemical transformations in the global carbon, nitrogen, and sulfur cycles (1). Sulfur is found in every cell in the body and is involved in a wide range about 15 function of biochemical functions e.g. Cellular energy production/ metabolism, maintaining blood glucose levels etc (2). Copper (Cu) have a various roles in biological electron transport and oxygen transportation (3). Also important in integrity of the C.N.S, elastin and collagen synthesis, melanin production and metabolism of iron (4). High levels of Mo can interfere with the body's uptake of Cu, creating copper deficiency by prevents plasma proteins from binding to Cu, and it also increases the amount of Cu that is excreted in urine, so the consumption of high amounts of molybdenum result in the development of symptoms including stunted growth, diarrhea, anemia and achromotrichia (loss of hair stain) (5). The antagonism between Mo and Cu is affected by the sulfate content of the diet. Sulfate reduces the retention of Mo by reducing its absorption from the gastrointestinal tract and increasing its elimination with the feces and the urine (6). This study was planning for understanding Mo and sulfate overload in outcome of Cu deficiency in rat, studying the clinical and biochemical toxic effect of Mo and S alone and in combination overload in rats and understanding the mechanism of toxicity due to Mo – Cu – sulfate interaction.

Materials and Methods

Twenty eight adult Albino male rats at weight range of (316-350 g) and age of (4-5 months) were randomly divided into four equal groups, they were housed in optimum condition of raising at the animal house/college of veterinary medicine. Special pellet diet and water were provided ad libitum for the experiment groups which were administered orally for 60 days by using special gavage needle as the following: Control group (C) received ordinary tap water, group (T1) rats were administrated 50 mg/kg

B.W of sodium molybdate, group (T2) rats were administrated 500 mg/kg B.W of sodium sulfate, group (T3) rats were administrated 25 mg/kg B.W of sodium molybdate + 250 mg/kg B.W of sodium sulfate. Fasting blood (for 8-12 hrs.) samples were collected at different times 0, 30 and 60 days of the experiment. Blood was drawn by cardiac puncture technique from anesthetized rats using Ketamine hydrochloride and xylazine hydrochloride at dose 90 mg/Kg B.W. 40 mg/kg B.W., respectively. Blood samples were let with and in for 30 min. at room temperature, and centrifuged at 2500 rpm for 15 minutes, and then serum samples were liquated and frozen at -20℃ until estimation of following parameters by using available commercial kits. The serum samples were subjected to assessment of the following parameter by using available commercial kits: Glutathione level (GSH), according to the method of (7), Bilirubin (8), blood urea nitrogen (BUN) (9), Creatinine (10) and Copper level in blood, by atomic absorption system, according to the (11). Furthermore, the weight change and clinical signs that developed and appear on the animal during the period of experiment in each group was recorded. The Statistical Analysis System as used to effect of different factors (group and day) by using ANOVA-2Ways in study parameters. Least significant difference-LSD test was used to significant compare between means in this study (12).

Results and Discussion

The result showed a higher significant reduction (P <0.05) in level of serum copper in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than in group T2 dosed with sodium sulfate (500 mg/kg B.W). The least significant reduced levels were recorded in T3 group that were given combined half doses of sodium molybdate and sodium sulfate in comparison with control group. All treated groups showed more significant reduction (P<0.05) at day 60 in comparison with that of day 30 but both showed significant reduction levels than that of pretreatment period (Table, 1). Glutathione serum (GSH) level result showed a higher significant reduction (P<0.05) in level of serum glutathione (GSH) in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than in group T2 dosed with sodium sulfate (500 mg/kg B.W). The least significant reduction levels were recorded in T3 group that were given combined half doses of sodium molybdate and sodium sulfate in comparison with control group. All treated groups showed more significant reduction (P<0.05) at day 60 in comparison with that of day 30 but both showed a significant reduction levels than that of pretreatment period (Table, 2).

Table, 1: The effect of oral intubation for two months with sodium molybdate, sodium sulfate and their combination on serum copper level (mg/dL) of adult male rats.

Group	Day of experiment			LSD value
	0	30	60	
C	1.96 ±0.06 a A	1.96 ±0.06 a A	1.96 ±0.06 a A	0.162 NS
T1	1.99 ±0.04 a A	1.13 ±0.02 b B	0.561 ±0.02 c D	0.093 *
T 2	2.02 ±0.07 a A	1.06 ±0.03 b B	0.684 ±0.01 c C	0.144 *
T 3	1.94 ±0.03 a A	1.72 ±0.06 b B	1.561 ±0.04 c B	0.134 *
LSD value	0.114 NS	0.114 *	0.114 *	---

* (P<0.05) , NS: Non-significant.

Table, 2: The effect of oral intubation for two months with sodium molybdate, sodium sulfate and their combination on serum Glutathione (GSH) (mg/dL) concentration of adult male rats.

Group	Day of experiment			LSD value
	0	30	60	
C	51.90 ±0.42 a A	52.30 ±0.43 a A	51.51 ±0.72 a A	1.615 NS
T1	53.54 ±1.24 a A	45.68 ±0.81 b B	38.35 ±0.65 c C	2.795 *
T2	51.60 ±0.73 a A	46.72 ±1.05 b B	39.65 ±1.01 c B	2.806 *
T3	52.47 ±0.36 a A	47.45 ±0.84 b B	43.82 ±1.94 b C	3.700 *
LSD value	2.267 NS	2.389 *	3.507 *	---

* (P<0.05) , NS: Non-significant.

The result showed a higher significant increase (P<0.05) in level of serum creatinine in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than in group T2 dosed with sodium sulfate (500 mg/kg B.W). The least significant increased levels were recorded in T3 group that were given combined half doses of sodium molybdate and sodium sulfate after 60 days of treatment only in comparison with control group. All treated groups showed more significant increasing (P<0.05) at day 60 in comparison with that of day 30 but both showed more significant increasing levels than that of pretreatment period (Table, 3).

Table, 3: The effect of oral intubation for two months with sodium molybdate, sodium sulfate and their combination on Total Serum Creatinine concentration (mg/dL) of adult male rats.

Group	Day of experiment			LSD value
	0	30	60	
C	0.350 ± 0.01 a A	0.392 ± 0.02 a B	0.378 ± 0.01 a C	0.038 *
T1	0.345 ± 0.01 c A	0.575 ± 0.02 b A	1.098 ± 0.06 a A	0.110 *
T2	0.377 ± 0.01 c A	0.541 ± 0.02 b A	1.040 ± 0.04 a A	0.085 *
T3	0.362 ± 0.01 c A	0.405 ± 0.01 b B	0.747 ± 0.03 a B	0.056 *
LSD value	0.031 *	0.045 *	0.121 *	---

* (P<0.05) , NS: Non-significant.

The result showed a higher significant increasing (P<0.05) in level of total serum bilirubin in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than group T2 dosed with sodium sulfate (500 mg/kg B.W). The least significant increase levels were recorded in T3 group that were given combined half doses of sodium molybdate and sodium sulfate in comparison with control group. All treated groups showed more significant increased (P<0.05) at day 60 in comparison with that of day 30 but both showed significant increasing levels than that of pretreatment period (Table, 4).

Table, 4: The effect of oral intubation for two months with sodium molybdate, sodium sulfate and their combination on Total Serum Bilirubin (TSB) concentration (mg/dL) of adult male rats.

Group	Day of experiment			LSD value
	0	30	60	
C	0.495 ± 0.05 c A	0.515 ± 0.01 a D	0.492 ± 0.02 a D	0.068 NS
T1	0.497 ± 0.02 c A	0.922 ± 0.01 b A	2.884 ± 0.01 a A	0.044 *
T2	0.485 ± 0.02 c A	0.728 ± 0.02 b B	1.535 ± 0.02 a B	0.056 *
T3	0.512 ± 0.02 c A	0.685 ± 0.02 b C	0.954 ± 0.02 a C	0.056 *
LSD value	0.065 NS	0.048 *	0.052 *	---

* (P<0.05) , NS: Non-significant.

The result showed a higher significant increasing (P <0.05) in level of BUN in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than group T2 dosed with sodium sulfate (500 mg/kg B.W). The least significant increased levels were recorded in T3 group that were given combined half doses of sodium molybdate and sodium sulfate in comparison with control group. All treated groups showed a more significant increasing (P<0.05) at day 60 in comparison with that of day 30 but both showed a more significant increasing levels than that of pretreatment period (Table, 5).

Table, 5: The effect of oral intubation for two months with sodium molybdate, sodium sulfate and their combination on (BUN) concentration (mg/dL) of adult male rats.

Group	Day of experiment			LSD value
	0	30	60	
C	14.92 ± 0.11 a A	14.65 ± 0.17 a C	14.72 ± 0.16 a C	0.449 NS
T1	15.24 ± 0.17 c A	18.94 ± 0.13 b A	22.50 ± 0.23 a A	0.546 *
T2	15.00 ± 0.15 c A	18.31 ± 0.38 b A	21.88 ± 0.21 a A	0.793 *
T3	15.25 ± 0.26 c A	17.18 ± 0.21 b B	19.25 ± 0.29 a B	0.771 *
LSD value	0.539 NS	0.708 *	0.676 *	---

* (P<0.05) , NS: Non-significant.

There are clear clinical signs in the treated groups when compared with the control one and/or pretreated group especially in groups treated with 50 mg/kg/day orally of sodium molybdate and 500 mg/kg/day orally sodium sulfate (T1 and T2) respectively. Less clinical signs appeared in combined treated group (T3) of 25 mg/ kg B.W sodium molybdate and 250 mg/kg B.W. orally sodium sulfate. Nearly the same symptoms appeared including hair loss in back area, hair roughness, loss of shiny appearance, and also loss of appetite, emaciation, restlessness, incoordination, lameness and paleness of ear. The sequences and severity of symptoms listed below in table according to time of appearance and intensity of sign in each treated group (Table, 6).

Table, 6: The development of clinical signs of adult male rats during the experiment period in all treated groups compared with control.

Group	Day Zero time	30 days	60 days
Control	Normal	Normal	Normal
T1	Normal	Significant loss of body weight and roughed hair	Oblivious emaciation, alopecia, incoordin -ation and paleness of ear and mucus membrane
T2	Normal	Moderate restlessness and loss of appetite	Mild loss of body hair in back area, emaciation and restlessness
T3	Normal	There are no frank changes on the animal when compared with pretreated period	Less loss of hair with lesser emaciation and other toxicity signs

The result showed a higher significant reduction ($P<0.05$) in body weight in the treated group T1 dosed with sodium molybdate (50 mg/kg B.W) more than group T2 that dosed with sodium sulfate (500 mg/kg B.W). The least significant reduced levels were recorded in T3 group given combined half doses of sodium molybdate and sodium sulfate in comparison with control group. All treated groups showed more significant reduction ($P<0.05$) at day 60 in comparison with that of day 30 but both showed more significant reduction levels than that of pretreatment period (Table, 7).

Table, 7: Show the development of body weight of adult male rats during the experiment period in all treated groups compared with control.

Group	Day of experiment			LSD value
	0	30	60	
C	341.57 ± 2.67 a A	353.42 ±19.67 a A	367.28 ±17.52 a A	45.429 NS
T1	339.42 ± 2.81 a A	320.71 ± 7.20 b B	293.71 ± 6.96 c B	17.85 *
T2	335.57 ± 4.83 a A	326.00 ± 3.24 a A	299.85 ± 6.16 b B	14.54 *
T3	338.42 ± 4.41 a A	329.00 ± 3.75 a A	315.85 ± 3.52 b B	11.63 *
LSD value	11.10 NS	31.42 *	29.408 *	---

* ($P<0.05$) , NS: Non-significant.

There are many studies dealing with Cu importance for body organisms, such as Cu have various roles in biological electron transport and oxygen transportation (3). Copper was necessary in integrity of the CNS elastin and collagen synthesis, melanin production and metabolism of iron etc. (4). Cu levels in body were related with other trace and/or essential elements ratio in diet, such as Zn and Mo which interfere with the body's uptake of Cu, so when Mo present in high level in diet it would create a state of copper deficiency by preventing plasma proteins from binding to Cu and increasing the amount of Cu excreted in urine. This resulted in the development of Cu deficiency symptoms including stunted growth, diarrhea, anemia and achromotrichia (loss of hair stain) (13).

Mo and sulfate were similarly shaped anions sharing some physicochemical characteristics, this lead to interactions of these anions in different eukaryotic systems by cross-inhibition of sulfate transport by molybdate, this explain why the combination of two anion at the half dose causing less Cu deficiency than each one alone (14). Sulphate, Mo and other group IV oxyanions have been shown to compete for sites on a common active transport system in rat and ovine ileum (15).

Moreover, the stomach was the site of significant interactions between Cu, sulfate and Mo. It also displays reactions among Cu,

sulfate and Fe. The interaction between Mo and S results in the creation of tetrathiomolybdates (TTM), which in the lack of sufficient amounts of stomach Cu are absorbed and bind to Cu in biological complexes. This was the cause of thiomolybdate toxicity. Tetrathiomolybdate was a powerful and selective Cu chelater that is used as a therapeutic agent for Wilson disease (16).

Thus, copper deficiency where noticed both after molybdenum and sulphate overload, but in T3 the half dose of sulphate and molybdate at ration 10:1 showed lesser toxicity symptom possibly due to their antagonism due to their completion as reported for absorption and distribution, the disposition of copper probably because of similarity of charge (anion) for Mo and sulfate with Cu, that's why the lesser serum. Cu deficiency was mainly reported at the end of experiment indicating such antagonism but this was not enough to overcome Mo and sulphate overload dose with their Cu deficiency consequence

Also, Cu act as a redox metal (Fenton reactant), so it play an chief role in inducing ROS and RNS synthesis creating oxidative change especially in parenchymal cells like liver, kidney and spleen. These oxidative changes usually opposed by glutathione interaction (the main important internal antioxidant element) to overcome their oxidative changes, those leading to their depletion (17). Also Cu was known as a source of superoxide dismutase which is one of the important enzymes in synthesis of oxidative radicals that also consider as the main cause of depletion of glutathione (18).

Naturally from the results can be conclude that the reduction in serum level of GSH in each treated group were proportional with their serum and tissue levels of Cu and the oxidative change induced by them in these organ which were more occur in T1 than T2 while the combination showed less effect probably due to less dose of Mo and sulphate which cause less Cu deficiency and less oxidative change. In other hand, the depletion of antioxidant defense system leads to tissue damage by different mechanisms including promoting lipid peroxidation, and protein modification, these processes have been implicated in the pathogenesis of several systemic diseases including liver and kidney documented in this study by changes of liver and kidney biomarkers (Creatinine, total serum bilirubin and blood urea nitrogen) (19).

In the present study the symptoms in different treated groups representing different dosing with Mo and sulphate and their combination at ratio of 10:1 at half dose. These toxicity symptoms could be attributed to the Cu deficiency induced in serum and important organs that manifested by its consequence on biochemical results. Since Cu consider as important and essential element that enter in all these processes.

Nearly same symptoms were noticed in oral intubation with a molybdenum salt caused a reduction in body weight. Typical symptoms of molybdenum toxicity included weight loss or growth retardation, anorexia**,** anemia, incoordination, achromotrichia, dyspnea, incoordination, and planes and irritation of mucous membranes. Molybdenum also disturbs bone metabolism, giving rise to lameness (20). Hair changes (roughing and losing) are recorded in cases with secondary Cu deficiency that was because the Cu content of the hair and nail was decreased in cases of Cu deficiency (21).

The present results were indicative of such conclusion, since the severity of symptoms in all treated groups T1, T2 and T3 were dose and period dependent with higher toxicity symptoms appearing in T1 dosed with Mo. That showed the highest serum Cu reduction, while T2 group showed lesser severity symptoms and to lesser extent the combined half dose in T3 group. So, the intensity of toxicity symptoms was higher in all treated groups at 60 days than at day 30 due to Cu deficiency level manifested at 60 days than at 30 days. The result of biochemical difference treated groups was support of such conclusion.

The clinical signs of Cu deficiency observed in different treated rats in the current study strongly act as a stress factors on their body weight gains accordingly with treatment coarse of experiment, probably due to anemia, loss of appetite, reduced nutrition supply and disturbed the normal biologically and physiological process as a result of different dosing system in T1, T2 and T3 and period.

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تأثير الإعطاء المفرط للموليبدات والسلفات إعطاءً مفرداً وخليطة على حصيلة النحاس في ذكور الجرذان البالغة

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الخلاصة

صُممت هذه الدراسة للتحقق من تأثير الموليبدات والكبريتات كلاً على حدة، وعند الجمع بينهما حين تناولها الزائد على حصيلة النحاس في ذكور الجرذان البالغة. استعمل ثمانية وعشرون من ذكور الجرذان البالغه وقسمت عشوائياً ألى أربع مجاميع متساوية، جرعت لمده 60 يوماً: مجموعة السيطرة جرعت ماء، المجموعة الثانية (T1) جرعت موليبدات الصوديوم (بجرعة 50 ملغ/كلغ من وزن الجسم) والثالثة (T2) جرعت سلفات الصوديوم (بجرعة 500 ملغ/كلغ من وزن الجسم) في حين جرعت حيوانات المجموعة الرابعة (T3) خليطاً من موليبدات الصوديوم وسلفات الصوديوم بنصف الجرعة للمادتين مقارنة مع المجموعتين الأولى والثانية. إنَ شدة الأعراض السمية التي لوحظت في نهاية التجربة كانت اكثر في مجموعةT1 تليها مجموعة T2، في حين أظهرت مجموعة T3 أقل شده في ظهور تلك الاعراض من T1 وT2، كما سُجَل تغيير في وزن الجسم حيث أن فقدان الوزن في أثناء كان نفسه وفقاً للترتيب الآتي T1، T2 تليهما T3 بالمقارنة مع مجموعة السيطرة التي أظهرت الزيادة الطبيعية في الوزن. وأظهرت النتائج البيوكيميائية انخفاض كبير في الجلوتاثيون في الدم والزيادة في كرياتينين المصل والبيليروبين ونتروجين يوريا الدم على نفس النمط في جميع المجموعات المعالجة متناسبة طردياً مع مسارالتجربة. ويعزى ذلك إلى النقص المسجل في مستوى النحاس في المصل الذي ربما حث بالإستمرارعلى حدوث فقر الدم وتغييرات الأكسدة فيT1 وT2 لكن إضافة الكبريتات للموليبدات في نصف الجرع قد سجل أقل نقص في مستوى النحاس في المصل وأقل سمية وهذا يدل على نوع من التأثير المضاد بينهما.

الكلمات المفتاحية: نحاس، موليبدات، سلفات.