WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 1057-1065. Research Article ISSN 2277– 7105

ALTERATIONS IN BIOCHEMICAL INDICES DURING REPEATED ORAL ADMINISTRATION OF MOLYBDENUM ALONE AND IN CONJUNCTION WITH COPPER SULFATE IN GOATS

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Article Received on
24 Dec 2014,

Revised on 18 Jan 2015,
Accepted on 12 Feb 2015

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ABSTRACT

Molybdenum is an essential trace element existing in the animal body in varying concentrations in different tissues and its toxicity is a serious problem in many parts of the globe. To access the alterations in the various biochemical indices during subacute toxicity of molybdenum and its amelioration by concurrent exposure with copper sulphate in goats. In the present study, ammonium molybdate at 20 mg/kg/day alone and in conjunction with copper sulphate (II), pentahydrate at 7.9 mg/kg/day was administered orally for 30 consecutive days in healthy goats of group I and II respectively. In group I, significant ($P<0.05$) alterations in plasma proteins profile, blood urea nitrogen and creatinine were observed on different days of exposure from pre-exposure values. However, no significant changes were observed in plasma calcium, phosphorous, magnesium, sodium and potassium on different days of exposure of ammonium molybdate. However, concurrent exposure with the copper sulphate (Group II) no significant changes were observed on different biochemical indices. Observation from the present study suggested that ammonium molybdate alone produced significant alterations in some biochemical indices and concurrent administration with copper sulphate reversed the alterations induced from the ammonium molybdate exposure.

KEYWORDS: Copper sulphate; biochemical indices; goats; molybdenum; subacute.

INTRODUCTION

Molybdenum (Mo) toxicity is a serious livestock health problem in many parts of the globe. Forages grown on molybdenum rich soil absorb and accumulate molybdenum more than their normal requirement and animals consuming such forages develop molybdenosis.[1] Mo essentially regulates copper absorption in animals and also effects blood serum levels of iron, calcium, sulphur and phosphorous.[1, 3] The biological function of Mo in animals is of a prosthetic group along with other groups in enzymes like xanthine oxidase / dehydrogenase, aldehyde oxidase, and sulphite oxidase, all of which catalyze oxidation reduction reactions.[4-6] It is further required in amino acid and protein metabolism, sulphur metabolism, hydrolysis of phosphate esters and transport and utilization of iron.[7-8] High levels of Mo accumulate in kidneys, liver, adrenal glands erythrocyte membrane and bones and excessive accumulation lead to the toxicity in animals.[9-11] Various ameliorative agents for the Mo toxicity have been tried, because most of symptoms of Mo toxicity are due to deficiency of the copper. Thus, keeping these points in mind, the present study aimed at studying the alterations in the biochemical profile during subacute Mo toxicity and possible ameliorative potential on biochemical indices on concurrent exposure with copper sulphate in goats.

MATERIALS AND METHODS

Experimental Design: Eight healthy female crossbred goats weighing 25-40 kg of 2-2.5 yrs of age were procured from local farmer of R. S. Pura, Jammu. They were acclimatized for two weeks in the Animal shed of Faculty of Veterinary Sciences and Animal Husbandry, R S Pura, Jammu under hygienic conditions before the commencement of the experiment. The animals were maintained on *ad lib* feed and water. They were divided into two groups of four each. Goats of group 1 were used to study the on non-enzymatic parameters, in which ammonium molybdate [$(NH_4)_6$ $Mo_7O_{24}.4H_2O$ AR, Qualigenes Fine Chem. Ltd. INDIA] alone was administered orally at the dose rate of 20mg/kg/day (equivalent to 10.86 mg of Mo) for 30 consecutive days. Whereas goats of group II were used to study the efficacy of copper sulphate (II), pentahydrate on the effect of subacute oral toxicity of molybdenum, in which same dose of ammonium molybdate along with copper sulphate (II) pentahydrate ($CuSO_4.5H_2O$ AR, Hi-media Laboratories Ltd. INDIA) at the dose rate of 7.9 mg $CuSO_4$/Kg/day (equivalent to 2mg of Cu) was administered orally for 30 consecutive days. Copper sulphate (II), pentahydrate was provided 30-40 minutes before ammonium molybdate administration. The daily administration of salts was made between 9.30-10.00 A.M. after dissolving them in adequate amount of tap water based on the body weights of goats. The

animals were weighed weekly and dosage of salts was corrected for changes in body weight. The experimental protocol was approved by the Institutional Ethics Committee.

Sample Collection: Blood samples (7-8 ml) were collected in clean sterile glass tubes containing heparin at 5-10 IU/ml blood on days 0, 1, 3, 7, 14, 21, 28, 30 of treatment and on days 7 and 14 after termination of treatment by jugular vein puncture. The heparinized blood samples were centrifuged at 3,000 rpm for 15 min. Plasma was separated and stored in glass vials at 4°C until the parameters were analyzed.

Parameters Estimation and Statistical Analysis*:* Total plasma proteins, albumin and calcium were estimated using the Bayer's Autopak kit based on Biuret method, Bromocresol green, and Cresolphthalein complexone methods respectively. Globulin was determined by subtracting albumin from total protein, where as Albumin/Globulin (A/G) ratio was determined by dividing albumin by globulin. Blood urea nitrogen (BUN), creatinine was determined and was expressed as mg/dl. Phosphorous level was determined by using Crest's kit based on Mod Gomorri's method. Sodium, magnesium and potassium levels were determined by using the Span's SP Twin Electrolyte kit which was based on Trinder's method and Tetraphenyl boron method, respectively. Parameters estimated by using different kits were measured on chemistry analyzer RT-1904C (Rayto). Values of different days were compared to pre-exposure (0 day) value of the same group and probability levels of $P<0.05$ and $P<0.01$ were considered statistically significant.[12]

RESULTS

The alterations in biochemical indices on different pre and post treatment days in different groups on exposure of ammonium molybdate alone and along with copper sulphate were presented in table 1 and table 2. The plasma BUN levels (mg/dl) increased significantly ($P<0.05$) from day 0 to day 21 with maximal increase ($P<0.01$) on day 30 of administration of ammonium molybdate and remained increased up to day 7 after termination of ammonium molybdate exposure. The plasma creatinine (mg/dl) was significantly ($P<0.05$) increased on day 28 of exposure in group I. These values returned to normal values ($P<0.01$) within 7 days of post treatment. The plasma total protein (gm/dl) was decreased significantly ($P<0.01$) on day 30 of molybdenum administration from day 0 level. This decreasing trend in the values was present even after one week of cessation of molybdenum treatment. However, the level returned to normal pre-treatment values after two weeks of molybdenum treatment. The plasma albumin (gm/dl) also decreased significantly ($P<0.01$) from day 0 to day 21 of

molybdenum administration and returned to its pre-treatment values within a week's time after cessation of molybdenum treatment. Similarly, the value of plasma globulin (gm/dl) decreased significantly ($P<0.05$) on day 21 of dosing. These values returned to pre-treatment value within two weeks of post treatment. The Albumin/Globulin ratio after an initial non significant decline increased significantly ($P<0.01$) from the 0 day values to 21 days of dosing. The values remained elevated even after last day of administration. The plasma calcium (mg/dl), magnesium (mg/dl), phosphorous (mg/dl), sodium (milliequiv/lit), and potassium (milliequiv/lit) did not show significant deviation during the entire period of study. In the ameliorative group (group II), blood biochemical analysis revealed non-significant alterations in the levels of mean plasma BUN, creatinine, total plasma proteins, albumin, globulin, albumin/ globulin ratio, calcium, magnesium, phosphorous, sodium and potassium.

Table 1: Effect of repeated oral administration of ammonium molybdate (20mg kg^{-1}b.wt.day^{-1}) alone for 30 consecutive days on biochemical indices in goats (mean ± SE; n=4).

Parameters	Days									
	Treatment days								Post treatment days	
	0	1	3	7	14	21	28	30	7	14
BUN (mg/dl)	7.44± 0.95	8.42± 2.45	10.51± 1.86	13.08± 2.34	13.29± 2.28	13.92[a]± 1.84	14.69[b]±1.54	17.71[b]±1.88	17.17[b]±2.14	13.52±2.29
Creatinine (mg/dl)	0.79± 0.04	0.79± 0.09	0.77± 0.06	0.77± 0.09	0.74± 0.09	0.78± 0.02	0.83[a]±0.02	0.91[a]±0.02	0.46 [b]±0.02	0.44 [b]±0.04
Total Protein (g/dl)	5.78± 0.30	5.95± 0.17	5.96± 0.25	6.7± 0.54	7.17± 0.58	4.01 [b]± 0.34	4.15 [b]±0.15	4.33 [b]±0.23	4.62 [a]±0.22	6.02±0.29
Albumin (g/dl)	3.72± 0.24	3.77± 0.19	3.71± 0.23	3.74± 0.51	4.15± 0.52	2.78 [b]± 0.08	2.84±0.28	3.31±0.09	3.25±0.19	3.43±0.12
Globulin (g/dl)	2.06± 0.11	2.18± 0.11	2.25± 0.36	2.96± 0.80	3.03± 0.38	1.23 [a]± 0.27	1.31±0.39	1.02 [a]±0.31	1.37±0.26	2.59±0.27
A/G ratio	1.81± 0.11	1.75± 0.16	1.80± 0.34	1.80± 0.77	1.43± 0.24	2.52 [b]± 0.43	3.23±1.17	4.39±1.29	2.65±0.53	1.37 ±0.16
Magnesium (mg/dl)	2.65± 0.22	2.9± 0.27	2.675± 0.25	2.4± 0.13	2.375± 0.21	2.225± 0.15	2.15±0.202	2.175±0.125	2.325±0.103	2.675±0.31
Calcium (mg/dl)	11.62± 0.52	11.3± 0.35	12.46± 1.13	13.51± 1.30	11.09± 1.16	10.56± 0.74	10.06±0.34	9.83±0.40	9.27±0.69	10.31±1.12
Phosphorus (mg/dl)	4.61± 0.21	4.24± 0.40	4.62± 0.33	4.65± 0.85	4.81± 0.53	4.96± 0.45	3.85±0.35	3.84±0.29	3.31±0.59	2.65±0.88
Sodium (mEq/L)	146.19±10.12	134.28± 4.79	127.54±7.56	124.05±9.61	116.74± 0.63	115.91±7.31	111.85±9.49	113.97±6.35	118.02±8.06	131.51±4.71
Potassium (mEq/L)	4.48± 0.39	4.33± 0.12	4.64± 0.50	3.34± 0.501	6.22± 0.90	5.025± 0.32	5.18±0.34	4.57±0.31	4.88±0.30	4.71±0.45

Values having superscript [a & b] differ significantly $P < 0.05$, $P < 0.01$as compared to pre-exposure (0 day) value of the same group respectively.

Table 2: Effect of repeated oral administration of ammonium molybdate (20 mg kg^{-1}b.wt.day^{-1}) along with Copper sulphate (7.9 mg kg^{-1}b.wt.day^{-1}) for 30 consecutive days on biochemical parameters in goats (mean ± SE; n=4).

Parameter	Days									
	Treatment days								Post treatment days	
	0	1	3	7	14	21	28	30	7	14
BUN (mg/dl)	15.70±0.35	15.67±1.00	15.14±1.37	14.81±1.06	12.87±1.09	12.89±1.19	14.59±1.32	14.44±1.84	12.71±1.25	13.17±1.23
Creatinine (mg/dl)	0.47±0.03	0.44±0.05	0.45±0.10	0.45±0.08	0.46±0.08	0.47±0.02	0.51±0.03	0.59±0.09	0.44±0.02	0.40±0.02
Total Protein (g/dl)	8.11±0.36	8.07±0.33	8.12±0.12	7.47±0.11	7.63±0.10	7.38±0.34	8.16±0.13	8.30±0.21	8.56±0.32	8.16±0.28
Albumin (g/dl)	4.71±0.31	4.49±0.21	4.40±0.43	4.02±0.20	4.64±0.10	3.83±0.19	4.73±0.09	4.87±0.17	5.02±0.29	4.72±0.17
Globulin (g/dl)	3.40±0.45	3.58±0.29	3.72±0.53	3.45±0.13	2.99±0.20	3.55±0.18	3.43±0.18	3.43±0.07	3.54±0.19	3.44±0.11
A/G ratio	1.52±0.36	1.28±0.13	1.29±0.26	1.18±0.09	1.58±0.14	1.08±0.04	1.39±0.09	1.42±0.044	1.43±0.12	1.37 ± 0.01
Magnesium (mg/dl)	2.63±0.17	2.73±0.25	2.56±0.29	2.33±0.18	2.93±0.26	2.90±0.26	2.80±0.24	2.83±0.26	2.85± 0.34	2.83±0.32
Calcium (mg/dl)	11.30±1.29	11.99±0.73	9.54±0.90	10.13±1.24	10.84±0.46	10.79±0.47	11.15±0.97	10.02±0.43	10.18±1.59	8.92±0.40
Phosphorus (mg/dl)	6.47±0.56	5.88±0.91	6.81±0.78	6.57±0.18	5.31±0.65	5.12±0.45	4.24±0.13	3.84±0.24	3.72±0.35	4.04±0.54
Sodium (mEq/L)	144.92±10.70	163.04± 16.23	157.71±23.90	137.40±11.92	153.26±7.26	158.30±14.62	164.0± 2.31	168.09±15.17	184.54±10.37	135.18±12.47
Potassium (mEq/L)	4.23±0.38	3.54±0.56	4.40±0.43	3.83±0.20	3.69±0.51	5.21±0.22	5.37±0.30	5.11±0.14	5.10±0.27	5.39±0.36

DISCUSSION

The absorbed molybdenum after its repeated oral administration of toxic dose in group I is excreted via urine through kidney and faeces.[11, 13-14] The significantly increased mean plasma BUN level in molybdenotic goats could be attributed to renal damage, muscular damage or an over activity of enzyme xanthine oxidase.[15-16] It is likely that in the present study there has been increased activity of xanthine oxidase leading to increased oxidation of xanthine to uric acid in tissues of goats which in turn come to the blood circulation causing rise in plasma BUN levels. This is also reported by the fact there was not tremendous increase in plasma creatinine values although the mean plasma creatinine level increased significantly from day 28th onwards of Mo administration. However, there was a significant decrease in the level of creatinine within a week of termination of Mo administration. Significant increase in the creatinine levels of molybdenotic goats might be attributed to nephrotic changes caused by Mo toxicity. Kaneko[16] reported that about 75% of the nephrons must be non functional, for the occurrence of increased BUN and creatinine values. Similar increase in levels of plasma BUN and creatinine has been also observed in molybdenotic buffalo calves.[17]

Mean value of total plasma proteins, albumin and globulin decreased significantly from 21 days onwards after dosing however; there was a significant rise on day 21 in A/G ratio followed by a non significant increase during rest of the experimental period of dosing. These findings are in agreement with the reported observations in molybdenotic goats and buffalo calves.[17-18] Plasma proteins are mainly synthesized in the liver and by immune system comprising of reticuloendothelial tissue, lymphoid tissue and plasma cells. The decrease in plasma proteins levels is suggestive of damage to these organs. Further some impairment of renal function might have contributed to the decreased levels of plasma proteins owing to the increased loss of albumin through urine, due to its small size and osmotic sensitivity to fluid movements.[16] The mean plasma value of calcium, magnesium, phosphorous, sodium and potassium manifested non-significant alterations in there levels during treatment and post treatment period. Similar finding was reported in copper deficient adult cows with normal levels of zinc, iron, calcium and phosphorous along with high sulphur content.[3] Similarly, in molybdenotic and copper supplemented buffalo calves the mean plasma phosphorous, calcium, magnesium, zinc, potassium, sulphur, manganese, and sodium did not show any significant alterations.[17] The

animals of group II, in which ammonium molybdate was administered along with copper sulphate (II) pentahydrate manifested non-significant alterations in different parameters on biochemical indices.

CONCLUSION

The results of the study indicates that repeated exposure of Mo causes significant alterations in biochemical parameters and exposure along with the copper sulphate maintains the normal activity of biochemical enzymes. Therefore, it can be concluded that supplementation of copper sulphate reversed the biochemical indices induced by the Mo toxicity in small ruminants.

ACKNOWLEDGMENTS

Authors thanks to the Dean, Faculty of Veterinary Sciences and Animal Husbandry, R S Pura, Jammu for providing necessary facilities for conducting the research.

Conflict of interest: Nil

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