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Clinical manifestations and oxidative profile in sub-chronic oral molybdenum toxicity in buffalo calves*

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Molybdenum toxicity creates critical health risks in exposed animals (McGuirk and Semrad 2005). In Punjab, areas around Sutlej belt (Ludhiana) are rich in molybdenum (Mo), so forages grown on such soils are considered as the most important source of molybdenosis in dairy animals (Radostits *et al.* 2006). An association of molybdenosis with increased oxidative stress had already been reported in cattle (Sharma 2002). Oxidative stress indicates impairment of antioxidant defence mechanism and generalised immune function thus is a point of major concern as it contributes to production and health losses in affected animals (Sordillo and Aitke 2009)**.** Nonetheless**,** any detailed information about the effect of high molybdenum content in diet for a prolonged period on the oxidative stress and antioxidative enzyme status in buffalos is still obscure. Therefore, the present study was planned to evaluate the clinical manifestations associated with sub-chronic oral molybdenum toxicosis and to determine the relationship of such molybdenosis with specific antioxidant status markers of buffalo calves.

Eight male buffalo calves (80–150 kg and 6–12 months age) were randomly divided in to 2 groups of 4 animals each. Group 1 served as control, whereas group 2 animals were treated with sodium molybdate (purity 99% +) @ 3 mg/kg/day, orally for 90 consecutive days. Animals were closely monitored for nature and degree of toxic symptoms. Blood sample were collected in heparinised test-tubes via jugular venepuncture on 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 days from all the calves. Further, various parameters, viz. lipid peroxidation, catalase, superoxide dismutase, glutathione, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase were analysed using previously described standard procedures (Sharma 2002). The data collected were subjected to the statistical analysis using t-test and significance was considered at $P \leq 0.05$. Results were expressed as mean±SE (Snedecor and Cochran 1994).

Sodium molybdate @ 3 mg/kg/day produced varying degree of toxic symptoms characterized by rough hair coat, diarrhoea, dullness and stiffness of joints in all the treated calves. This observed finding, adds validity to the earlier observations of Sharma (2002) and Randhawa *et al.* (2003) in molybdenotic cow calves and buffalo calves, respectively. The high incidence of diarrhoea could be attributed to rapid turnover of intestinal epithelium which makes this tissue especially responsive to Mo induced secondary hypocuprosis. Because diarrhoea is not a major sign in natural primary copper deficiency, it is plausible due to the conditioning factor that reduces the availability of copper (Radostits *et al.* 2006) and may represent a specific toxic effect of molybdenum.

The data pertaining to the effect of long-term exposure to sodium molybdate @ 3 mg/kg/day on oxidative profile of buffalo calves are presented in Table 1. In the current investigation, sodium molybdate administration produced a nonsignificant change in the extent of lipid peroxidation and is in accordance with the finding of Sharma (2002) in cow calves. The catalase activity remained steady up to 70th day, however, thereafter a significant increase in catalase activity (Table 1) was seen. Further, compared with control group superoxide dismutase (SOD) activity showed variable results as the value declined ($P<0.05$) on 40th day while a marked increase ($P<0.01$) was recorded at the end of treatment period. In support to the findings of Sharma (2002) and Ahmed *et al.* (2009) in cow calves and buffalos, respectively, the decline observed could be attributed to secondary copper deficiency, which results in decline of SOD activity in several tissues

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Table 1. Effect of the subchronic oral toxicity of sodium molybdate (3 mg/kg/day) on lipid peroxidation and antioxidant enzymes in buffalo calves

	Time (days)									
	0	10	20	30	40	50	60	70	80	90
	Lipid peroxidation (nmol malonyldialdehyde/g Hb/hr)									
Control	6.67±1.44	5.57±1.48	8.97±2.93	14.08±2.38	12.95±0.55	12.11±0.58	8.43±0.41	9.73±0.45	11.66±0.61	10.19±1.18
Treatment	4.07±1.57	9.87±2.09	8.80±1.94	12.51±0.73	10.74±1.79	9.74±0.63	8.38±0.61	8.60±0.85	9.31±1.71	8.30±0.86
	Catalase (mmol H_2O_2 decomposed/min/mg Hb)									
Control	1369 ±30.87	1567 ±35.37	1438 ±32.73	890 ±14.00	1231 ±82.25	1230 ±106.37	1202 ±65.50	1056 ±55.25	1131 ±32.25[a]	1383 ±16.75[a]
Treatment	1327 ±15.62	1523 ±21.87	1398 ±30.25	875 ±29.25	1398 ±13.50	1390 ±27.75	1060 ±49.00	1371 ±114.6	1365 ±59.37[b]	1127 ±111.3[b]
	Superoxide dismutase(EU/mg Hb)									
Control	2.37±0.15	2.61±0.31	3.47±0.04	2.56±0.19	6.82±0.23[a]	8.26±0.40	5.02±0.37	1.51±0.12	3.16±0.12	4.08±0.45[a]
Treatment	2.65±0.11	2.52±0.20	3.28±0.22	2.85±0.41	6.49±0.42[b]	7.09±0.50	5.55±0.83	1.97±0.16	2.97±0.16	6.13±0.35[b]
	Glutathione(mg/ml)									
Control	597.3 ±46.08	648.5 ±14.75	765.0 ±37.78	219.8 ±35.96	205.5 ±26.03	88.4 ±16.58	251.6 ±14.97	308.7 ±14.53[a]	219.7 ±11.98[a]	232.6 ±18.40[a]
Treatment	539.6 ±17.96	552.1 ±63.15	779.8 ±67.77	294.0 ±18.88	155.7 ±8.76	84.7 ±21.18	223.0 ±19.36	217.4 ±16.58[b]	133.6 ±13.36[b]	137.2 ±9.88[b]
	Glutathione peroxidase (EU/mg Hb)									
Control	2.83±0.14	1.60±0.08	3.92±0.11	3.53±0.18	3.64±0.17	2.56±0.18	3.29±0.27	3.25±0.12	3.20±0.11	3.18±0.08[a]
Treatment	2.88±0.23	2.11±0.25	3.69±0.16	3.52±0.15	3.59±0.08	3.25±0.20	3.61±0.24	3.26±0.17	3.32±0.14	3.47±0.05[b]
	Glutathione reductase (mmoles of NADPH oxidised/min/mg Hb)									
Control	0.77±0.04	1.10±0.05	0.83±0.06	0.63±0.07	0.79±0.05	1.16±0.09	1.10±0.05	1.15±0.03[a]	0.70±0.05	0.91±0.11
Treatment	0.54±0.08	1.04±0.13	0.86±0.16	0.63±0.15	0.98±0.14	1.13±0.06	1.10±0.06	0.95±0.03[b]	0.69±0.05	1.06±0.04
	Glutathione-S-transferase (mmoles of GSH-CDNB conjugate formed/min/mg Hb)									
Control	804 ±27.37	914 ±156.2	925 ±32.25	1250 ±449.2	1005 ±64.00	627 ±75.87	621 ±36.25	812 ±42.87	1010 ±190.3	687 ±43.00
Treatment	888±59.62	808±93.75	830±96.62	482±57.62	1279±228.1	535±16.50	574±30.25	773±160.1	1324±128.0	746±84.87

Values (mean±S E), [a,b]within a column with different superscripts alphabets differ significantly ($P < 0.05$).

and erythrocytes, thus indicating an increase in the occurrence of oxidative damage to cellular components (Sulalski *et al.* 1997). In line with the earlier studies (Sharma 2002) sodium molybdate exerted a marked depression ($P<0.01$) in the blood glutathione (GSH) concentration on the 80th day of treatment period in treated buffalo calves (Table 1) as compared to control cows. A tendency for increase in catalase, glutathione peroxidises (GP) and SOD activity at the end of experiment might be a mere reflection of body's adaptability behaviour towards chronic Mo administration (Majak *et al.* 2006).

Glutathione reductase (GR) on 70th day after start of Mo administration was lower ($P<0.05$) than control group and agrees with work on molybdenotic cow calves (Sharma 2002) and hypocuprosis buffalos (Ahmed *et al.* 2009). However, no effect of administering sodium molybdate was recorded on glutathione-S-transferase which is evidenced by its similar concentration in both the groups and contrary to the observation in crossbred cow calves (Sharma 2002). It is well documented that glutathione redox cycle consists of GSH, GR and GP, posses a major role in antioxidant defence mechanism (Denisov and Afan 2005). Investigating the oxidative status in molybdenotic buffalo calves herein indicated that sub-chronic oral molybdenosis induced alterations in glutathione redox cycle. Consequently, these effects may impair the cell immune functionality, affecting the bactericidal capacity and rendering the animals more susceptible to infection (Sordillo and Aitke 2009). Hence, in order to improve the overall production and health performance in buffalo species, long term exposure to excess dietary molybdenum should be avoided.

SUMMARY

Repeated oral administration of sodium molybdate @ 3 mg/kg/day for 90 consecutive days produced characteristic clinical signs of sub-chronic molybdenois, i.e. rough hair coat, diarrhoea, dullness and stiffness of joints in buffalo calves. Although, sodium molybdate did not cause significant alterations in the extent of lipid peroxidation and concentration of glutathione-S-transferase, blood glutathione levels were depleted. Similarly, significant decline in the values of superoxide dismutase and glutathione reductase was observed. In conclusion, sub chronic molybdenosis produced varying degree of toxic signs and exerted oxidative stress in buffalo calves.

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