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Haematological profile of subacute oral toxicity of fluoride and ameliorative efficacy of aluminium sulphate in goats

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Abstract

High affinity of the fluoride to the bone causes intoxication of bone marrow resulting into alterations in normal hematological profile. In present study sodium fluoride alone and with aluminium sulphate (ameliorative agent) was administered orally daily for 30 days in healthy goats of group 1 and 2 respectively to access the effect on the hematological profile on different days of exposure. Exposure of sodium fluoride alone produced significant reduction in hemoglobin (Hb), packed cell volume (PCV), total leucocytes count (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and increase in blood clotting time. However, values of total erythrocyte count (TEC) and differential leucocytes count (DLC) were not significantly changed. On comparing the observations of ameliorative group with the group 1 goats, it is concluded that the use of the ameliorative salt Al2(SO4)3 has beneficial effects on the alterations in values of Hb, PCV , TLC, MCV, MCH and MCHC except blood clotting time produced by the subacute exposure of sodium fluoride in goats.

Keywords: Aluminium sulphate, Goats, Haematological profile, Sodium fluoride.

Introduction

Fluorine is the most reactive nonmetal and most electronegative element of the periodic table. Due to its high reactivity and electronegativity, it combines directly or indirectly with other elements, except oxygen and nitrogen under ordinary temperature and pressure (Banks and Goldwhite, 1966), to form complexes called fluorides. It almost never occurs in its elemental state in nature. Fluorine is considered ubiquitous in food, air and water and as a trace element it plays an important role in imparting strength to teeth and bone. High concentration of fluoride in environment affects the health of humans and animals as well as plant life (Yur *et al.,* 2003); therefore, it is of important public health concern all over the globe. In India, toxicity of fluoride in man and animals is very common and at least 20 states, including Jammu and Kashmir (J&K), are considered endemic to fluoride intoxication. Water is the source of fluoride in the endemic areas of J&K and animals are as severely affected as humans are. In humans, both forms dental and skeletal fluorosis has been reported (Majeed, 2007). Bone marrow is one of the tissues which are vulnerable to its intoxication (Blood and Radostits, 1989). Therefore, it alters the normal hematological profile, which can be rectified and/ or prevented by using ameliorative agent (Marconi, 2000). Literatures regarding fluoride toxicity mediated hematological alterations are available in sheep (Mohiuddin and Reddy, 1989), buffaloes (Swarup and Singh, 1989) and cows (Singh and Swarup, 1994), but there is lack of such study in goats. Keeping this in mind, the present study has been undertaken to investigate the alterations in hematological parameters during subacute oral toxicity of fluoride.

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Materials and Methods

Eight healthy crossbred goats of age 1.5-2 years of 25-30 kg body weight were procured from local farmers of R.S. Pura, Jammu. They were acclimatized for two weeks in the divisional animal shed under standard conditions before the

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commencement of experiment. The animals were maintained on *ad lib* feed and water. The experimental protocol was approved by institutional ethics committee. The experimental goats were divided into two groups of 4 each. Goats of Group 1 were used to study the changes in haematological parameters during subacute oral toxicity of fluoride, in which sodium fluoride (NaF, SD Fine- Chem. Ltd.) alone was administered orally @ 20 mg/kg b.wt. (providing 9 mg/kg b.wt. fluorine) daily for 30 days and goats of Group 2 were used to study the efficacy of aluminium sulphate on the changes produced in haematological parameters by subacute oral toxicity of fluoride, in which same dose of NaF along with aluminium sulphate [Al2(SO4)3.16H2O, SD Fine- Chem. Ltd.] @ 150 mg/kg b.wt. was administered orally daily for 30 days. Al2(SO4)3 was provided 30 minutes before the administration of NaF. Different salts were dissolved separately in 100 ml of distilled water and drenched to the animals daily between 9.00 to 10.00 a.m. All the animals were weighed weekly and doses of NaF and $Al_2(SO_4)_3$ were corrected according to the changes in body weight. All animals were closely observed for clinical signs and mortality.

Blood samples were collected in plastic vials containing dipotassium salt of EDTA (Himedia Mumbai) @ 2 mg/ml of blood on day 0, 1, 3, 7, 14, 21, 28, 30 of treatment and on day 3, 7, 14 after termination of treatment by jugular vein puncture for haematological parameters. Haemoglobin concentration (Hb) was determined by Sahli's hemometer method and packed cell volume (PCV) was determined by microhaematocrite method. Blood clotting time was determined by capillary method. Total leucocyte count (TLC), total erythrocyte count (TEC), differential leucocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined by using the standard reference methods of Benzamin (1985).

Different hematological parameters on different days of exposure of fluoride were compared to pre-exposure (0 day) value of the same group and a probability level of $P<0.05$ was considered statistically significant (Senedecor and Cochran, 1989).

Results and Discussion

Repeated administration of sodium fluoride alone daily for 30 days produced varying degree of toxic symptoms characterized by inappetance, lethargy, weight loss, decreased ruminal motility, decreased milk production, hemorrhagic diarrhoea, muscle weakness and decreased movement. The toxic symptoms were not seen in ameliorative (group 2) animals. The details of the haematological profile of Group 1 and Group 2 are presented in Table 1 and 2 respectively. Repeated oral administration of sodium fluoride alone produced significant ($P<0.05$) decrease in mean value of Hb (g/dl) from day 14 (10.60 ± 0.47) of exposure till day 30 of exposure from pre exposure values (11.85 ± 0.13). The mean value of PCV (%) also showed significant ($P<0.01$) decrease on day 14 (30.00 ± 0.82) of exposure from pre exposure values (35.25 ± 1.03). The mean blood clotting time (min) increased significantly ($P<0.01$) from 2.38 ± 0.24 on different days of exposure. However, the mean value of TLC ($x10^3/mm^3$) decreased significantly on day 7 (7.4 ± 0.28) after termination of exposure from pre exposure (9.04 ± 0.30) value, but the mean value of TEC ($x10^6$/mm3) and different leucocytes on different days of treatment showed no significant deviation from their pre treatment values.

The mean value of MCV (fl) was decreased significantly from 33.84 ± 1.36 on day 0 to 29.19 ± 0.64 on day 7 ($P<0.05$) and 28.25 ± 0.52 on day 14 ($P<0.01$) of exposure. The mean value of MCH (pg) also decreased significantly ($P < 0.05$) on day 14 (9.38 ± 0.36) following termination of exposure from day 0 (11.39 ± 0.51) value. A significant ($P<0.05$) reduction in MCHC (%) value was observed on day 28 (28.28 ± 1.56) of exposure and day 7 (31.52 ± 0.40) after termination of exposure.

In the ameliorative group, decreased value of Hb during first week of treatment and later on significantly ($P<0.05$) increased value on day 21 (12.65 ± 0.30) of exposure from its day 0 value was observed. The mean value of PCV showed significant ($P<0.01$) increase on day 21 (39.75 ± 0.85) when compared to day 0 value. The mean time of blood clotting increased significantly

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Table 1. Effect of repeated oral administration of NaF (20 mg.kg^{-1} b.wt.) alone daily for 30 days on hematological parameters. (mean±SE) (n=4).

Parameter	Days										
	Treatment days								Post Treatment days		
	0	1	3	7	14	21	28	30	3	7	14
Hb(gm/dl)	11.85 ±0.13	11.75 ±0.17	11.05 ±0.38	10.90 ±0.58	10.60[a] ±0.47	10.75[a] ±0.40	10.75[a] ±0.41	11.05[a] ±0.22	11.65 ±0.35	11.40 ±0.29	10.75 ±0.17
PCV(%)	35.25 ±1.03	35.75 ±2.10	34.75 ±1.89	31.25 ±1.25	30.00[b] ±0.82	34.00 ±2.42	38.25 ±1.93	35.75 ±1.49	37.25 ±1.97	36.25 ±1.25	37.50 ±1.55
Clotting time (minutes)	2.38 ±0.24	2.88 ±0.51	4.48[b] ±0.35	3.33 ±0.99	4.30[a] ±0.55	3.68[b] ±0.22	6.75[b] ±0.88	9.73[b] ±1.21	4.83[b] ±0.26	4.80[b] ±0.60	7.88 ±1.38
TLC ($\times10^3$/mm^3)	9.04 ±0.30	8.61 ±0.37	8.68 ±0.40	9.00 ±0.39	8.72 ±0.28	8.06 ±0.20	8.17 ±0.21	8.10 ±0.24	8.13 ±0.41	7.48[b] ±0.28	8.15 ±0.70
TEC ($\times10^6$/mm^3)	10.47 ±0.53	11.33 ±0.41	11.18 ±0.35	10.70 ±0.21	10.63 ±0.29	10.32 ±0.47	11.79 ±0.69	10.26 ±0.47	11.69 ±0.45	10.94 ±0.43	11.21 ±0.25
Lymphocytes	56.75 ±1.11	58.00 ±0.82	56.75 ±1.70	59.00 ±0.71	59.25 ±0.75	59.00 ±1.87	57.00 ±1.29	56.75 ±1.70	55.50 ±1.32	57.00 ±1.29	56.25 ±1.31
Neutrophils	29.25 ±1.11	29.25 ±0.75	30.50 ±1.85	28.50 ±1.44	29.75 ±0.25	27.75 ±1.44	28.50 ±1.32	30.50 ±1.44	31.25 ±1.75	29.00 ±1.47	31.00 ±1.00
Monocytes	8.50 ±0.65	8.50 ±0.65	8.00 ±0.41	7.00 ±0.41	7.25 ±0.48	9.00 ±1.08	9.25 ±0.48	8.00 ±0.91	7.50 ±0.65	9.00 ±0.91	7.25 ±1.03
Eosinophils	2.00 ±0.41	1.50 ±0.29	1.50 ±0.65	1.50 ±0.65	1.00 ±0.41	1.25 ±0.48	1.75 ±0.25	2.00 ±0.41	2.00 ±0.41	1.75 ±0.25	2.25 ±0.48
Basophils	3.50 ±0.65	2.75 ±0.25	3.25 ±0.48	3.50 ±0.65	2.75 ±0.48	3.00 ±0.41	3.50 ±0.65	2.75 ±0.63	3.75 ±0.48	3.25 ±0.75	3.25 ±0.85
MCV (fl)	33.84 ±1.36	31.47 ±0.83	31.31 ±2.52	29.19[a] ±0.64	28.25[b] ±0.52	32.89 ±1.47	32.51 ±0.58	33.33 ±0.66	32.02 ±0.58	33.80 ±0.25	32.65 ±1.24
MCH (pg)	11.39 ±0.51	10.41 ±0.44	9.91 ±0.45	10.19 ±0.49	10.00 ±0.50	10.45 ±0.40	9.22 ±0.65	10.81 ±0.56	10.00 ±0.20	10.65 ±0.06	9.38[a] ±0.36
MCHC (%)	33.67 ±0.62	33.24 ±2.12	31.98 ±1.42	35.00 ±2.06	35.33 ±1.24	31.91 ±1.46	28.28[a] ±1.56	32.40 ±1.31	31.27 ±1.18	31.52[a] ±0.40	28.87 ±2.00

[a, b] Significantly different as compared to pre-exposure (0 day) value of the same group at 5% ($P<0.05$) and 1% ($P<0.01$) respectively.

($P<0.01$) from 2.40±0.34 on day 0 to 7.78±1.19 on day 14 of exposure and remained significantly increased till day 14 (4.37±0.64) after termination of exposure. The mean values of TLC and TEC (x 10^3/mm^3) showed no significant deviation during the study. The mean values of the different leucocytes showed no much significant alterations from their pre exposure values. Although a reduction in number of lymphocytes and monocytes was observed on 7th day of exposure. However, the mean value of MCH was significantly ($P<0.05$) increased only on day 1 (11.11±0.31) of exposure, but the mean values of MCV and MCHC showed no significant deviation from pre exposure values.

The reduction in Hb after repeated administration of fluoride may be an indication that it would lead to anaemia as there could be suppression of various haemopoietic system of the body in goats. Similar findings were reported in sheep (Mohiuddin and Reddy, 1989), buffalo (Swarup and Singh, 1989) and cows (Singh and Swarup, 1994). Similarly decrease in PCV was observed by Donald *et al.* (1979), Swarup and Singh (1989) and Gujrathi *et al.* (1991) during fluorosis in cattle and buffaloes. The increase in the clotting time may be due to the fact that NaF is an anticoagulant and also chelates calcium ions from the blood, which is pre-requisite for blood clotting. Similar findings have been reported in buffalo calves (Marconi, 2000). Similar findings of

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Table2. Effect of repeated oral administration of NaF (20 mg.kg^{-1} b.wt.) along with $Al_2(SO_4)_3$ (150 mg.kg^{-1} b.wt.) daily for 30 days on hematological parameters. (mean±SE) (n=4)..

Parameter	Days										
	Treatment days								Post Treatment days		
	0	1	3	7	14	21	28	30	3	7	14
Hb(gm/dl)	11.10 ±0.39	10.50 ±0.53	10.70 ±0.37	10.90 ±0.40	11.70 ±0.17	12.65[a] ±0.30	11.80 ±0.32	11.73* ±0.52	11.53* ±0.48	11.47* ±0.55	11.53* ±0.37
PCV(%)	34.25 ±1.75	28.50 ±2.06	29.75 ±1.44	35.75 ±2.66	36.75 ±0.75	39.75[b] ±0.85	35.75 ±1.31	34.67* ±1.45	35.00* ±1.00	35.67* ±2.03	35.33* ±0.88
Clotting time (minutes)	2.40 ±0.34	2.68 ±0.54	2.98 ±0.47	2.98 ±0.52	7.78[b] ±1.19	8.15[b] ±1.04	9.13[b] ±0.73	9.80*[b] ±0.12	6.40*[b] ±1.13	3.77*[a] ±0.32	4.37*[a] ±0.64
TLC ($\times10^3$/mm^3)	8.94 ±0.31	8.96 ±0.49	8.85 ±0.35	8.76 ±0.23	9.24 ±0.38	9.18 ±0.43	9.55 ±0.43	9.77* ±0.32	9.74* ±0.08	9.85* ±0.52	9.56* ±0.48
TEC ($\times10^6$/mm^3)	11.26 ±0.66	9.49 ±0.68	10.66 ±0.22	11.60 ±0.39	11.73 ±0.27	12.61 ±0.18	11.37 ±0.37	11.48* ±0.41	11.53* ±0.44	11.85* ±0.69	11.89* ±0.57
Lymphocytes	55.25 ±1.11	52.50 ±1.71	52.75 ±0.48	50.75[a] ±1.11	53.75 ±2.17	54.75 ±1.38	56.00 ±2.16	54.67* ±0.88	53.67* ±1.76	51.00* ±2.08	52.33* ±1.20
Neutrophils	31.25 ±1.11	31.25 ±1.49	32.67 ±0.58	33.25 ±1.11	32.00 ±1.47	31.25 ±1.49	29.50 ±1.94	30.33* ±0.33	33.00* ±0.58	33.67* ±1.86	33.67* ±0.67
Monocytes	8.50 ±0.65	10.25 ±0.85	10.00 ±0.41	10.50[a] ±0.29	8.25 ±0.85	9.25 ±0.48	10.00 ±1.08	8.67* ±0.33	8.33* ±0.88	8.33* ±0.33	8.67* ±0.33
Eosinophils	1.75 ±0.48	2.25 ±0.25	1.75 ±0.25	2.00 ±0.41	2.00 ±0.41	2.00 ±0.41	1.75 ±0.25	2.33* ±0.33	2.33* ±0.33	2.67* ±0.67	2.00* ±0.58
Basophils	3.25 ±0.48	3.75 ±0.48	3.50 ±0.50	3.50 ±0.50	4.00 ±0.41	2.75 ±0.48	2.75 ±0.48	4.00* ±0.58	2.67* ±0.67	4.33* ±0.33	3.33* ±0.33
MCV (fl)	30.62 ±1.76	30.04 ±0.73	27.87 ±0.86	30.74 ±1.69	31.34 ±0.37	31.53 ±0.52	31.48 ±1.12	30.18* ±0.20	30.38* ±0.36	30.11* ±0.28	29.84* ±1.47
MCH (pg)	9.90 ±0.24	11.11[a] ±0.31	10.03 ±0.16	9.40 ±0.25	9.99 ±0.30	10.03 ±0.16	10.38 ±0.07	10.21* ±0.09	10.00* ±0.04	9.69* ±0.11	9.72* ±0.17
MCHC (%)	32.59 ±1.57	37.01 ±0.81	36.04 ±0.58	30.81 ±1.49	31.89 ±1.02	31.87 ±1.03	33.10 ±1.09	33.84* ±0.11	32.93* ±0.52	32.19* ±0.46	32.67* ±1.12

[a, b] Significantly different as compared to pre-exposure (0 day) value of the same group at 5% ($P<0.05$) and 1% ($P<0.01$) respectively.

decreased value of TLC were observed by Behera (1993) in buffaloes and Swarup and Singh (1989) in cows. In contrast, an increase in TLC was reported by Gujarathi *et al.* (1991). The finding of TEC is in agreement with work of Mohiuddin and Reddy (1989) in sheep. However, decreased TEC was reported in cattle (Behera, 1993) and increased TEC was reported in buffalo calves (Gujarathi *et al.,* 1991). No significant deviation in DLC was reported by Mohiuddin and Reddy (1989) in sheep. In contrast, increase in eosinophil level in fluorotic cattle (Hoogstratten *et al.,* 1965; Hillman *et al.*, 1979; Jagadish *et al.,* 1998), slight decrease in neutrophils in sheep (Tiwary *et al.,* 1978) and increase in neutrophils in buffalo calves (Gujarathi *et al.,* 1991) was observed. Similar decrease in MCV was observed by Donald *et al.* (1979).

On comparing the observations of ameliorative group with the observations of group 1 goats, it is concluded that the use of the ameliorative salt $Al_2(SO_4)_3$ has beneficial effects on the alterations in values of Hb, PCV , TLC, MCV, MCH and MCHC except blood clotting time produced by the subacute exposure of sodium fluoride in goats.

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