HAEMATOLOGICAL ALTERATIONS INDUCED BY SUBCHRONIC ORAL EXPOSURE OF BUFFALO CALVES TO FIPRONIL AND FLUORIDE

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SUMMARY: A study is reported on haematological alterations in buffalo calves from subchronic oral exposure to the pesticide fipronil, sodium fluoride alone, and the two in combination. Twenty-four male buffalo calves, 6 to 12 months old, were divided into 4 groups of 6 animals each. Group I without treatment served as the control. Groups II and III were orally administered commercial fipronil at 0.5 mg/kg bw/day and sodium fluoride (NaF) at 6.67 mg/kg bw/day, respectively, for 98 days. An additional group IV was co-administered fipronil and NaF at the same dosages as groups II and III. Although blood haemoglobin and total erythrocyte count were not appreciably altered by fipronil alone in group II, they were lowered by NaF in group III and in the combined group IV. On the other hand, total leukocyte count increased in the NaF group III and in the combined group IV but not in the fipronil alone group II. The present study indicates that fipronil aggravates the toxic effects of NaF on the hematopoietic system as haematological alterations were more pronounced with concomitant exposure to fipronil and NaF.

Keywords: Buffalo calves; Fipronil; Haematological alterations; Sodium fluoride and fipronil.

INTRODUCTION

Fipronil, a member of a relatively new class of phenylpyrazole pesticides, is a highly effective, broad-spectrum insecticide used for the control of a wide range of agricultural, public health, and veterinary pests.[1] Haematological alterations have been reported from fipronil in experimental studies conducted in rats and beagle dogs.[2]

Endemic skeletal fluorosis is widely prevalent in India and is a major public health problem. Chronic toxicity of fluoride (F) is caused by prolonged ingestion of F in food and water. About 40–70% of the districts in Punjab, India are affected in some degree by skeletal and dental fluorosis.[3] Many studies on toxic effects of F on blood and blood constituents of different animal species have also been reported.[4-9]

It is now well recognized that human beings and animals are exposed concurrently to more than one chemical in the environment from various sources. The potential hazard due to pesticide residues on the health of livestock, in particular, and interaction of pesticides with the toxic constituents in water and soil is a growing concern. In a recent report, co-exposure of fluoride and deltamethrin was shown to produce more severe oxidative stress in rats in comparison to their individual effects, indicating interaction between the two toxicants in promoting

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free radical damage.[10] However, interactive effects of pesticides and heavy metals and F have had limited attention.[11-18]

Buffaloes, which contribute to the bulk of milk production in several South Asian countries, are an important dairy animal of India. The quality of buffalo milk and its contamination with various toxicants has considerable bearing on human health. The present research was designed to elucidate interactive effects of fipronil and F on haematological parameters in buffalo calves.

MATERIALS AND METHODS

The study was performed on twenty-four healthy male buffalo calves 6–12 months old weighing between 80 and 120 kg, procured from the University dairy farm. The animals were maintained under standard experimental conditions with *ad libitum* access to feed and water. All animals were subjected to regular clinical examination and treated with anthelmintics before the start of the study. The experimental protocol followed the ethical guidelines on the proper care and use of animals and was approved by the Institutional Animal Ethics Committee.

Fipronil (Reagent^{R}, 5% SC) insecticide was purchased from Bayer Crop Science Ltd. Mumbai. Sodium fluoride (NaF) was obtained from Sisco Research Laboratories Pvt Ltd. All other chemicals/reagents used in this study were of analytical grade, procured from reputable companies. The buffalo calves were divided into four groups. Group I receiving no treatment served as control. Group II animals were orally administered fipronil at 0.5 mg/kg bw/day, selected on the basis of recommended concentrations used for crop protection. Similarly, NaF at 6.67 mg/kg bw/day (providing 3 mg/kg bw/day fluoride) was administered to group III animals. The dose of fluoride was selected on the basis of similar doses used by previous workers in published reports on chronic F toxicity in cattle.[19,20] Group IV animals received both fipronil and NaF at the same dosages as groups II and III, respectively. The requisite amount of commercial fipronil and NaF was suspended in 50 mL of water and administered orally by a drenching tube (like a syringe) daily for 98 consecutive days. In order to minimize possible instability, the aqueous suspensions of both toxicants were prepared fresh each day. All the calves were weighed weekly to correct the dosages according to body weight.

Blood samples were collected in EDTA vials from the jugular vein of the animals at weeks 0, 2, 4, 6, 8, 10, 12, and 14 of the experiment and at two weeks post-treatment. Haematological parameters including haemoglobin concentration (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and were determined by using standard methods.[21]

Statistical analysis: Statistical analysis of the data was done by one-way ANOVA using SPSS® 16.0 software package. Significance was assessed at $p<0.05$.[22] Tukey's test was applied for multiple comparisons.

RESULTS

Changes in Hb: Fipronil exposure in the group II calves did not produce any significant alteration in the blood Hb levels. However, exposure to NaF alone and in combination with fipronil in groups III and IV, respectively, resulted in a significant ($p<0.05$) decline in Hb levels (Figure 1) by week 10 and thereafter. The 28.1% Hb decrease in group III (NaF treatment) increased to 30.9% in group IV (co-exposed to fipronil and NaF). By week two of post treatment, the Hb levels in the treated groups did not differ significantly ($p<0.05$) from the control group.

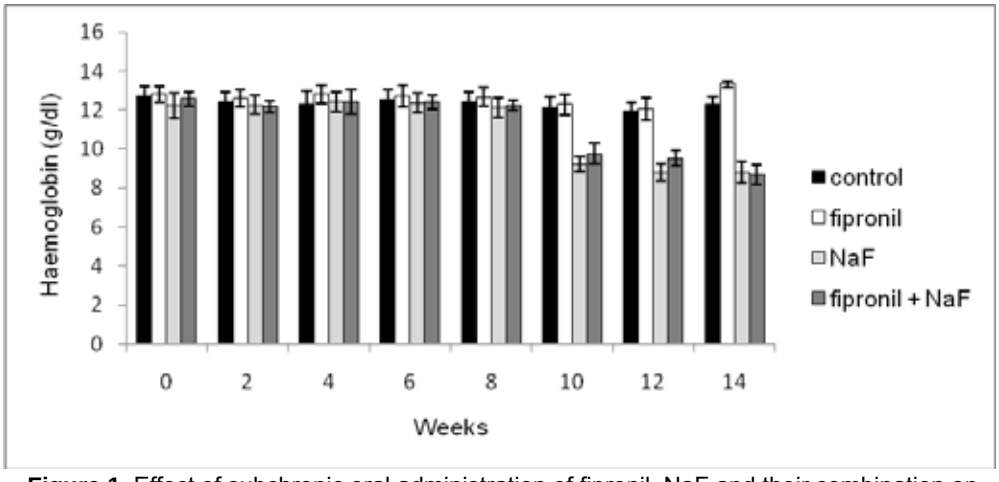

Figure 1. Effect of subchronic oral administration of fipronil, NaF and their combination on blood haemoglobin (Hb) in buffalo calves.

Changes in PCV and ESR: As shown in Table 1, no significant alterations were observed in PCV and ESR in any of the three toxicant-exposed groups.

Changes in erythrocyte indices: Exposure to either of the toxicants failed to produce any significant change in any of the three erythrocyte indices, viz., MCV, MCH, and MCHC (Table 2).

As seen in Figure 2, a 54.3% elevation in TLC was observed in the group IV calves co-exposed to both the toxicants compared to 47.6% in group III. A significant ($p<0.05$) 28% and 41% decline in TEC was observed in groups III and IV, respectively (Figure 3). Changes in TLC and TEC were significant only after the 10th week of treatment. However, exposure to fipronil alone failed to produce any significant alteration in TLC and TEC. By week 2 in the post- treatment period, these two parameters did not differ significantly ($p<0.05$) from those in the control group.

Table 1. Effect of repeated oral administration of fipronil, NaF and their combination on PCV and ESR in buffalo calves (n = 6). (Values are mean ± SEM.)[a]

Group	Treatment (weeks)								
	Treatment								Post treatment
	0	2	4	6	8	10	12	14	2
	Packed cell volume (%)								
I	36.9 ± 1.32[a]	36.4 ± 1.75[a]	34.9 ± 1.65[a]	33.2 ± 2.11[ab]	34.7 ± 1.30[ab]	36.5 ± 2.18[a]	32.8 ± 1.86[a]	33.2 ± 2.28[a]	32.9 ± 1.53[a]
II	44.1 ± 3.26[ab]	43.2 ± 3.36[a]	36.8 ± 2.71[a]	31.3 ± 2.79[a]	29.65 ± 2.84[a]	31.5 ± 3.74[a]	31.6 ± 1.31[a]	29.0 ± 3.32[a]	31.5 ± 2.88[a]
III	44.1 ± 2.38[ab]	40.0 ± 2.26[a]	38.4 ± 1.56[a]	37.3 ± 1.35[ab]	35.7 ± 2.10[ab]	36.3 ± 1.31[a]	33.6 ± 2.10[a]	31.0 ± 2.06[a]	32.9 ± 1.48[a]
IV	42.1 ± 1.34[ab]	43.1 ± 0.94[a]	39.1 ± 1.28[a]	38.0 ± 1.08[b]	36.2 ± 1.34[b]	32.0 ± 2.10[a]	31.5 ± 2.06[a]	32.1 ± 1.34[a]	30.8 ± 0.93[a]
	Erythrocyte sedimentation rate (mm/24hr)								
I	106.7 ± 3.78[a]	106.2 ± 5.14[a]	98.6 ± 3.16[a]	93.6 ± 4.31[a]	98.4 ± 4.84[a]	105.6 ± 4.50[a]	97.8 ± 3.67[a]	124.0 ± 7.49[a]	116.0 ± 8.37[a]
II	105.0 ± 7.11[a]	107.0 ± 7.98[a]	94.6 ± 7.22[a]	92.6 ± 4.26[a]	96.0 ± 7.04[a]	109.2 ± 6.32[a]	101.5 ± 5.42[a]	128.7 ± 6.84[a]	120.3 ± 4.84[a]
III	108.7 ± 5.26[a]	102.8 ± 4.45[a]	100.5 ± 2.57[a]	92.6 ± 2.89[a]	96.8 ± 5.63[a]	102.2 ± 8.35[a]	98.0 ± 6.44[a]	125.4 ± 8.19[a]	117.8 ± 8.78[a]
IV	103.7 ± 4.25[a]	107.2 ± 4.74[a]	95.8 ± 3.83[a]	94.7 ± 4.67[a]	96.8 ± 6.06[a]	102.2 ± 6.54[a]	98.2 ± 4.21[a]	120.0 ± 8.45[a]	113.3 ± 7.20[a]

[a]Means with at least one common superscript do not differ significantly ($p<0.05$).

I = control, II = fipronil, III = NaF, IV = fipronil and NaF combined

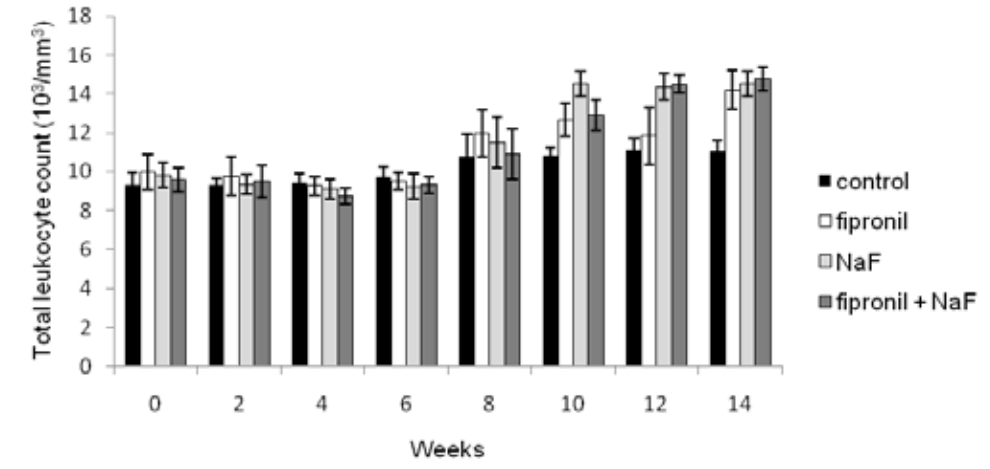

Figure 2. Effect of subchronic oral administration of fipronil, NaF and their combination on total leukocyte count in buffalo calves.

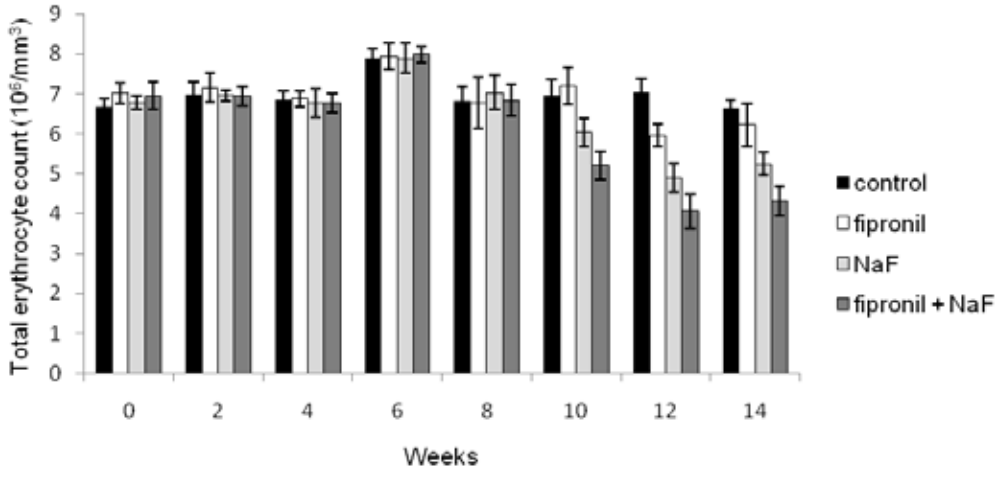

Figure 3. Effect of sub chronic oral administration of fipronil, fluoride and their combination on total erythrocyte count in buffalo calves.

DISCUSSION

The circulatory system plays an important role in maintaining homeostasis in animals. The cellular constituents, plasma protein, and chemical composition of blood have vital roles in the different metabolic activities. Any toxicant gaining access into the body that is not eliminated by the liver is distributed throughout the body by the circulatory system. When the concentration of a toxicant increases sufficiently, it will cause qualitative and quantitative abnormalities in the exposed animals. Various changes in blood parameters have been reported in F-exposed mice[23] and camels.[24] However, WBC, MCV, MCH, and MCHC were not affected by F toxicosis in rats.[25]

Table 2. Effect of repeated oral administration of fipronil, NaF and their combination on erythrocyte indices in buffalo calves (n = 6). (Values are mean ± SEM.)[a]

Group	Time (weeks)								
	Treatment								Post treatment
	0	2	4	6	8	10	12	14	2
	Mean corpuscular haemoglobin (pg/dL)								
I	19.3 ± 1.18[a]	17.9 ± 1.03[a]	18.1 ± 1.20[a]	15.9 ± 0.65[a]	18.5 ± 1.18[a]	17.6 ± 1.18[a]	17.2 ± 1.18[a]	18.6 ± 1.09[a]	19.0 ± 1.21[a]
II	18.3 ± 0.90[a]	17.9 ± 1.36[a]	18.6 ± 0.47[a]	16.2 ± 1.26[a]	19.2 ± 1.95[a]	17.2 ± 1.25[a]	20.3 ± 1.02[a]	21.7 ± 1.70[a]	19.4 ± 0.72[a]
III	18.9 ± 1.29[a]	17.7 ± 0.83[a]	18.8 ± 1.66[a]	15.7 ± 0.73[a]	17.6 ± 1.70[a]	15.6 ± 1.37[a]	18.5 ± 2.00[a]	16.0 ± 2.38[a]	24.6 ± 0.90[a]
IV	18.2 ± 0.83[a]	17.6 ± 0.60[a]	18.5 ± 1.20[a]	15.6 ± 0.54[a]	18.2 ± 1.24[a]	19.2 ± 1.90[a]	23.2 ± 2.02[ab]	20.2 ± 1.13[a]	24.1 ± 1.62[a]
	Mean corpuscular haemoglobin concentration (g/dL)								
I	34.6 ± 1.37[a]	34.5 ± 1.98[a]	35.7 ± 3.08[a]	38.5 ± 3.51[a]	36.0 ± 1.59[a]	33.5 ± 1.50[a]	36.6 ± 1.43[a]	37.6 ± 2.20[a]	37.4 ± 1.24[a]
II	29.5 ± 1.89[ab]	29.7 ± 1.91[a]	35.4 ± 2.13[a]	41.9 ± 4.23[a]	43.8 ± 9.92[a]	41.1 ± 6.25[a]	38.3 ± 1.80[a]	47.3 ± 5.30[a]	42.5 ± 3.79[a]
III	29.5 ± 2.61[ab]	31.3 ± 2.54[a]	32.6 ± 2.01[a]	33.3 ± 1.81[a]	34.4 ± 2.35[a]	25.4 ± 0.71[a]	26.4 ± 1.63[a]	30.3 ± 0.60[a]	39.0 ± 2.31[a]
IV	30.6 ± 1.91[ab]	28.2 ± 0.60[a]	32.0 ± 1.94[a]	32.8 ± 1.45[a]	34.0 ± 1.59[a]	31.5 ± 3.07[a]	31.2 ± 2.92[a]	27.1 ± 1.38[a]	40.0 ± 1.58[a]
	Mean corpuscular volume (fL)								
I	55.9 ± 3.38[a]	52.3 ± 2.70[a]	51.6 ± 4.01[a]	42.1 ± 2.33[a]	51.5 ± 3.30[a]	52.9 ± 4.06[a]	47.6 ± 4.57[a]	50.3 ± 4.63[a]	51.2 ± 4.70[a]
II	63.4 ± 5.87[a]	61.6 ± 7.00[a]	53.5 ± 3.61[a]	39.9 ± 4.62[a]	44.3 ± 4.10[a]	44.4 ± 5.93[a]	53.1 ± 0.66[a]	48.2 ± 9.94[a]	46.1 ± 2.43[a]
III	64.9 ± 3.11[a]	57.5 ± 3.29[a]	57.2 ± 2.46[a]	47.4 ± 1.02[a]	51.0 ± 2.78[a]	60.9 ± 4.00[a]	70.6 ± 7.96[ab]	56.4 ± 5.24[a]	64.4 ± 5.97[a]
IV	67.1 ± 3.8[a]	62.5 ± 2.42[a]	58.4 ± 3.43[a]	47.6 ± 1.00[a]	54.0 ± 3.96[a]	62.0 ± 4.66[a]	76.8 ± 8.29[ab]	74.7 ± 7.53[ab]	64.1 ± 6.19[a]

[a]Means with at least one common superscript do not differ significantly ($p<0.05$).

I = control, II = fipronil, III = NaF, IV = fipronil and NaF combined

The significant decline in the Hb levels and TEC in the F-treated group II in the present study is in agreement with the previous reports.[26,27] It is known that F intoxication depresses bone marrow activity in cattle,[28] and F-induced disorders in hematopoietic organs in mice and in human hematopoetic proginator cells are on record.[29]

Administration of NaF to rats has been found to cause oxidative injury manifested by increase in lipid peroxidation and decrease in superoxide dismutase, catalase, and glutathione peroxidase activities of erythrocytes.[10] The decrease in Hb observed here from repeated administration of F to the buffalo calves may be an indication that F leads to anaemia involving suppression of the hematopoietic system. On the other hand, no significant alteration was noticed in MCHC since the derivation of MCHC also takes into consideration the packed cell volume. Likewise, erythrocyte indices are used to predict the extent and type of anemia under various conditions. MCV expresses the average volume of individual erythrocytes and is calculated by dividing the hematocrit (Hct) by the red blood cell count whereas MCH is the average amount of Hb per red blood cell and is calculated by dividing the hemoglobin by the red blood cell count. MCHC is the concentration of haemoglobin in the average erythrocyte or ratio of weight of haemoglobin to volume in which it is contained. No significant change in the erythrocyte indices in the F-treated calves of group III in the present study might be suggestive of normochromic normocytic type of anemia caused by NaF. Although the dosage of F used in present study was based on previous reports,[19,20] it has been found not to be suitable and have adverse effects for bovines conceived and reared under field conditions.[30,31] Therefore the increase in TLC might be due to stressful condition caused by exposure to toxicants like F. However, the lack of significant alteration in any of the haematological parameters in the fipronil-exposed calves of group II may indicate that fipronil can be used safely at its present dose rates without causing any adverse effect on the hematopoietic system.

As found here, haematological alterations were more pronounced with concomitant exposure to fipronil and NaF. Thus, the results of present study indicate that fipronil aggravates the toxic effects of NaF on the hematopoietic system. In support of this interpretation, NaF and deltamethrin have been reported to aggravate the oxidative injury of each another to rat erythrocytes.[10]

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