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Original Article

Hematobiochemical Evaluation of Dermal Subacute Cypermethrin Toxicity in Buffalo Calves

Shabir Ahmad Dar, Rajdeep Kaur

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

ABSTRACT

Dermal exposure of cypermethrin, a type II synthetic pyrethroid insecticide, at dose rate of 0.25% for 14 consecutive days produced mild signs of toxicity in buffalo calves. It produced significant elevation in the levels of alanine aminotransferase (ALT; 39.5%), aspartate aminotransferase (AST; 32.0%), blood urea nitrogen (BUN; 57.7%), and plasma creatinine (30.0%). Cypermethrin also produced significant decrease in the hemoglobin (Hb) concentration (5.4%), packed cell volume (PCV; 3.4%), and total erythrocytic count (4.0%). Additionally, there was a significant increase in erythrocytic sedimentation rate (ESR; 3.1%). On the basis of the present study, it can be concluded that cypermethrin induces significant biochemical and hematological alterations in buffalo calves when exposed dermally.

Key words: Biochemical, buffalo calves, cypermethrin, dermal, hematology, toxicity

INTRODUCTION

It has been said that "no pesticide is perfect, but the pyrethroids come close".[1] Pyrethroid pesticides have gained popularity over other conventional pesticides due to their high efficacy against target species; they have relatively low mammalian toxicity and rapid biodegradability. Synthetic pyrethroid pesticides account for over 30% of the global pesticide use and these are preferentially used in place of organophosphates and organochlorines.[2]

Serum content of these enzyme activities reflects the overall status of the animal when subjected to exogenous modulants such as toxins, infection, or injury. A decremental effect on the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was observed in cypermethrin-exposed rats (41 mg/kg bwt) by Nagarjuna *et al*., (2008).[3] It was hypothesized by Manna *et al*., (2004)[4] that an increase in ALT activity with concomitant decrease in the activity of free radical scavengers may be representative of α-cypermethrin induced pathological changes in the liver.

An induction of deoxyribonucleic acid (DNA) damage in the hematopoietic system, viz., bone marrow and lymphocytes was also observed by Patel *et al*., (2006).[5] Due to the hydrophobic nature and small molecular size, cypermethrin passes through the cell membrane, and reaches the nucleus. It is suggested that within the nucleus cypermethrin binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be a possible mechanism for its genotoxicity.[6] The increased use of α-cypermethrin has increased the risk of environmental contamination and the ensuing intoxication of non-target organisms in different ecosystems.[7] The present investigation was undertaken to evaluate the toxic effects of subacute dermal exposure of cypermethrin in buffalo calves.

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Address for correspondence: Dr. Rajdeep Kaur, Assistant Toxicologist, Department of Pharmacology and Toxicology, College of Veterinary Science, GADVASU, Ludhiana - 141 004, Punjab, India. E-mail: rajksidhu@yahoo.com

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MATERIALS AND METHODS

The experiment was conducted on eight healthy male buffalo calves (6–12 months; 60–120 kg), procured from the University Dairy Farm and local market. The animals were acclimatized in the animal shed of department under uniform conditions for 2 weeks, prior to the commencement of study. The animals were dewormed, fed seasonal green fodder and wheat straw, and water was provided *ad libitum*. The permission to conduct the experiment was duly taken from the University Animal Ethics Committee. The animals were randomly divided into two groups of four animals, each. Animals of Group I served as healthy control, whereas, Group II animals were dermally sprayed with 0.25% cypermethrin for 14 consecutive days. Blood samples were collected in heparinized vials via jugular venipuncture on days 0, 3, 7, 10, and 14 in dermally exposed animals.

Plasma lactate dehydrogenase (LDH) estimation in blood was done by ultraviolet (UV) Kinetic (French Society of Clinical Biology (SFBC)) method by using Bayer Autopak kits on Photometer 5010 (Nicholas Piramal) as described by Vassault *et al*. (1982).[8] Plasma estimation of gamma-glutamyl transpeptidase (GGT) was done by Kinetic method using Bayer Autopak kits as described by Szasz (1976). Blood urea nitrogen (BUN) estimation was done as described by Talke and Schubert (1965)[9] by UV method using Bayer Autopak kits. Plasma creatinine and total protein were estimated by picrate method and biuret method, respectively, using Bayer Autopak kits as described by Henry *et al.* (1974).[10] AST and ALT levels in blood was measured by the method of Expert Panel of International Federation of Clinical Chemistry (1976)[11] with Bayer Autopack kit on Photometer 5010 (Nicholas Piramal).

Erythrocyte sedimentation rate (ESR), PCV, hemoglobin (Hb) concentration, total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume, mean corpuscular Hb concentration, and mean corpuscular Hb were done by method of Benjamin (1985).[12] The differences between two means based on individual observation were determined by Student's*t*-test using Statistical Package for Social Sciences (SPSS)® 16.0 software package.

RESULTS

Short-term dermal exposure of 0.25% cypermethrin in buffalo calves did not produce any significant changes in LDH and GGT levels [Table 1]. These findings are in contrast with those of Aslam *et al*., (2010)[13] and Remya *et al*.,(2010)[14] who found increased serum LDH and GGT activity in cypermethrin treated poultry and rats, respectively.

The levels of AST significantly increased from 0 day level of 157.8 ± 16.52 U/L to 187.4 ± 16.43 U/L by the 14th day of treatment as depicted in Table 1. Similarly, there was 39.5% increase in ALT by 14th day of exposure [Table 1]. The enzymatic activity of ALT, however, returned to normal within 7 days of withdrawal of treatment.

The repeated dermal exposure to 0.25% cypermethrin in buffalo calves produced significant changes in BUN as presented in Table 1. There was 57.7% increase in BUN on 14th day of cypermethrin exposure. However, there was a remarkable recovery during 7 days of posttreatment period. In addition, cypermethrin treatment produced significant

Table 1: Effect of repeated dermal exposure of 0.25% cypermethrin on lactate dehydrogenase, gamma glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase and blood urea nitrogen in buffalo calves

Time (days)	0	3	7	10	14	7
Treatment						Post-treatment
Lactate dehydrogenase (U/L)						
Control	1455±42.00	1447±36.25	1502±44.37	1474±22.98	1487±38.56	1494±36.17
Treatment	1503±40.00	1474±39.40	1529±41.20	1508±32.94	1522±35.80	1505±24.49
Gamma glutamyl transpeptidase (U/L)						
Control	20.2±2.35	17.4±±1.21	17.8±2.23	19.8±1.74	20.6±1.89	19.4±1.46
Treatment	22.8±2.35	18.2±1.21	22.0±2.23	22.4±1.74	23.2±1.89	21.4±1.46
Aspartate aminotransferase (U/L)						
Control	149.0±15.41	140.0±17.83	139.8±18.62	144.0±19.46	142.0±18.24	139.8±16.92
Treatment	157.8±16.52	168.2±14.61	170.5±21.42	174.4±19.64	187.4±16.43**	145.6±17.78
Alanine aminotransferase (U/L)						
Control	37.4±4.25	40.8±4.91	35.8±4.86	40.6±4.35	39.0±4.12	37.6±4.54
Treatment	40.4±4.94	44.6±4.29	43.2±4.13	47.9±4.27	54.4±4.82**	43.4±4.57
Blood urea nitrogen (mg/dl)						
Control	4.6±1.17	5.4±0.76	5.4±0.73	5.8±1.02	5.2±0.86	5.2±0.66
Treatment	5.2±1.07	5.0±0.71	5.6±0.68	6.8±0.86	8.2±0.66**	6.2±0.59

Values given represent the Mean±S.E. of 5 animals unless stated. Values with superscript in a given column differs significantly from each other. (*P<0.05 and **P<0.01)

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increase in plasma creatinine levels on 10th and 14th days of treatment and a maximum of 30% increase in plasma creatinine was observed on 14th day of exposure [Table 2]. These observations are in agreement with those of Padma and Ashok (2010),[15] who also reported increase in BUN after cypermethrin treatment.

The repeated dermal exposure of 0.25% cypermethrin in buffalo calves did not produce any significant changes in total proteins as presented in Table 2. These find in gsare not consistent with those of Nagarjuna *et al*.,(2008)[3] and also Yousef *et al*.,(2003)[16] who found decrease in total protein in cypermethrin treated rats and rabbits, respectively.

Significant decline in the levels of Hb concentration was found from 10th day onwards [Table 2] and the maximum decrease was observed to be 5% on 14th day of exposure. The Hb content was however recouped within 7 days of treatment withdrawal. A significant decline in the levels of PCV with a maximum decrease of 3.4% on 14th day of treatment [Table 2] was observed following dermal cypermethrin exposure. The ESR values declined from 0 day value of 132.0 ± 1.5 mm/24 h to 137.4 ± 0.8 mm/24 h by the 14th day of cypermethrin treatment [Table 2].

The maximum decrease observed in TEC was 4% after 14th day of treatment as shown in Table 3. Dermal exposure

Table 2: Effect of repeated dermal exposure of 0.25% cypermethrin on creatinine, total proteins, haemoglobin, packed cell volume and erythrocyte sedimentation rate in buffalo calves

Time (days)	0	3	7	10	14	7
Treatment						Post-treatment
Creatinine (mg/dl)						
Control	1.21±0.10	1.18±0.09	1.11±0.09	1.13±0.08	1.13±0.06	1.16±0.08
Treatment	1.23±0.08	1.29±0.07	1.31±0.08	1.36±0.09**	1.47±0.08**	1.21±0.10
Total proteins (g/dl)						
Control	7.60±0.29	7.70±0.35	7.62±0.33	7.46±0.35	7.46±0.35	7.51±0.31
Treatment	7.50±0.27	7.52±0.25	7.32±0.22	7.25±0.32	7.40±0.34	7.44±0.25
Haemoglobin (g/dl)						
Control	9.96±0.21	9.85±0.16	9.82±0.14	9.81±0.15	9.74±0.16	9.88±0.13
Treatment	9.86±0.20	9.59±0.21	9.53±0.18	9.31±0.16**	9.21±0.17**	9.67±0.17
Packed cell volume (%)						
Control	36.83±0.35	36.72±0.34	36.81±0.34	36.52±0.30	36.63±0.30	36.66±0.38
Treatment	36.56±0.37	36.44±0.36	36.33±0.31	35.50±0.26**	35.40±0.42**	36.63±0.28
Erythrocyte sedimentation rate (mm/24 hour)						
Control	132.0±1.5	133.0±1.6	132.6±1.4	134.4±1.5	133.2±1.3	131.8±1.4
Treatment	132.0±1.5	131.6±1.4	134.2±1.3	136.8±1.2	137.4±0.8**	133.4±1.2

Values are the Mean±S.E. of 5 animals unless stated and units are expressed as mg/dl. Values with superscript in a given column differs significantly from each other (*P<0.05 and **P<0.01)

Table 3: Effect of repeated dermal exposure of 0.25% cypermethrin on total erythrocytic count, total leukocytic count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in buffalo calves

Time (days)	0	3	7	10	14	7
Treatment						Post-treatment
Total erythrocytic count (10^6/mm^3)						
Control	7.23±0.06	7.23±0.05	7.21±0.06	7.21±0.06	7.22±0.07	7.178±0.08
Treatment	7.22±0.07	7.17±0.06	7.13±0.05	6.97±0.06**	6.93±0.06**	7.15±0.09
Total leukocytic count (/mm^3)						
Control	11760±996	11750±774	11790±871	11800±690	11760±1047	11740±674
Treatment	11740±828	11750±791	11760±548	11780±598	11800±853	11760±744
Mean corpuscular volume (fl)						
Control	50.87±2.93	50.82±2.21	50.72±1.97	51.25±2.02	51.38±1.80	51.11±0.87
Treatment	51.30±3.26	50.73±1.77	51.30±2.49	51.00±1.60	51.38±1.26	51.55±1.93
Mean corpuscular haemoglobin (pg/dl)						
Control	13.83±0.75	13.66±0.28	13.63±0.24	13.81±0.86	13.70±0.76	13.81±0.22
Treatment	13.50±0.22	13.42±0.40	13.41±0.32	13.19±0.28	13.21±0.21	13.59±0.37
Mean corpuscular haemoglobin concentration (g/dl)						
Control	27.28±0.97	26.99±0.76	27.02±0.98	26.87±0.82	26.62±0.81	27.038±0.47
Treatment	27.01±0.97	26.52±0.80	26.28±0.83	25.93±0.66	25.75±0.485	26.415±0.48

Values given represent the Mean±S.E. of 5 animals unless stated. Values with superscript in a given column differs significantly from each other. (*P<0.05 and **P<0.01)

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Table 4: Effect of repeated dermal exposure of 0.25% cypermethrin on differential leukocyte count in buffalo calves

Time (days)	Control					Treatment				
	N	L	M	E	B	N	L	M	E	B
Treatment										
0	28.2±1.0	64.2±1.7	6±0.6	1.8±0.7	0.0±0.0	24.0±1.0	68.0±2.4	6.2±0.9	2.2±0.7	0.0±0.0
3	27.8±1.0	65.6±1.4	4.6±0.4	2.6±0.8	0.2±0.2	25.6±1.0	65.8±2.1	5.8±0.3	2.8±0.2	0.2±0.2
7	27.0±1.2	65.2±1.6	5.0±0.4	2.6±0.4	0.2±0.2	27.0±1.2	66.2±1.2	4.4±0.2	2.0±0.5	0.0±0.0
10	26.8±1.2	67.4±1.7	4.0±0.4	2.2±0.2	0.0±0.0	26.2±1.2	66.0±1.4	5.2±0.4	2.4±0.4	0.2±0.2
14	25.4±1.0	66.6±1.4	5.4±0.4	2.6±0.4	0.2±0.2	22.8±1.1	70.8±2.8	4.8±0.6	1.6±0.5	0.0±0.0
Post-treatment										
7	23.2±1.0	66.6±1.7	7.8±0.9	1.6±0.4	0.0±0.0	18.4±2.3	73.2±2.6	7.4±0.6	1.8±0.7	0.0±0.0

Values given are expressed as % and represent the Mean±S.E. of 5 animals unless stated. Values with superscript in a given column differs significantly from each other. (*P<0.05 and **P<0.01)

of cypermethrin (0.25% for 14 consecutive days) produced no significant changes in TLC and DLC [Tables 3 and 4, respectively]. There were no changes observed in DLC during any time of the cypermethrin exposure in buffalo calves. Cypermethrin at the concentration of 0.25% for 14 consecutive days produced no significant changes in erythrocytic indices [Table 3].

DISCUSSION

The increase in plasma LDH activity in present investigation reflects damage to a range of tissues including skeletal or cardiac muscles, kidneys, and liver. Although the exact cause of increased GGT level in the present study could not be ascertained, yet cholestatic disorders of all species are associated with increased GGT activity.[17] Cardiac and skeletal muscles have high concentrations of AST,[18] its elevation in present investigation suggests muscular damage. In large domestic species, the activity of ALT in the liver is low and in case of liver injury, the ALT is not remarkably elevated.[19] Leakage of ALT is indicative of hepatocellular damage, so its level increases in plasma in various species and is usually elevated in disorders of inflammatory, toxic, or degenerative origin.[20] Increase in plasma creatinine and BUN levels probably indicate renal damage, which may be attributed to urinary obstruction, which potentiates decreased secretion of urea from the body and increased protein catabolism.[21]

Significant reduction of Hb content during later period of investigation could be due to decreased synthesis of red blood cells in bone marrow, or reduced biosynthesis of heme in bone marrow.[22] This could probably be explained by the effect of cypermethrin on erythropoiesis. The subsequent recovery of Hb after termination of cypermethrin treatment indicates that the damage produced was of reversible nature. The decrease in TEC, Hb concentration, and PCV observed in this study could be due to the disruptive action of the cypermethrin on the erythropoietic tissue as a result of which the viability of the cells might have been affected. One of the most important factors to be considered in reduction of TEC is the production of the hormone erythropoietin. This could be due to the reason of kidney damage caused by cypermethrin.

The findings of the present investigation strongly suggest that cypermethrin induces significant biochemical and hematological alterations in buffalo calves when exposed dermally.

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