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Research Article

HAEMOBIOCHEMICAL PROFILE OF GOAT FOLLOWING SINGLE OR COMBINED APPLICATION OF FLUMETHRIN AND IMIDACLOPRID ON SKIN

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ABSTRACT: The haemobiochemical effects of two insecticides, flumethrin and imidacloprid were studied alone and in combination in goats following dermal application weekly for eight weeks. Thirty two animals were divided into four groups of eight animals each. The first group was treated as control and second, third and fourth group received flumethrin, imidacloprid and both insecticides dermally respectively. Haemoglobin level and total erythrocyte count did not change in any group but changes in total leucocytes count were observed in groups II and IV from seventh week and no changes in III group were found. Glucose level was also not altered in any group. The serum protein level was changed significantly seventh week onwards in group II and IV but not in group III. Similarly activities of aspartate (AST) and alanine transaminase (ALT) were increased sixth week onwards in II and IV group, whereas no changes observed in the third group. Weekly dermal application of flumethrin and also flumethrin and imidacloprid combination produced mild toxicity but imidacloprid alone showed no such effect as revealed from haemobiochemical study on goat.

Keyword: Flumethrin, Imidacloprid, Haemobiochemical profile, Goat,

INTRODUCTION

Pesticides are heterogeneous group of substances used for preventing, destroying or repelling pests. Animals are infested by a number of parasitic insects and acarine species causing major economic losses in agriculture and livestock industry. Pesticides have consistently demonstrated their worth by increasing productivity, reducing insect born endemic diseases and protecting and restorating plantation. Therefore, information relating to toxicity to biological system is essential for safe utilization of pesticide. Synthetic pyrethroids are most widely used ecto-parasiticides today as they found comparatively lesser toxic to animals than that of other insecticides (Deo and Krishnakumary 1991). Flumethrin is a lipid soluble neurotoxic insecticide, under Type II

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pyrethroids, used to control ecto-parasites infesting on various species of animals. It is a neuro-poison for insects and its main target of action is on nerve membrane sodium channel. It inactivates the Na+ channel causing long lasting prolongation of transient increase in Na^+ ion permeability of nerve membrane producing a persistent depolarization and frequency dependent conduction block in sensory and motor neurons and long lasting repetitive firing of sensory nerves organ and muscle fibre producing killing effect on insects ((Hayes and Laws 1991). In veterinary medicine, it is applied topically as 1% w/v pour-on and 6% w/v as a plunge dip. The neonicotinoids, the newest major class of insecticide, have outstanding potency and systemic action for crop protection against piercing, sucking pests and also they are highly effective for flea control on cats and dogs. Imidacloprid has been marketed worldwide by the applicant as advantage (10 % Imidacloprid) as spot-on treatment for control of fleas on dogs and cats. Imidacloprid represents the new generation of neurotoxic insecticides, which exhibit more selective toxicity for insects relative to mammals. It is one of the fastest sold insecticides across the world because of its high selective toxicity in insects and apparent safety in humans. It acts on the nervous system by blocking postsynaptic acetylcholine receptors, which kills the insect (Tomizawa and Casida 2005). Both the insecticides imidacloprid and flumethrin are used alone and in combination to control ectoparasites in animals (Dik and Uslu 2008, Drothee, 2012, Genchi 2012). Continuous exposure of insecticides to animal may affect the haemobiochemical status and ultimately the health of animal (Dewangan *et al*., 2012). Goat is a multi-purpose animal and act as good source of income by producing meat, milk, hide, fibre and manure, also called poor man's cow. Therefore, flumethrin and imidacloprid were selected separately and in combination to study their adverse effect on goat health status by measuring haemobiochemical parameters .

MATERIALS AND METHODS

Flumethrin: Technical grade flumethrin was provided by M/S Gharda chemicals Ltd. Mumbai, (India) and the purity was 92%.

Imidacloprid: Technical grade imidacloprid was obtained from M/S United Phosphorus Limited, Ankleshwer, Gujrat and the purity was 97.3%.

All other chemicals used in this study were obtained from E. Merck (India), Rankem (India) and Sigma Chemicals Co., USA.

Experimental animal

Goat is easily available docile animal, easy in handling and it is a prototype of ruminant therefore clinically healthy Black bengal adult goats (1-1½ years age) weighing between 12-14 kg were used in this experiment. They were caged individually in custom made stainless steel metabolic cage. The animals were stall-fed and water was provided *ad libitum*. The composition of feed was 2 part wheat husk, 1 part crushed maize, 1 part crushed gram and 2 part green. The temperature of the animal room was maintained at 22 ± 3^0C and provided with artificial lighting facilities. Before starting the experiment, the animals were dewormed once with a mixture of albendazole and rafoxanide (Vetalben-R, Indian Immunologicals) at the dose rate of 7.5 mg/ kg. body weight. After 21 days of deworming, the animals were acclimatized in experimental environment for

Table 1: Effect of flumethrin and imidacloprid alone and combination on hemoglobin level (g/dl) at different days following weekly dermal application for 56 days in goats.
(Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	8.47±0.11	7.72±0.19	8.02±0.2	7.88±0.11
7	8.45±0.13	8.00±0.18	7.70±0.21	8.33±0.12
14	8.11±0.34	7.08±0.11	8.07±0.19	8.1±0.49
21	7.97±0.24	7.85±0.35	7.65±0.13	8.20±0.01
28	8.47±0.17	7.92±0.23	8.17±0.19	9.1±0.19
35	8.27±0.27	7.99±0.44	8.22±0.28	8.10±0.26
42	8.74±0.11	8.45±0.18	8.17±0.13	7.65±0.18
49	7.62±0.19	7.90±0.20	7.92±0.28	7.87±0.18
56	8.66±0.14	8.00±0.31	7.87±0.68	8.9±0.19

Table 2: Effect of flumethrin and imidacloprid alone and combination on total erythrocyte count (x10^6 cells/L) at different days following weekly dermal application for 56 days in goats.
(Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	10.94±0.06	10.34±0.57	10.27±0.13	10.18±0.11
7	10.85±0.18	10.71±0.18	9.66±0.39	10.17±0.76
14	10.9±0.22	10.13±0.10	10.41±0.54	10.32±0.15
21	10.57±0.17	10.08±0.46	10.65±0.31	10.19±0.08
28	10.38±0.05	10.71±0.08	10.53±0.15	10.60±0.20
35	10.29±0.29	10.43±0.17	10.42±0.59	10.92±0.10
42	10.66±0.42	10.71±0.09	10.24±0.57	10.89±0.13
49	10.28±0.79	10.93±0.67	10.32±0.53	10.98±0.10
56	10.96±0.22	10.30±0.32	10.47±0.24	10.22±0.53

7 days. Institution Animal Ethics Committee (IAEC) approved experimental protocol before starting the experiment.

Thirty two goats of either sex were divided into four groups (I, II, III and IV) each consisting of eight animals.

Dose and Mode of application

Flumethrin @ 2 mg/kg b.wt ,1% in acetone CVMP 2000) and imidacloprid @ 10 mg/kg b.wt, 10% in acetone (http://www.animalhealth.bayer.com /4897.0.html, Study No. 21091, 1986) alone and in combination

Table 3: Effect of flumethrin and imidacloprid alone and combination on total leucocyte count (x10^3 cells/L) at different days following weekly dermal application for 56 days in goats.

(Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	8.41^{ax}± 0.41	8.9^{ax}±0.15	8.34^{ax}±0.47	8.87^{ax}±0.58
7	8.22^{ax}± 0.25	8.84^{ax}±0.10	8.2^{ax}±0.32	8.96^{ax}±0.5
14	8.11^{ax} ±0.13	8.19^{ax}±0.27	8.15^{ax}±0.51	8.00^{ax}±0.98
21	7.97^{ax}± 0.54	8.94^{ax}±0.22	8.36^{ax}±0.24	8.83^{ax}±0.33
28	8.90^{ax} ±0.19	8.79^{ax}±0.21	8.65^{ax}±0.01	8.87^{ax}±0.38
35	8.45^{ax} ±0.75	8.89^{ax}±0.84	8.43^{ax}±0.08	8.34^{ax}±0.29
42	8.03^{ax}± 0.51	8.31^{ax}±0.25	8.46^{ax}±0.26	8.25^{axy}±0.22
49	8.03^{ax}± 0.51	7.11^{ay}±0.81	8.40^{ax}±0.26	7.4^{ayz}±0.28
56	8.00^{ax}± 0.05	6.33^{by}±0.34	8.39^{ax}±0.12	6.71^{bz}±0.56

Mean value with dissimilar superscript (abc) in the row vary significantly (P < 0.05)
Mean value with dissimilar superscript (xyz) in column vary significantly (P < 0.05)

were applied dermally along the backline for eight weeks to animals of groups II ,III and IV respectively while group I was treated as control and received vehicle (acetone) dermally.

Collection of samples

Blood : Blood samples were collected from jugular vein of each animal of Groups-I, II, III and IV at '0' (before application of test drugs), 7, 14, 21, 28 , 35, 42, 49 and 56th day of treatment. One ml of blood was kept in EDTA vials for haematological study, 0.5 ml of blood in sodium fluoride for glucose estimation, 3 ml of blood was allowed to clot for separation of serum, and stored at –20^{0}C for further use. From collected serum, estimation of protein, AST and ALT was carried out.

Blood biochemical parameters

Hematology

Haemoglobin level was determined by indirect acid haematin method (Coffin 1953) and expressed as g/dl. Total erythrocyte count, total leucocyte count were done following standard method of Schalm *et al.* (1975) and expressed as SI unit.

Blood Glucose

Blood glucose was determined by Glucose Assay kit by GOD- POD method using *in vitro* Diagnostic kit (Trinder 1969)

Serum protein

Protein content of serum was estimated by Bi-Uret method (Wooton 1975).

Table 4: Effect of flumethrin and imidacloprid alone and combination on Blood Glucose level (n mole/L) at different days following weekly dermal application for 56 days in goats. (Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	2.88±0.19	3.26 ±0.20	3.48 ± 0.20	3.36±0.29
7	2.95±0.19	3.16 ± 0.20	3.44± 0.29	3.40±0.30
14	2.67±0.17	3.56 ±0.14	3.77±0.53	3.25±0.26
21	2.89±0.18	3.22±0.23	3.46±0.3-0	3.45±0.30
28	2.91±0.16	3.24 ± 0.19	3.47±0.30	3.35±0.29
35	2.92±0.17	3.31 ± 0.22	3.51± 0.29	3.36±0.30
42	2.89±0.19	3.25 ±0.18	3.55±0.29	3.41±0.29
49	2.66±0.14	3.49 ±0.18	3.39±0.43	3.28±0.35
56	2.90±0.18	3.30 ± 0.16	3.52± 0.29	3.42±0.30

Table 5: Effect of flumethrin and imidacloprid alone and combination on Serum protein (g/L) level at different days following weekly dermal application for 56 days in goats. (Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	67.00^{ax}±0.16	58.00^{bx}±1.3	61.20^{bcx}±0.69	63.25^{cx}±1.71
7	67.97^{ax}±0.39	57.00^{bx}±0.47	61.36^{cx}±0.63	63.71^{cx}±1.9
14	68.12^{ax}±0.16	$57.76.^{bx}$±1.1	61.22^{bcx}±0.65	62.25^{cx}±1.3
21	67.02^{ax}±0.16	57.47^{bx}±1.0	61.31^{cx}±0.56	63.10^{cx}±0.33
28	67.40^{ax}±0.15	58.41^{bx}±1.0	60.31^{cx}±0.45	62.37^{cx}±0.33
35	66.8^{ax}±0.71	58.10^{bx}±1.1	61.5^{bcx}±0.55	63.62^{acx}±1.59
42	67.5^{ax}±0.42	58.15^{bx}±1.1	60.72^{bx}±0.53	59.8^{bxy}±1.35
49	67.47^{ax}±0.2	55.85^{by}±0.41	60.32^{cx}±1.4	57.9^{bcy}±1.6
56	67.5^{ax}±0.37	55.14^{by}±0.35	61.17^{cx}±0.17	57.77^{bcy}±2.4

Mean value with dissimilar superscript (abc) in the row vary significantly (P<0.05)
Mean value with dissimilar superscript (xy) in column vary significantly (P<0.05)

Serum Aspartate and alanine transaminase

Aspartate and alanine transaminase activities were measured in serum sample by the method of Yatazidis (1960) and expressed as mg pyruvic acid ml^{-1} hr^{-1}.

Statistical analysis

Statistical analysis was done by one-way ANOVA using SPSS software version 10.0. Level of significance was determined at p<0.05.

Table 6: Effect of flumethrin and imidacloprid alone and combination on aspartate transaminase activities (ug Pyruvic acid $ml^{-1}hr^{-1}$) at different days following weekly dermal application for 56 days in goats.

(Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	90.81^{ax}±0.40	90.36^{ax}±0.64	91.95^{ax}±0.35	98.2^{bx}±0.68
7	90.47^{ax} ±0.51	90.1^{abx}±0.49	91.85^{bx}±0.98	98.95^{cx}±0.32
14	90.63^{ax} ±0.51	91.34^{ax}±0.63	91.54^{ax}±0.68	99.87^{bx}±0.15
21	90.28^{ax} ±0.47	91.68^{ax}±0.61	92.00^{ax}±0.48	98.99^{bx}±0.16
28	90.44^{ax} ±0.41	91.24^{ax}±0.61	91.90^{ax}±0.48	99.10^{bx}±0.18
35	89.87^{ax}±0.91	91.05^{ax}±0.32	91.59^{ax}±0.20	99.29^{bx}±0.49
42	90.24^{ax}±0.38	94.74^{by}±0.36	91.4^{ax}±0.55	106.82^{cy}±1.0
49	90.63^{ax}±0.42	$95.36.^{by}$±4.6	91.1^{ax}±0.42	110.50^{cz}±7.85
56	90.03^{ax}±0.24	102.5^{bz}±5.3	91.9^{ax}±0.28	111.25^{cz}±6.26

Mean value with dissimilar superscript (abc) in the row vary significantly (P<0.05)
Mean value with dissimilar superscript (xyz) in column vary significantly (P<0.05)

Table 7: Effect of flumethrin and imidacloprid alone and combination on alanine transaminase activities (ug Pyruvic acid $ml^{-1}hr^{-1}$) at different days following weekly dermal application for 56 days in goats.

(Mean of 8 replicates with SE, n=8)

Day	I	II	III	IV
0	28.27^{ax}±0.82	30.70^{bx}±0.43	30.2^{bx}±0.29	30.35^{bx}±0.21
7	28.00^{ax}±0.87	31.31^{bx}±0.19	29.82^{bx}±0.19	30.00^{bx}±0.18
14	28.7^{ax}±1.12	31.35^{bx}±0.39	29.96^{abx}±0.22	30.59^{bx}±0.74
21	28.15^{ax}±1.01	30.84^{bx}±050	29.72^{abx}±0.38	30.4^{bx}±0.39
28	28.78^{ax}±1.3	30.98^{bx}±0.30	29.90^{abx}±0.26	30.61^{bx}±0.49
35	27.9^{ax}±1.12	31.01^{bx}±0.39	29.8^{abx}±0.22	30.45^{bx}±0.43
42	28.55^{ax}±0.69	33.5^{by}±0.58	30.00^{abx}±0.29	33.90^{by}±0.68
49	28.95^{ax}±0.70	33.8^{by}±0.25	29.65^{ax}±0.22	34.9^{by}±036
56	28.37^{ax}±0.56	34.6^{by}±0.28	30.20^{cx}±0.18	35.08^{by}±0.60

Mean value with dissimilar superscript (abc) in the row vary significantly (P < 0.05)
Mean value with dissimilar superscript (xy) in column vary significantly (P < 0.05)

RESULTS AND DISCUSSION

Heamatology

No significant change in values of haemoglobin level, and total erythrocyte count were observed in groups I, II, III and IV at different days of collection compared to '0'day (Table 1, 2). However, total leucocyte count was decreased significantly ($p<0.05$) from seventh week in groups II and IV compared to '0'day. But, no significant changes were noticed in group III up to 8 weeks (Table 3).

Blood glucose level

Significant changes were not recorded in glucose level in group I, II, III and IV at different days (Table 4).

Serum Protein

Protein level was significantly ($p<0.05$) reduced in group II and group IV significantly ($p<0.05$) on seventh and eighth week compared to respective '0' day value (Table 5).

Serum aspartate transaminase (AST) and alanine transaminase (ALT)

AST activity increased significantly ($p<0.05$) in group II and group IV on 6^{th}, 7^{th} and 8^{th} week compared to '0'day value however no changes in AST value recorded in group III. Similarly, ALT activity increased significantly ($p<0.05$) in group II and IV on 6^{th}, 7^{th} and 8^{th} week but in group III no significant ($p<0.05$) difference was observed at different days, compared to '0' day value (Table 6 and 7).

The present study was conducted to observe the effect of two insecticides flumethrin and imidacloprid alone and in combination on the hematobiochemical profile in goat. Therapeutic doses of both insecticides (flumethrin at dose rate 2mg/kg b.wt. and imidacloprid 10 mg/kg b.wt.) were administered weekly through dermal route for 56 days, hematological parameters, haemoglobin, total erythrocyte count, total leucocyte count, total protein and AST, ALT level have been investigated in the present study. Both the insecticides did not affect the hemoglobin, total erythrocyte count and differential leucocyte count neither alone nor in combination but, the total leucocyte count was reduced in flumethrin treated group and in group treated with both the insecticides 49 day onwards which may be due to flumethrin only as imidacloprid could not produced the same when given alone. Reduction in total leucocyte count was may be due to bone marrow depression or liver intoxication effect of flumethrin. A significant reduction in total leucocyte count was found following oral administration of cypermethrin, (a synthetic pyrethroid of same group of flumethrin) at the dose of 40mg/kg for 90 days in rats (Varshneya *et al*., 1992). Both the insecticides could not alter the blood glucose level neither alone nor in combination. Protein level was lowered in flumethrin treated group and in group treated with both insecticides on 49 day onwards which may be due to flumethrin only because protein level did not change in imidacloprid treated group. Results suggest that flumethrin reduced the serum protein level on long term weekly dermal application in goat producing hypoproteinamia. Hypoproteinamia was observed in rats following oral administration of cypermethrin. The reduction in total protein may be due to hepatotoxicity induced by flumethrin, which was evident in group II and IV from elevated serum ALT and AST activities. A significant increase in AST and ALT was found 42 day onwards in group treated with flumethrin alone and in group treated with both the insecticides together. This may be due to flumethrin only (Manna *et al*.,2004) as there

was no increase recorded in group treated with imidacloprid alone which suggests that flumethrin may have some toxic effect on liver when given alone and also when given along with imidacloprid. This effect may be due to degeneration and necrosis of hepatocytes, which attributes an increased permeability of cell membrane that results in release of transaminases into the blood stream (Shah and Gupta 1997). In our study, imidacloprid is not found to alter AST or ALT level. Elevation in AST and ALT level was observed after oral administration of imidacloprid in female rats at the rate of 5, 10 and 20 mg/kg body wt for 90 days (Bhardwaj *et al.*, 2010), but in present study no such changes were found. This may be due to difference in route, frequency of administration or species difference. Increase in activity of ALT in rats was found following oral administration of alpha cypermethrin, a synthetic pyrethroid (Manna *et al.*, 2004).

CONCLUSION

The present study shows that flumethrin, a synthetic pyrethroid insecticide at therapeutic dose, alone and in combined therapy can produce leucopenia and hypoproteinaemia when used for more than six weeks continuously which is indicative of immunosuppressive effect of flumethrin in long term use. An increased level of AST and ALT is suggestive of liver injury by flumethrin alone and in combined therapy following prolong weekly dermal application in goats. Imidacloprid, a member of the neonicotinoids group of insecticides did not produce any alteration in haemo-biochemical parameters alone and in combination and so it can be considered as nontoxic even after prolong weekly dermal application.

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