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EFFECT OF FLUMETHRIN ON HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN RATS

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ABSTRACT : The effect of daily oral administration of flumethrin on the blood and tissue enzyme activity in albino rats was investigated. In the present study 12 (6 female and 6 male) rats were used and divided in to two groups. The first group served as the control group; the second group received flumethrin (1% pour on formulation) at dose rate of 2 mg/kg bw orally daily for 14 days. On 15th day, animals were sacrificed and blood and liver samples were collected. Flumethrin neither altered the hemoglobin level significantly nor the blood cell counts of rats. Flumethrin significantly altered the enzymatic activity of serum and liver tissue and also the serum and tissue protein. Flumethrin leads to increased MDA level, SOD and catalase activity in liver and blood samples of rats. The present study suggests that flumethrin is having toxic effect, producing oxidative stress in animal's body.

Key words: Albino rats, Flumethrin, Enzymes, Serum, Tissue, Oxidative stress.

INTRODUCTION :

Flumethrin is a lipid soluble insecticide used to control ecto-parasites on cattle, sheep, goats, horses and dogs. In veterinary medicine, it is applied topically as 1% w/v pour-on and 6% w/v as a plunge dip. Flumethrin is a neuro-toxic poison for insects and its main target of action 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 fiber producing killing effect on insects. On the other hand, flumethrin was found to have toxic effects in a variety of experimental animals. Anadon *et al*. (1995) studied the effects of repeated exposure to the pyrethroid insecticide flumethrin (40 mg/kg intraperitoneally once a day for 6 days) on the activity of cytochrome P450-dependent monooxygenases and UDP-glucuronosyltransferase as well as on antipyrine disposition in male Wistar rats and concluded that

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flumethrin exposure diminishes hepatic enzyme levels and catalytic activities of monooxygenase systems. Also this flumethrin is used as an insecticide in field animals. On dermal application it may penetrate the skin and on continuous use may get accumulated in the body, if this treated animal is consumed by the human being it can produce undesirable effect. The amount of flumethrin entering the food chain is at very low concentration level. That is why this work was conducted with the aim to see any toxic effect of flumethrin at very low dose or at therapeutic dose (1/10th of LD50) on the hematological and biochemical parameters of albino Wistar rat following daily oral administration for 14 days.

MATERIALS AND METHODS :

Test compound: Tikkil-Power® (flumethrin 1% w/v pour on solution, Indian Immunologicals Ltd.) was purchased from local market. All other chemicals used were of analytical grade from E. Merck (Germany and India); Sigma Chemicals, USA and SRL Chemicals (India).

Experimental animal: Young Wistar albino male rats (150-200g) were obtained from a r*egi*stered laboratory animal breeder. The animals were grouped and housed in polyacrylic cages and maintained in an air conditioned Lab. Animal House attached to the Department of Pharmacology & Toxicology. All animals were fed with standard laboratory animal diet with free access to clean drinking water. The animals were acclimatized to the laboratory conditions for 10 days before commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEA).

Twelve male rats were divided into two groups (I and II) each consisting of six animals. Group I (control) was treated with vehicle (arachis oil). In group II flumethrin (1% pour-on formulation) was dissolved in arachis oil and given orally daily at a dose rate of 2 mg/kg b.w. (1/10 of LD50) (Schmidt 1984) by oral gavage for 14 days.

Collection of sample : Blood was collected on 15th day after daily oral administration of flumethrin from both control group as well as test group in separate sterile vials for hematological and blood biochemical estimation. One ml of blood was collected in sterile vial previously containing EDTA at 1 mg/ml of blood for hematological assays like hemoglobin (Coffin 1953), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) following standard method of Wintrobe as described by Schalm (1975). The remaining 2.5 ml of blood collected without any anticoagulant was allowed to clot in a larger and bigger dimension test tube keeping in slanting position for sufficient times.

After clotting, the serum was aspirated with the help of Pasteur pipette in a separate sterile glass vial and then centrifuged by Remi Centrifuge machine at 5000 r.p.m. for 15 minutes. The clear supernatant thus obtained was pipetted out and collected in a separate sterile glass vial labeled properly, corked tightly and then preserved in a deep freezer (-20°C) for ALT, AST (Reitman and Frankel 1957) and protein estimation (Wooton 1974).

A part of EDTA blood was centrifuged at 2500 rpm for 15 min at 40C. Compact RBCs were washed twice with equal volume of normal saline and centrifuged again at 2500 rpm for 20 min. Plasma was removed from RBCs and kept separately for the estimation of lipid peroxidation (Bu*eg*e and Ausf 1976). Supernatant saline was removed and from compact RBCs 5% haemolysate was prepared for blood biochemical parameters like superoxide dismutase (Mishra and Fridovich 1972) and catalase (Aebi 1974) activity.

A portion of liver was collected for estimation of tissue protein (Wooton 1974) and various biochemical parameters like lipid peroxidation (Nair and Turner 1984), superoxide dismutase (Mishra and Fridovich 1972) and catalase (Bergmeyer 1984) activity. A small portion of liver was also

collected for estimation of aspartate (AST) and alanine transaminase (ALT) (Yatazidis 1960).

STATISTICAL METHODS:

The results were expressed as mean standard error (S.E.). The data were analyzed statistically using general linear model with univariate data in SPSS 10.0 version of software.

RESULTS AND DISCUSSION :

Physical parameters :

Significant changes were observed in the treated groups of rat. Little mortality was observed till the end of the dosing period in treated group of rats when compared to the control group. The most prominent clinical signs were manifestations of central nervous system toxicity, such as reduced motor activity and altered gait, in which animal showed circling movement. Skin lesions were ulcerative and scabbed patches were seen on the head, neck, shoulder girdle, and front extremities. Immediately after the starting of dose, animals showed aggressive behavior and frequent scratching movements.

Haematological parameters:

No significant changes were observed in hemoglobin, total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) in all the treated groups as compared to respective control group. (Table 1)

Blood biochemical parameters :

Significant reduction was observed in serum protein. However significant induction in ALT, AST, lipid peroxidation, catalase and superoxide dismutase was observed (Table 2).

Tissue biochemical parameters:

No significant changes were observed in serum protein. However significant induction in ALT, AST, lipid peroxidation, catalase and superoxide dismutase was observed. (Table 3)

Administration of flumethrin to rats (orally) for 14 days showed visible changes which includes ulcerative and scabbed skin lesions which were in correlation with hematological changes like differential leucocyte count showed non-significant induction in the count of lymphocyte, with a corresponding increase in the neutrophils, an indicator of inflammation. The findings are in accordance with the findings of McGr*eg*or (1996) who studied short term toxicity of flumethrin in rats.

Alanine and aspartate transaminase activity showed significant reduction in both liver and serum indicating hepatotoxic effect of flumethrin. There is also a non-significant reduction in tissue protein

Table 1. Hematological parameters in rats after daily oral administration of flumethrin for 14 days

Parameters	Group I	Group II
Hemoglobin (g/dl)	12.78±0.40	11.98±0.50
TEC (x10^6cmm)	6.13±0.11	5.40±0.25
TLC (x10^3cmm)	6.70±0.16	7.25±0.13
Neutrophil (x10^3cmm)	1.68±0.68	2.05±0.10
Lymphocyte (x10^3cmm)	4.04±0.11	5.13±0.47
Monocyte (x 10^3cmm)	0.25±0.02	0.38±0.01
Eosinophil (x 10^3cmm)	0.40±0.01	0.41±0.01

Mean value with dissimilar superscript vary significantly ($P < 0.05$)

Table 2. Biochemical parameters of blood in rats after daily oral administration of flumethrin for 14 days.

Parameters	Group I	Group II
Serum protein(g/l)	5.71±0.24	4.26*±0.22
ALT(U/ml)	72.97±0.59	81.00*±0.99
AST(U/ml)	4.91±0.47	11.99*±0.66
Lipid peroxidation(nmoles MDA/ ml)	1.51±0.14	5.25*±0.24
Catalase(k/gHb)	3.16±0.19	4.77*±0.63
Superoxide dismutase(U/gHb)	1145.00±3.17	1500.00*±5.00

Mean value with dissimilar superscript vary significantly ($P < 0.05$)

Table 3. Biochemical parameters of tissue in rats after daily oral administration of flumethrin for 14 days.

Parameters	Group I	Group II
Serum protein(g/l)	0.83±0.01	0.60±0.01
ALT(U/mg of protein/hr)	148.25±1.90	276.50*±1.03
AST(U/mg of protein/hr)	141.14±0.87	154.65*±0.78
Lipid peroxidation (nmoles malonaldehyde/ gm of tissue)	10.70±0.57	17.95*±0.40
Catalase(mM H_2O_2 decomposed /min/mg of protein)	8.49±0.22	9.44*±0.22
Superoxide dismutase(units/mg of protein)	862.50±4.78	879.33*±2.41

Mean value with dissimilar superscript vary significantly ($P < 0.05$)

but significant reduction in plasma protein that might be due to poor condition of rats or may be due to skin lesions. In the present study there is significant rise in the SOD and catalase activity, which are the scavengers of free radicals formed during various enzymatic and non-enzymatic reactions in the cells. SOD catalytically scavenges the superoxide radicals and hence the first line of defense against free radical induced injury. Superoxide radicals are converted to H_2O_2 by SOD by dismutation reaction (Barber and Bernhein 1967). When free radical production increases initially there is induction of endogenous antioxidant enzymes to remove the continuously generated free radicals. The increased activity of SOD in the present study could therefore be due to excess formation of superoxide radicals. H_2O_2 formed is decomposed to water and molecular oxygen by the action of catalase (Yasminch 1994). In our study there is induction in catalase activity.

The H_2O_2 formed in dismutation reaction could possibly have caused the induction of catalase to overcome its deleterious effect. These superoxide radicals are involved in the mechanism of lipid peroxidation. These free radicals are capable of damaging nucleic acid, protein, lipids and carbohydrates but lipids are probably the most susceptible (Cross *et al*. 1987). In the response to oxidative stress when lipid peroxidation occurs a great variety of aldehydes are formed (Esterbauer *et al*. 1991). Some of which are highly active and considered as second messenger that disseminate and augment initial free radical reaction. Among many different aldehydes malondialdehyde is the most extensively studied. In our study there is significant induction in the malondialdehyde formation indicating oxidative stress.

The H_2O_2 produced by dismutation reaction could possibly convert to hydroxyl radicals by iron released from hemoglobin of lysed erythrocytes and augment further oxidative stress (Fantone and Ward 1982). The decrease in blood hemoglobin and reduction in erythrocytic count is also one of the findings of our study, which is further supported by the above explanations.

CONCLUSION :

From the above experiment it can be concluded that rats treated with flumethrin for 14 days produces significant changes in the biochemical parameters showing induction in anti-oxidative status and oxidative stress and a non-significant rise in blood chemistry (cell count) suggesting mild toxic effect of flumethrin at this dose level. Thus even at this low therapeutic dose level of flumethrin mild toxic effect is developed. This paved the path for future studies in higher animals.

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