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EFFECT OF FLUMETHRIN ON TISSUE BIOCHEMISTRY FOLLOWING ORAL ADMINISTRATION IN WISTAR ALBINO RATS

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ABSTRACT

The effect of daily oral administration of flumethrin on the tissue enzyme activity on albino rats was investigated. In the present study twenty albino Wister rats were used and divided into two groups. The first group (10 rats) served as the control group; the second group (10 rats) both male and female received flumethrin (1% pour on formulation) at the rate of 5 mg/kg bw orally daily for 14 days. On day 15, animals were sacrificed and liver tissue samples were collected. Flumethrin leads to increased MDA, ALT, AST levels and decreased catalase, SOD and protein level. The present study suggests that flumethrin is having hepato-toxic effect, producing oxidative stress in animal's body.

KEYWORDS

Flumethrin; oxidative stress; superoxide dismutase; lipid peroxidation; aspartate transaminase; alanine transaminase

1. INTRODUCTION

Pesticides include insecticides, acaricides, rodenticides, fungicides herbicides and fumigants. Synthetic pyrethroids are most widely used ecto-paraciticides today as they are found comparatively less toxic to animals than other insecticides (Deo and Krishna kumary,1991). Synthetic pyrethroids are classified into two groups viz., non cyano (Type I) and cyano (Type II) groups containing pyrethroids. Type II pyrethroids includes flumethrin, cypermethrin, deltamethrin, fenvalerate etc. Flumethrin is a lipid soluble insecticide used to control ecto-parasites on cattle, sheep, goats, horses and dogs (Hayes *et al.,* 1991). 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-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 fibre producing killing effect on insects (Hayes *et al.,* 1991).

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 P_{450}-dependent monooxygenases and UDP-glucuronosyltransferase as well as on antipyrine disposition in male Wistar rats and concluded that flumethrin exposure diminishes hepatic enzyme levels and catalytic activities of monooxygenase systems as well as oxidative metabolism of antipyrine.

Keeping in view of above, the present research work has been undertaken with following objective:

To study the effect of Flumethrin on tissue bio-chemistry following daily oral administration for 14 days in albino Wister rats.

2. MATERIALS AND METHODS

2.1. Test compound:

Flumethrin

rade name : TIKKIL (1% pour on formulation)

Manufacturer: Makam pharmachem, A-81, 6th Milan, IInd stage, peenya ind. Estate, Banglore-560058, India.

2.2 Expeimental animal

Twenty Adult Wistar albino rats of either sex (100-150g) were obtained from a registered 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 7 days before beginning of the experiment. All the experimental protocol´s were approved by the Institutional Animal Ethical Committee (IAEA) and were in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of` Forests and Environment, Government of India. Twenty male and female rats were

divided into two groups (I and II) each consisting of ten animals.

2.3. Dose and Mode of application

Group I (control) was treated with vehicle (Tween 80). In group II flumethrin (1% pour-on formulation) was given orally at a dose rate of 5 mg/kg body weight per day for 14 days.

2.4. Collection of sample

Experimental rats were anaesthetized with chloroform vapor and dissected on 15th day of the experiment. A portion of the liver was collected for estimation of tissue protein and various biochemical parameters like lipid peroxidation, superoxide dismutase and catalase activity. A small portion of liver was also collected for estimation of aspartate and alanine transaminase.

2.5. Tissues biochemical parameters

Preparation of tissue homogenates for the estimation of catalase, superoxide dismutase, lipid peroxidation, alanine amino transaminase (ALT), aspartate amino transaminase (AST) and total protein.

A part of tissue was minced with stainless steel blade, washed in chilled distilled water and blotted. Tissue homogenate (10%) was prepared with chilled distilled water by using tissue homogenizer (Remi RQ127A). The crude homogenate (10%) was divided into two portions. To one portion of homogenate, required amount of chilled distilled water was added in the ratio of 1:1, so that the final concentration of homogenate would be 5%. The tissue homogenate (5%) was centrifuged in refrigerated centrifuge (Remi C 24) at 4^{0}C at 6000 rpm for 20 minutes. The supernatant was utilized for the estimation of catalase (Bergmayer, 1984) total protein, (Wooton, 1974), AST and ALT (Yatazidis, 1960)

A portion of tissue homogenate (10%) was utilized for estimation of superoxide dismutase (Misera and Fridovich, 1972).

Lipid peroxidation level

A part of liver tissue was minced with stainless steel blade, washed in chilled distilled water and blotted. Tissue homogenate (10%) of liver was prepared by adding 0.1 ml of 2% Butylated hydroxy toluene (BHT) in ethanol and 8.9 ml of 1 mM of ethylene diamintetra acetic acid (EDTA) to 1gm of tissue. Above homogenate (4 ml) was taken in another test tube to which required amount of 10% trichloroacetic acid was added, and then kept in refrigerator for 10 min. Then this cooled mixture was centrifuged (Remi C 24) at 4^{0}C at 6000 rpm for 20 min. The supernatant was collected for the estimation of lipid peroxidation. Estimation of lipid peroxidation of liver tissue homogenate (10%) was carried out according to the method of Buege et al, 1976 and Nair and Turner, 1984.

Reagents:

i) Trichloroacetic acid (10% w/v)
ii) Thiobarbituric acid (0.67% w/v)
iii) Ethylene diamine tetraacetic acid disodium salt-1mM
iv) Butylated hydroxy toluene (BHT)- 2% in ethanol (w/v)
v) Malonaldehyde – 25 μm in distilled water
i) Ethanol

Procedure: Supernatant fraction (2 ml) of tissue homogenate (10%) was mixed with an equal volume of thiobarbituric acid (0.67% w/v) and placed in a boiling water bath for 10 min. The sample was cooled by placing in ice bath for 5 min and then absorbance was read at 532 nm. wavelength using UV-VIS spectrophotometer (Systronic-105). Then the concentration of malonaldehyde was calculated from the standard curve and expressed as n mol of malonaldehyde /gm of wet tissue.

Preparation of standard curve

A standard curve was prepared with concentration of malonaldehyde varying from 0.5 to 20 n mole. The concentrations were plotted against optical density on a graph paper

and the curve was subsequently utilized for measuring of unknown samples. The linearity of the standard curve was found to be maintained upto 10 nmole of malonaldehyde.

Catalase activity

Catalase activity in liver tissue homogenate (5%) was measured by the method described by Aebi (1974) using UV-VIS Spectrophotometer.

Reagent:

i) Sodium phosphate buffer (100 nM, pH-7).
ii) Hydrogen peroxide (420 mM) solution was standardized spectrophotometrically at 240 nm, using a molar extinction coefficient of E_{240} = 43.6 $M^{-1}cm^{-1}$.

Procedure: The samples (5% liver tissue homogenate) were diluted with cold 100 mM phosphate buffer to achieve a twenty fold dilution. Each 0.1 ml of the diluted sample was then placed in reference and test cuvettes. In the reference cuvette 1.5 ml of phosphate buffer (10 mM) and 1.4 ml of distilled water were added. Likewise, 1.5 ml of phosphate buffer (10 mM) and 1.3 ml of distilled water were taken in a test cuvette. The reaction was started by adding 0.1 ml of 420 mM hydrogen peroxide in the test cuvette. The decrease in absorbance at 240 nm against the reference was followed for 60 sec using UV-VIS spectrophotometer (Systronic 108). The dilution made it easy to obtain sufficient sample for the assay and to achieve a stable rate of decrease of optical density between 0.04 and 0.20 per 60 sec. Catalase activity was expressed as millimoles of H_2O_2 decomposed/min/mg of protein.

Superoxide dismutase activity (SOD)

SOD activity in liver tissue homogenate (10%) was measured by the method of Misera and Fridovich (1972) using UV-VIS spectrophotometer.

Reagent:

i) Sodium carbonate buffer (100 mM, pH 10.2).
ii) Epinephrine (10 mM, pH 2.0)

Procedure: Each 0.2 ml of the 10% liver tissue supernatant (10%) samples were placed in reference and test cuvettes. In the reference cuvette, 1.5 ml of 100 mM sodium carbonate buffer (pH 10.2) and 1.3 ml of distilled water were placed. The test cuvette was filled with 1.5 ml of 100 mM sodium carbonate buffer and 1.1 ml of water. The reaction was started by adding 0.2 ml of epinephrine in the test cuvette so that the final volume of reaction mixture would be 3.0 ml. The increase in absorbance at 480 nm against the reference was followed till the attainment of maximum optical density. SOD activity was expressed in unit per milligram of protein. One unit SOD activity is approximately 50% inhibition of auto oxidation of epinephrine.

Estimation of tissue protein

Protein content of tissue was estimated by Bi-Uret method described by Wooton, 1974.

Reagent: 1) Bi-Uret Reagent

9.0 gm of sodium potassium tartarate and 3.0 gm of pentahydrate copper sulfate ($CuSO_4$. $5H_2O$) were dissolved in 500 ml of 0.2 N sodium hydroxide, and potassium iodide 5 gm was added. The final volume was made upto 1000 ml with 0.2 N sodium hydroxide.

2) Egg albumin: 5000 μg ml^{-1} in distilled water.

***Procedure*:** 0.2 ml of 5% tissue homogenate and 2.8 ml of distilled water was taken in a test tube and mixed well. 5.0 ml of Bi-Uret reagent was added to make the final volume 8 ml. Instead of tissue homogenate blank contained only distilled water. The optical density was read at 540 nm in Spectrophotometer (Systronic 105) after 30 min.

Standard curve:

From stock solution of egg albumin (5000 μg ml^{-1} of distilled water) 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 and 1.2 ml corresponding to 500 μg to 6 mg was taken to prepare the

standard curve. The concentrations were plotted against the optical density on a graph paper and were subsequently utilized for estimation of unknown protein sample. The protein content was expressed as gm/liter.

Aspartate and alanine transaminase

Aspartate and alanine transaminase activity in liver was determined by kit based on Reitman and Frankel's method manufactured by Span Diagnostic Ltd, Surat, India.

3. STATISTICAL METHODS

The results were expressed as Mean ± Standard error (S.E.). The data were analysed statistically using general linear model with univariate data in SPSS 10.0 version of software.

4. RESULTS AND DISCUSSION

4.1. Physical parameters

Significant changes were observed in the treated groups of rat. Two animals were died 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 that is animal showed circling movement. Skin lesions were ulcerative and scabbed patches and were seen on the head, neck, shoulder girdle, and front extremities. Immediately after the start of dosing, the animals at this dose showed aggressive behaviour and frequent scratching movements.

4.2. Biochemical parameters

Lipid peroxidation

Mean values with SE of lipid peroxidation in liver of rats of groups I (control) and II on day 15 following daily oral administration of flumethrin have been shown in Table 1 and Figure 1 .

Table 1 describes that mean values of lipid peroxidation in rats of groups I and II on day 15 were 11±0.82 and 13.8±1.04 nmol of malonaldehyde/gm of tissue respectively. It is apparent from table that significant alteration was observed when group II was compared to control. Lipid peroxidation is a well-defined mechanism of cellular damage that occurs *in vivo* during toxicity and in certain disease states. Lipid peroxides are unstable markers of oxidative stress which decompose to form complex, reactive by-products like malonaldehyde**.** Manna *et al* (2004) reported an increase in lipid peroxidation in rats following oral administration of α-cypermethrin for 30 days. In present study similar results were obtained with flumethrin. Flumethrin leads to increased plasma and tissue MDA levels, serum and tissue NO levels, tissue GSH-Px activities and decreased erythrocyte and tissue SOD and CAT activities, and erythrocyte GSH-Px activity, compared to the controls ($p< 0.05$) in Wister albino rats.

Table 1

Effect on tissue biochemical parameters in liver on 15th day following daily oral administration of flumethrin for 14 days in rats

Parameters	Units	Groups (Mean±SE)	
		Group I	Group II
Lipid peroxidation	**malonaldehyde/ gm of tissue**	11±0.82	13.8±1.04*
Catalase activities	**mMH_2O_2 decomposed /min/mg of protein**	8.316±1.56	2.95±0.92*
Superoxide	**units/mg of protein**	61±1.2	35±2.01*

dismutase			
Tissue Protein	**g/L**	0.75±0.23	0.62±0.11*
Alanine transaminase (ALT)	**Units/mg of protein/ hr**	141.38±1.47	283.51±1.89*
Aspartate transaminase (AST)	**Units/mg of protein/ hr**	140.38±0.97	151.06±1.42*

Group I (Control) rats received no insecticide.
Mean value with dissimilar superscript vary significantly (P < 0.05)

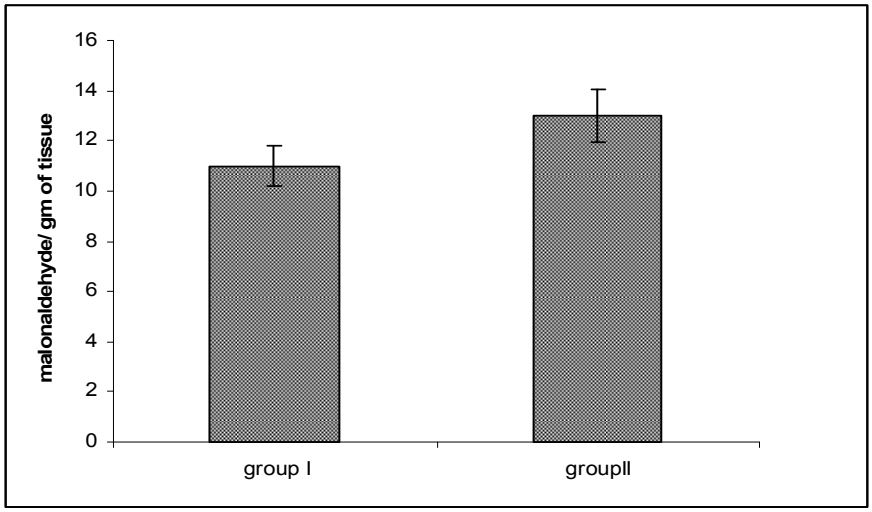

Figure1
Effect on lipid peroxidation in liver at 15th day following daily oral administration of flumethrin for 14 days in rats

Catalase

Mean values with SE of Catalase activities in liver of rats of groups I (control), and II on day 15 following daily oral administration of flumethrin have been incorporated in Table 1 and Figure 2.

Table 1 reveals that mean values of Catalase activities in liver of groups I and II 8.316±1.56 and 2.95±0.92 mMH_2O_2 decomposed /min/mg of protein respectively.

The table 1 shows that there is significant ($p<0.05$) reduction in catalase activity in group II when compared to control value. Hydrogen peroxide is a harmful by-product of many normal metabolic processes; catalase is frequently used by cells to catalyze the decomposition of hydrogen peroxide rapidly into less reactive gaseous oxygen and water molecules. Catalase level goes down during oxidative stress leading to cytotoxicity.

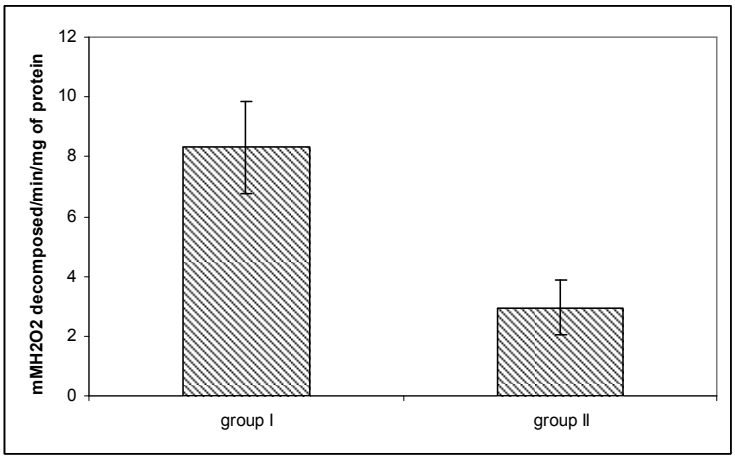

Figure 2
Effect of Flumethrin on Catalase activities (mMH_2O_2 decomposed /min/mg of protein) on 15th day following daily oral administration for 14 days in rats

Superoxide dismutase

Mean values with SE of superoxide dismutase activity in liver of rats of groups I (control), and II on day 15 following daily oral administration of flumethrin have been incorporated in Table 1 and Figure 3. The mean values of superoxide dismutase activities in rats of group I and II on 15th day were 61±1.2 and 35±2.01 units/mg of protein, respectively. It is evident from the table that superoxide dismutase activity reduced significantly ($p<0.05$) in groups II when compared to control group. Superoxide dismutases are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Inhibition of superoxide dismutase activity indicates the inability of the liver to get rid of the insecticide induced toxic radicals leading to their accumulation and subsequent cytotoxicity.

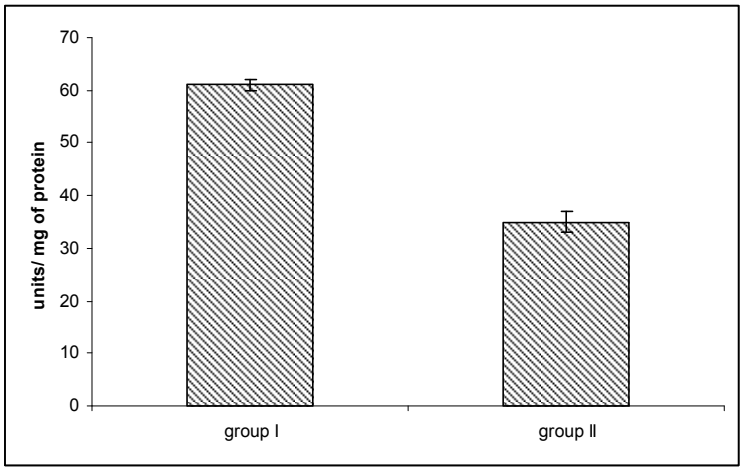

Figure 3
Effect of Flumethrin on superoxide dismutase (units/mg of protein) on 15th day following daily oral administration for 14 days in goats

Tissue Protein

Mean values with SE of protein in liver of rats of groups I (control), and II on day 15 following daily oral administration of flumethrin have been incorporated in Table 1 and Figure 4.

Table 1 depicts that mean values of protein in liver of rats of groups I and II were 0.75±1.63 and 0.62±2.31 g/L, respectively.

It is adduced from table 1 that value of protein activity in liver was decreased significantly ($p<0.05$) in group II compared to control group.

The results suggest that flumethrin have some effect on tissue protein. Reduction in protein content may be correlated with effect on immune system. Manna *et al* (2004) also observed hypoproteinemia in rats following oral administration of cypermethrin, a synthetic pyrethroid.

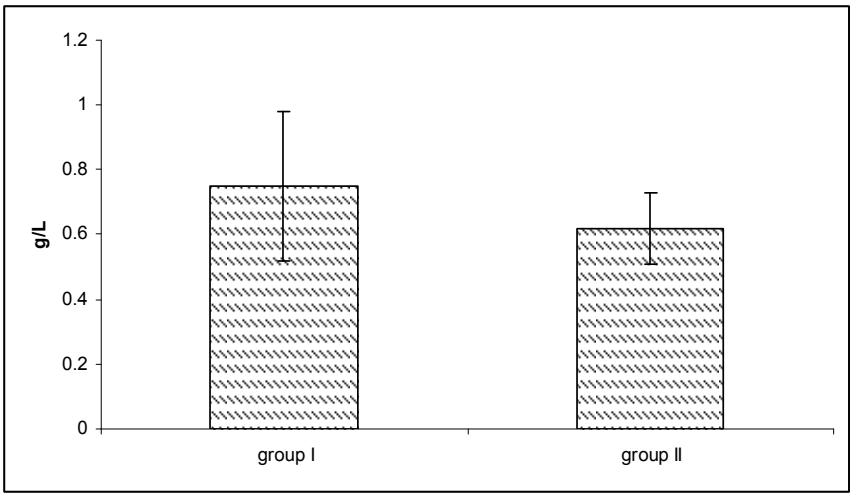

Figure 4
Effect of Flumethrin on tissue protein (g/L) at 15th day following daily oral administration for 14 days in rats

Alanine transaminase (ALT) and Aspartate transaminase (AST)

Mean values with SE of ALT in liver of rats of groups I (control), and II on day 15 following daily oral administration of flumethrin have been incorporated in Table 1 and Figure 5. Table 1 depicts that mean values of ALT in liver of rats of groups I and II were 141.3±1.47 and 283.51±1.89 U/mg of protein/hr respectively,

It is adduced from table 1 that value of ALT activity in liver was increased significantly ($p<0.05$) in group II compared to control group. The results suggest that flumethrin have some effect on ALT activity in liver.

Mean values with SE of AST in liver of rats of groups I (control) and II on day 15 following daily oral administration of flumethrin have been incorporated in Table 1 and Figure 6.

It is apparent from the Table 1 that mean values of AST in liver of rats in groups I and II were 140.38±0.97 and 151.06±1.42 U/mg of protein/hr, respectively.

Table 1 reveals that value of AST activity in liver was increased significantly ($p<0.05$) in group II compared to control group.

Increased serum ALT or AST activity is particularly marked in liver damage due to hepatotoxic drugs or chemicals (Wooton, 1974), tissue damage involve cellular necrosis or increased membrane permeability due to which there is increased leakage of AST or ALT in hepatocytes. Manna *et al*., (2004) also observed increase activity of ALT in rats following oral administration of alpha cypermethrin, a synthetic pyrethroid.

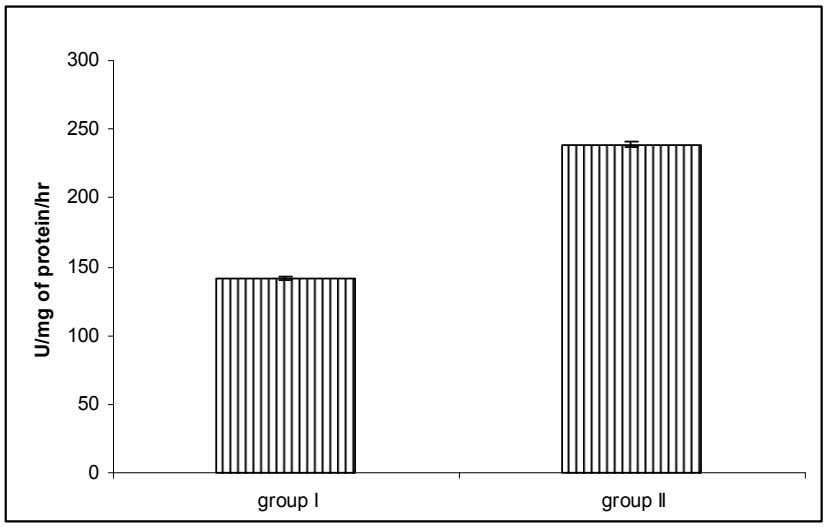

Figure 5
Effect of Flumethrin on Alanine transaminase activities(U/mg of protein/ hr) on 15th day following daily oral administration for 14 days in rats

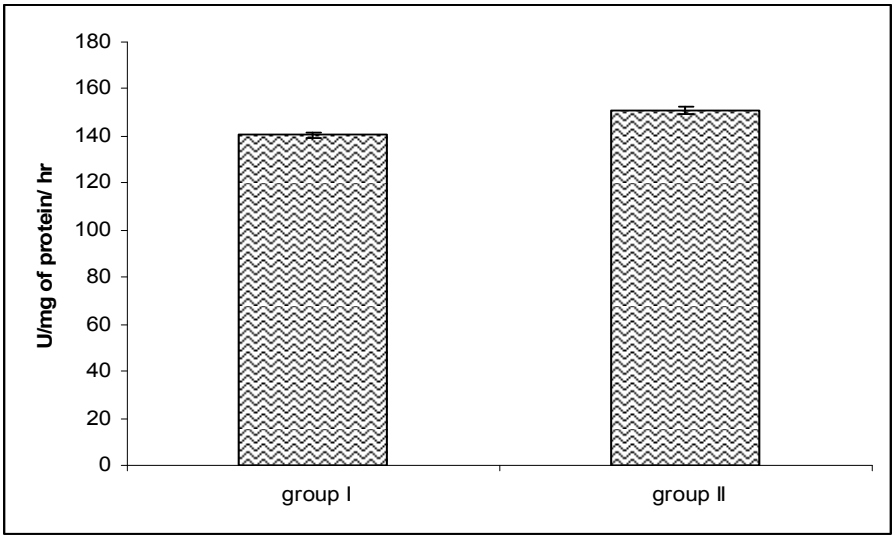

Figure 6
Effect of Flumethrin on Aspartate transaminase activities (U/mg of protein/hr) on 15th day following daily oral administration for 14 days in goats

5. CONCLUSION

Thus, it can be concluded from the above findings that the oral administration of flumethrin in rats may produce mild central nervous symptom while, skin lesion indicates accumulation of flumethrin in subcutaneous fat.

The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification of xenobiotics. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue SOD and catalase activities. In addition, serum levels of many biochemical markers like ALT and AST are elevated. In the present study, there was significant change in the levels of hepatic enzymes ALT and AST in flumethrin treated group of rats as compared to the control group suggesting the untoward reaction of this pesticide to the animal body

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