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RESEARCH ARTICLE
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Assessment of hemato-biochemical parameters on exposure to low level of deltamethrin in mouse model

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Abstract

Aim: In this study, sub-acute toxicity of deltamethrin on hematological and biochemical blood parameters of male albino Swiss mice was evaluated.

Materials and Methods: Generally, the maximum permissible residue level (MRL) of deltamethrin for food products lies between 0.01 to 0.5 mg/kg body weight. So the mice were exposed orally with two doses of pesticide i.e. 0.1 and 0.5 mg/kg body weight. The doses were given on a daily basis for a period of 15 days and 30 days respectively. Ground nut oil was used as control treatment. Samples of blood were collected at the end of the treatment. Hepatotoxicity was evaluated by quantitative analysis of the serum enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALKP), total bilirubin (TBIL) and serum urea. Alterations of hematological parameters were analysed by total leukocyte, differential leukocyte count and hemoglobin levels.

Results: Significant increase in the levels of hepatic enzymes (ALT, AST, ALKP) were observed for both doses, but no considerable differences were found by histological analysis. The hematological parameters showed significant alterations for 0.5 mg/kg body weight dose which is indicated by leukocytosis, lymphocytosis and neutropenia in long duration study.

Conclusions: The results indicated that even very low dose of deltamethrin can promote hematological and hepatic alterations. Thus it is imperative to do further studies on the detrimental effect of the low levels of pyrethroid commonly present in our food, which further necessitate the reduction of maximum permissible levels of residual synthetic pyrethroid levels in foods and feed.

Keywords: biochemical, deltamethrin, enzymes, hematological, mice, serum

Introduction

Deltamethrin is a broad-spectrum synthetic pyrethroid insecticide that is registered in the U.S. for direct application to a wide variety of food/feed crops, for use on stored grains, and for use in food/feed handling establishments [1]. It is commonly used pesticide in industry, agriculture and veterinary as well as for household activities. It is a synthetic derivative of pyrethrins which are obtained from the flowers of *Chrysanthemum cinerariaefolium* [2]. The neurotoxic mechanisms of deltamethrin include prolonging the opening of the voltage-sensitive sodium channels and inhibition of voltage gated chloride channels and GABAA (gamma amino butyric acid) receptors [3]. It has been employed as a substitute for organochlorines, organophosphates and carbamate insecticides. Because of its low persistence in the environment, low frequency of resistance development in insects and comparatively lower toxicity to humans and other non-target animals [4,5], it has become an insecticide of choice in most countries.

On average, 229 tonnes of pyrethroids were reportedly used annually for vector control at the global level during 2006–2007 [6]. Deltamethrin is one of the insecticides recommended by the World Health Organization (WHO) for indoor spraying [7] and is one of the insecticides being used for treatment of mosquito nets [8]. Among all pyrethroids, deltamethrin also has found wide acceptability for agricultural purposes. Even though it is already known that this insecticide is highly toxic to fish and various other aquatic organisms [9] a number of studies on the side effects of this insecticide have been reported, including neurotoxicity, immuno-suppression and allergy, hypertension and decreased testosterone levels [10]. According to some reports the liver was found to accumulate a greater concentration of metabolites since it is the major site of pyrethroid metabolism [10]. Deltamethrin was reported most significantly in the milk samples [11], vegetables [12] and even in soft drinks [13].

The oral LD_{50} of deltamethrin is 50 mg/kg in mice, so the concentration range reported to cause toxicity [14] is much higher than the concentrations (0.1 and 0.5 mg/kg/d) used in the current study. The doses given in the present study are equal to the maximum residue limits (MRLs) for deltamethrin permitted legally in animal food and feed (MRL: 0.5 mg/kg for fat, meat and liver and 0.1 mg/kg for chicken) [1]. Human beings

living near the areas where agricultural and pest control is operated would be subjected to continuous exposure to deltamethrin due to its high lipophilic accumulation in tissues [15]. Due to the extensive use of deltamethrin for public health purposes in developing countries and to check the given MRLs of deltamethrin in various animal products, it is important to investigate the subacute effects of deltamethrin on blood parameters. These parameters can serve as a useful tool for evaluation of the effects of pesticides on the cellular components of blood and vital organs of the body.

Therefore this study analysis can contribute to the assessment of deltamethrin on human health and also the habitat conditions. The mouse model is the one commonly used for studies of human toxicology, this study was aimed to evaluate the subtoxic effects of deltamethrin on hematological and biochemical parameters in mice.

Materials and Methods

Ethical approval: The experiment protocol used in this study was approved by Animal Ethical Committee.

Chemicals: The animals were treated using technical grade deltamethrin with 98.50 % purity, obtained from Kilpest India Ltd, Bhopal, India. All other chemicals used in the study were of the highest grade available commercially.

Animals: 36 Swiss albino male mice of 4 months age were used in this study. The animals were housed in special cages at an ambient temperature of 20^0 C. The animals were provided with ad libitum water and pellet feed.

Treatment and control groups: The treatment groups were randomly divided into six groups of six animals each. Desired concentration of deltamethrin was formulated in ground nut oil and was given orally to each mouse. The grouping of mice was done as, Group A: Dose 1- deltamethrin (0.1 mg/kg/day) administered for 15 days; Group A: Dose 2- deltamethrin (0.5 mg/kg/day) administered for 15 days; Group B: Dose 1- deltamethrin (0.1 mg/kg/day) administered for 30 days; Group B: Dose 2- deltamethrin (0.5 mg/kg/day) administered for 30 days. Control groups i.e. Group C_A and C_B were given only vehicle (ground nut oil) for 15 and 30 days, respectively.

Collection of blood samples: Mice from all groups were anaesthetized by intraperitoneal administration of anesthetics at 10 mg xylazine and 100 mg ketamine/kg body weight on 16^{th} and 31^{st} day of respective experiment. Blood samples were collected from the control group and the experimental groups. Blood samples were analyzed for hematological parameters. The remaining blood samples were centrifuged at 3000 rpm and the separated sera were transferred into Eppendorf tubes and analyzed for biochemical parameters.

Hematology: Hemoglobin (Hb), Total Leukocyte Count (TLC), lymphocyte and neutrophil count were estimated by following the standard protocols [16].

Biochemical parameters: 7 biochemical parameters were estimated using an auto analyzer (BIOTRAN BTR-830) and diagnostic reagent kits (Johnson & Johnson) supplied by Siemens Healthcare Diagnostics Ltd, Gujarat, India. The sera samples were used for liver function assessment by estimating the concentrations of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), and total bilirubin (TBIL), total protein (TP) and cholesterol (CHOL). Renal function was evaluated from sera samples by estimating the urea concentration.

Histopathological analysis: Samples of hepatic tissue were obtained from the animals by surgical excision following euthanasia. In all cases, a standardized 0.5 cm section of sample was removed from the same hepatic lobe. The samples were fixed using 10% neutral buffered formalin (pH 7.4), washed, dehydrated in alcohol, clarified using xylene, and mounted in paraffin blocks. The tissues were sectioned into 5 µm slices, stained with hematoxylin-eosin, and evaluated by light microscopy.

Statistical analysis: The comparison of the control group with the treated group was made by the one way ANOVA followed by Tukey test for post hoc multiple comparisons. The value of $P \leq 0.05$ was considered significant. Comparisons were done both on the basis of level of exposure i.e. dose of deltamethrin as well as duration of exposure i.e. number of days of exposure.

Results

It was observed that deltamethrin can affect hepatic metabolism and can cause important hematological alterations based upon concentration of pesticide and duration of exposure. Changes in the blood profiles in both control and deltamethrin-exposed Swiss albino mice for 15 days (Group A) and 30 days (Group B) exposure are depicted in the Table-1 and Table-2 respectively.

On comparisons between levels of exposure for 15 day exposed mice groups showed a statistically significant increase for ALT, AST and ALKP activity. Control, dose 1 (0.1 mg/kg) and dose 2 (0.5 mg/kg) groups differed significantly with each other for AST level. The dose 2 (0.5 mg/kg) group showed significantly higher ALKP level in comparison to the control group. Other parameters in Group A showed insignificant alteration in their concentrations. In Group B, ALT level was found significantly higher for both doses. ALKP and CHOL activity were found to be significantly higher in dose 1 (0.1 mg/kg) group as compared to control group in 30 day experiment, whereas for dose 2 (0.5 mg/kg) group, the difference was insignificant. There was also significant increase in the TLC for dose 2 (0.5 mg/kg) group mice as compared to dose 1 (0.1 mg/kg) group.

On comparison between different exposure group

Table-1. Changes in the blood profiles of control and deltamethrin-exposed Swiss albino mice group with 15 days exposure (n = 17).

Parameter	Group A (15 days)		
	Control (C_A)	Dose 1 (0.1 mg/kg)	Dose 2 (0.5 mg/kg)
AST (IU/L)	89.80 ± 7.99[a]	107.67 ± 6.53[a]	239 ± 39.88[a]**
ALT (IU/L)	44.8 ±1.93[b]	52.80 ± 1.74[b]*	58.6±1.90[b]**
ALKP (IU/L)	68.20 ± 4.89[c]	83.50 ± 5.27	98.67 ± 10.01[c]
TBIL (mg/dl)	0.40 ± 0.29	0.43 ± 0.03	0.67 ± 0.10**
TP (g/dl)	6.16 ±.0.41	6.25 ± 0.16	6.70± 0.27
CHOL (mg/dl)	133 ± 15.88	166.50 ± 22.59	199.17 ± 13.36**
Urea (mg/dl)	22.20 ± 3.06	24.00 ± 0.53*	20.33 ± 0.84
Hemoglobin (g %)	6.76 ± 0.34	6.50 ± 0.78	8.58 ± 0.57
TLC (cells/mm^3)	3740 ± 446.21	4891.67 ± 762.83	2983.33 ± 432.95**
Lymphocyte (%)	59.60 ± 10.07	71.50 ± 4.69	55.33 ± 4.40**
Neutrophil (%)	40.00 ±9.82	27.83 ± 4.67	44.67 ± 4.28**

[a,b,c] Means with common superscript differ significantly ($p \leq 0.05$). * Means differ significantly for Dose 1 between Group A and B ($p \leq 0.05$).
** Means differ significantly for Dose 2 between Group A and B ($p < 0.05$).

Table-2. Changes in the blood profiles of control and deltamethrin-exposed Swiss albino mice group with 30 days exposure (n = 19).

Parameter	Group B (30 days)		
	Control (C_A)	Dose 1 (0.1 mg/kg)	Dose 2 (0.5 mg/kg)
AST (IU/L)	84.67 ± 7.08	108.29 ± 7.68	115.67 ± 12.00**
ALT (IU/L)	49.8±1.89[a]	66.9± 1.27[a]*	68.33±1.38[a]**
ALKP (IU/L)	70.33 ± 2.65[b]	87.00 ± 6.03[b]	80.33 ± 2.97
TBIL (mg/dl)	0.32 ± 0.03	0.40 ± 0.05	0.43 ± 0.02**
TP (g/dl)	5.78 ± 0.16	6.03 ±0.19	6.22 ± 0.11
CHOL (mg/dl)	116.33 ± 9.12[c]	153.43 ± 11.12[c]	146.67 ± 9.07**
Urea (mg/dl)	19.00 ± 0.89	20.43 ± 1.11*	17.67 ± 1.52
Hemoglobin (g %)	6.78 ± 0.21	7.27 ± 0.35	5.83 ± 0.31
TLC (cells/mm^3)	3533.30 ± 241.75	3478.57 ±674.35[d]	5500.00± 1288.15[d]**
Lymphocyte (%)	74.67 ± 4.02	75.71 ± 3.94	79.00 ± 4.12**
Neutrophil (%)	25.33 ± 4.02	24.00 ± 3.93	21.00 ± 4.12**

[a,b,c] Means with common superscript differ significantly ($p \leq 0.05$). * Means differ significantly for Dose 1 between Group A and B ($p \leq 0.05$).
** Means differ significantly for Dose 2 between Group A and B ($p < 0.05$).

durations for the same dose level, it was observed that there was significant increase in the ALT level and decrease in serum urea [Group A: Dose 1 (0.1 mg/kg) mice compared to Group B: dose 1 (0.1 mg/kg)]. While a statistically significant decrease in AST, TBIL, CHOL and neutrophil count was observed in Group B: Dose 1 (0.5 mg/kg) mice as compared to Group B: Dose 2 (0.5 mg/kg) mice. TLC and lymphocyte count were significantly higher in Dose 2 (0.5 mg/kg) group mice exposed for 30 days.

There was no significant variation in TP and Hb levels in different exposure level groups as well as different duration of exposure groups. Histological analysis of the hepatic tissue did not reveal any significant differences between control group and for both doses and duration treatment groups (Figure-1).

Discussion

The present study was designed to investigate the adverse effects of low dose of deltamethrin insecticide exposure on hematological and biochemical parameters of male mice given repetitive oral doses of this pesticide for 15 and 30 days. A very low dose level of deltamethrin selected in this experiment was to mimic the permissible level of deltamethrin residue present in various foods and feed [17]. Pyrethroids tend to bio-accumulate in lipid compartments, becoming a potential source of human exposure through foodstuffs [18].

Liver is the first organ to face any foreign molecules that is carried through portal circulation and it is the one subjected to most damage. Transaminases (AST and ALT), ALKP and TBIL are important liver enzymes and responsible for detoxification processes [19]. These enzymes are secreted into the blood circulation during hepatocellular injury and their levels constantly increase in the blood. Changes in these enzyme levels might differ depending on the exposure time and dose. The increase in the activities of these enzymes in serum is indicative of liver, kidney and muscle damage and thus causes alteration in the organ functions [20,21].

During 15 day exposure period, deltamethrin significantly elevated serum AST and ALT enzyme levels at both dose levels in relation to control group values. Significantly increased AST level was observed in high dose group compared to low dose group. The increase in serum AST and ALT activities observed in this study is in harmony with the findings of Issam et al. [10], Vijayavel and Balasubramanian [22], Nayak et al. [23] and Rao [24]. There was statistically significant increase in serum ALKP level in high dose group as compared to control group. Yousef et al. [21] reported that the increase in the phosphatases and transaminases

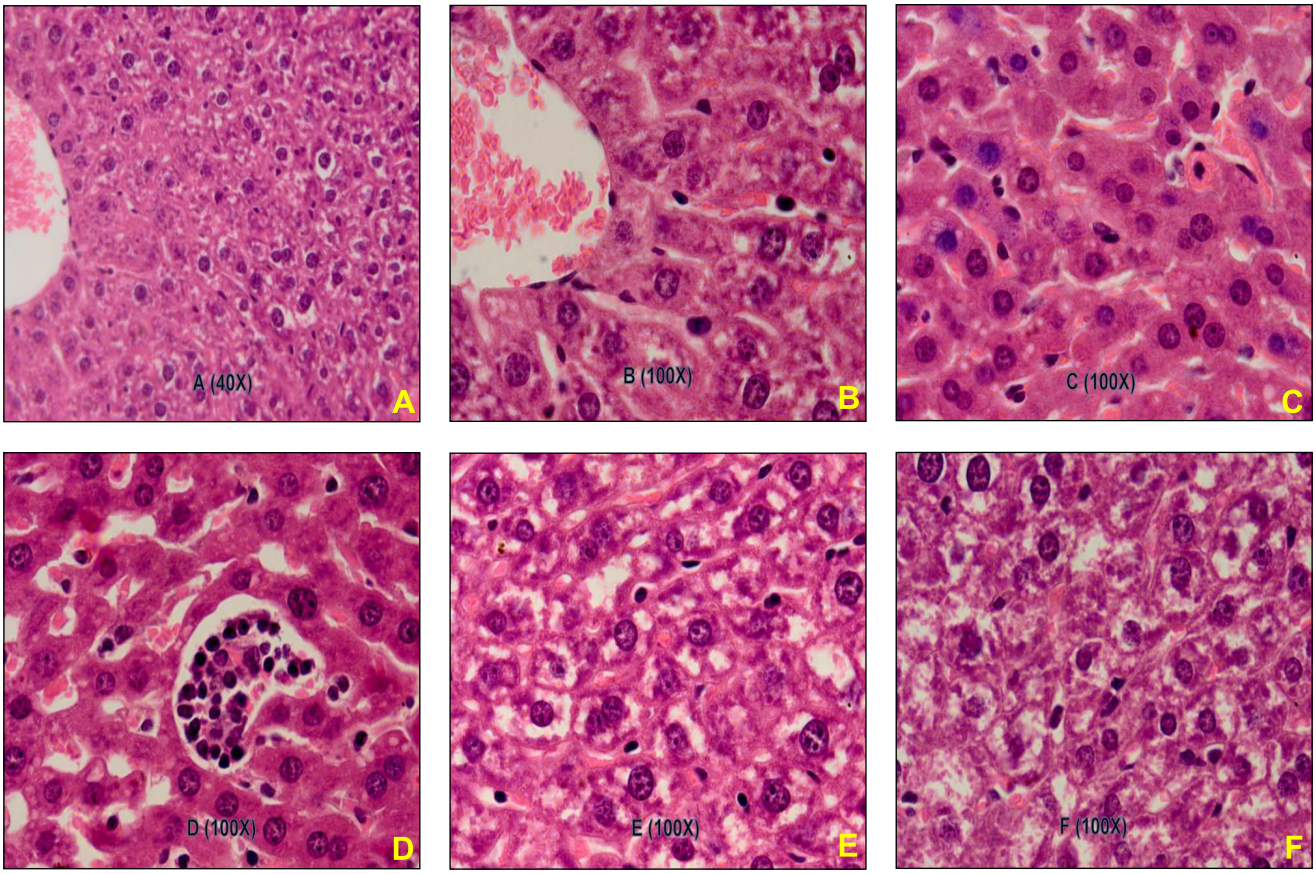

Figure-1. Histological analysis (at 40X and 100X magnification) of the liver tissues of male mice given oral treatment with deltamethrin for 15 days at a dose rate of 0.1 and 0.5 mg/kg body weight and for 30 days at a dose rate of 0.1 and 0.5 mg/kg body weight. (A) Control (40X), (B) Control (100X), (C) 0.1 mg/kg dose for 15 days, (D) 0.5 mg/kg dose for 15 days, (E) 0.1 mg/kg dose for 30 days, and (F) 0.5 mg/kg dose for 30 days.

activities in the blood might be due to the necrosis of liver, kidney and lung and the leakage of these enzymes in the blood stream corresponding to the stress levels of the treated animals. Similar results have been indicated by studies showing increased activities of transaminases in human and animals after exposure to pesticide [25,26]. Other hematological and biochemical parameters showed non-significant alterations in their activity. The probable reason might be due to the low dose of deltamethrin used and also given for a short period of time (15 day), which mainly effected liver, as liver is the first organ to encounter any foreign molecule.

In 30 day exposure group, significantly increased level of serum ALT enzyme activity found in both the dose groups compared to control group indicates liver damage. Serum AST activity was also elevated but alterations were insignificant. The increase in ALKP and CHOL level at lower dose was found to be significant in relation to control group, whereas the high dose group showed non-significant changes for both the enzymes. The significant elevation in the ALKP activity indicated damage to one or more of the organs producing this enzyme, such as the liver, kidneys, muscle and intestinal mucosa [27].

For both 15 and 30 day exposure groups, deltamethrin caused a significantly elevated leukocytes in mice administered with the high pesticide dose, whereas there was no significant change observed with respect to the percentage of peripheral blood lymphocytes and neutrophils in either case.

When comparisons were made between duration of exposure i.e. 15 days against 30 days exposure for low pesticide dose, ALT level was found to be significantly higher in 30 day group, which may indicate long duration daily exposure of even low level of deltamethrin can have detrimental effect on the liver. But serum urea level was significantly reduced in low dose group of 30 day mice group, which was also seen in caeses where reduced BUN level seen in high carbohydrate/low protein diet resulted in increased anabolic demand, malabsorption state and acute liver damage [28]. In case of 30 day high dose group, there was statistically significant decrease in activity of TBIL.

The liver processes bilirubin, it has been suggested that bilirubin is not only a waste end-product but also an antioxidant [29,30] that may protect against diseases associated with oxidative stress [31]. In several prospective studies, an inverse relationship has been reported between bilirubin and the following disease conditions: cardiovascular disease, coronary heart disease, myocardial infarction, ischemic heart disease and cancer [32]. The above findings support that the sub-acute exposure to pesticides can still have a detrimental effect on liver and other organs when an animal or human is continuously exposed to these chemicals.

There was also statistically significant decrease in activity of AST and CHOL for 30 day exposure experiment. These findings agreed with those of Bernard Lloyd et al. [33] and Ambali et al. [27] who reported decrease in the activity of AST and ALT in prolong exposure of pesticide. The reason for the low level of AST is not known and besides, the toxicological significance of this remains obscure. The deviation of normal cholesterol values in the blood serum is considered as symptoms of liver diseases [34]. In the present study the decreased cholesterol level implies liver damage, which is in accordance with increased alkaline phosphatase level discussed earlier. El-Sayed and Saad, [35] found increased level of CHOL after treatment with deltamethrin.

Assessment of hematological parameter revealed significant increase in TLC and lymphocytes (lymphocytosis) and significantly reduced neutrophil count (neutropenia). According to Santoni et al. [36], pyrethroids cause shifts in the pool of spleen and peripheral blood lymphocytes, which is manifested by lymphocytosis in the blood. The present study also showed significant lymphocytosis and neutropenia in 30 day exposure as compared to 15 day exposure to deltamethrin. Non-specific tissue irritation due to the toxicant and/or its metabolites results into formation of free radicals. Activated neutrophils may be said to play essential role in free-radical-mediated injury through the extracellular release of superoxide free radical [37], which is cytotoxic to the host cell including neutrophil itself, thereby resulting in its decreased count. These results are opposite to those of El-Sayed and Saad [35] who found significantly decreased lymphocyte and total leucocytic count in Nine Tilapia (fish) following exposure to deltamethrin.

Treating mice with deltamethrin caused slight rather non-significant variation in serum proteins and Hb level at the end of our experimental period in either dose levels or durations of exposure. This might be because of low pesticide dose or shorter duration of exposure. While El-Sayed and Saad [35] reported decrease level of TP and Hb in animals treated with cypermethrin and deltamethrin. Abbassy and Mossa [38] also found significant decrease in Hb concentration after cypermethrin and deltamethrin exposure for 90 days.

On histopathological examination of the liver, there was not any major changes were found as compared to the control samples for both doses and duration which may be due to the low dose and short duration of exposure. Similar kind of findings were reported by Jasper et al [39] that significant increase in the levels of hepatic enzymes (ALT, AST, and γ-GT) for glyphosate-Roundup® herbicide treatments without any considerable changes in histology of liver.

Conclusion

This study indicated that even very low dose and short duration of exposure of deltamethrin can cause hematological and hepatic alterations. The cumulative effect of these pyrethroids can change the function of certain targets following exposure of organisms to low doses which tends to accumulate during life. Thus it is imperative to do further studies on the detrimental effect of the low levels of pyrethroid usually present in our food and food products.

Authors' contributions

The present study was part of AT's PhD dissertation. JPSG approved the experimentation protocol. AT conducted the experiment, drafted the manuscript. JPSG critically reviewed the manuscript. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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