IJBPAS, July, 2022, 11(7): 3015-3028

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PERMETHRIN INDUCED BIOCHEMICAL ALTERATIONS IN SWISS ALBINO MICE

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Received 14th June 2020; Revised 18th July. 2020; Accepted 17th Oct. 2021; Available online 1st July 2022

https://doi.org/10.31032/IJBPAS/2022/11.7.6202

ABSTRACT

Type I pyrethroid, Permethrin is the most broadly used insecticide worldwide because of its high insecticidal potency and low mammalian toxicity. It is one of the most frequently used pyrethroid to control home pest, disease vector and protect food stuff. Although being considered safe pesticide, literature study reveals toxic implications of pyrethroid on non-target organism on various vital tissues. Therefore, it is necessary to evaluate toxic manifestation of permethrin in liver and kidney. Hence, the objective of the current investigation was to study the effect of permethrin on hepatic and renal tissues of Swiss albino mice. Animals were divided into 3 groups namely, control, vehicle control and permethrin treated. Permethrin (130mg/kg) was administered orally via feeding canula for 21 days. Gravimetric parameters revealed reduced body weight and organ weight after administration of toxicant. Biochemical parameters assayed in liver and kidney showed reduced Protein level, cholesterol content, ATPase, SDH and ALPase enzyme activity. ACPase activity was observed to increase after permethrin administration. Altered biochemical assays may lead to oxidative stress and obstruct the normal physiological function.

Keywords: Permethrin, Pyrethroid, Biochemical, Hepatotoxicity

INTRODUCTION

Pesticides are the chemicals used to control the insects and weeds population associated with variety of agricultural crops. The usage of pesticides is meant to improve the

crop yields to fulfill the food requirements of drastically increasing population of the world **[1]**. Among the most commonly used pesticides which are gaining importance are pyrethroids, synthetic analogs of naturally occurring pyrethrins, present in pyrethrum; components of extracts from the flowers of *Chrysanthemum* spp. They are mainly used to control insect pests of agriculture, horticulture, forestry and household. Pyrethroids are considered comparatively safe but their extensive and indiscriminate use makes them harmful for humans and animals **[2, 3]**. These are mainly classified as Type I and Type II depending on the presence of α cyano group.

Permethrin (3-Phenoxybenzyl (1RS)- cis, trans-3-(2,2-dichlorovinyl)-2,2 dimethylcyclo-propane carboxylate is one such synthetic pyrethroid belonging to Type I pyrethroid insecticide. It is one of the most widely used synthetic pyrethroid insecticides in agricultural, veterinary, medical, and household settings **[4]**. Other applications of permethrin include public health mosquito control programs, hair treatment for head lice infestations, and clothing impregnation as an ectoparasite repellant. Thus, human exposure to permethrin is quite likely. But Permethrin shows low toxic activity to mammals and is weakly absorbed via the skin according to few reports. The direct usage of Permethrin to control pests can result in remnant on soil and vegetables, and lead to exposure to mammals and rodents.

Although believed that Permethrin shows low mammalian toxicity, an increasing number of studies have shown that Permethrin can also cause a variety of toxicities in animals and humans, such as neurotoxicity **[5-10]**, immunotoxicity **[11, 12]**, cardiotoxicity **[13, 14]**. Genotoxic **[15-17]**, and haematotoxin effects **[18]**, fetotoxicity **[19]**, and cytotoxicity **[20]** in vertebrates and invertebrates.

Therefore, the present *in vivo* study was undertaken to gauge the effects of the said pesticide on the vital organs *viz.* liver and kidney of Swiss albino male mice after the oral administration of Permethrin for 21 days.

MATERIALS AND METHODS

Housing and care of animals

Adult, pathogen free, healthy, colony bred male albino mice (*Mus musculus*) of Swiss strain weighing between 30- 40 gm obtained from Cadila Health Care and Pharmaceutical, Ahmedabad, Gujarat, India was used in the present study. The experimental protocol was approved by the local animal ethics committee meeting under registration No. 167/GO/ReBi/S/99/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India and Committee for the Purpose of Control and Supervision of Experiments on Animals,

Chennai, India.

Chemicals and Dose

Technical grade Permethrin of 95% purity was procured from Nanjing Essence Fine chemicals, China. All the other chemicals used in different ways were procured from Merck or HiMedia.

Experimental Design

Permethrin was dissolved in Corn oil and administered via oral gavage. The dose was determined based on $LD_{50}$of permethrin in corn oil i.e., 130 mg/kg body weight **[21]**.

The animals were treated orally using a gavage. Control animals were provided distilled water and chow (*ad libitum*) and the vehicle control animals were given 0.2 ml corn oil. The duration of the treatment was 21 days.

At the end of each treatment, the animals were first weighed on digital balance (Reptech) and then were euthanized. Vital organs like liver and kidney were dissected out carefully, blotted free of blood, weighed to the nearest milligram, and utilized for study.

EXPERIMENTAL PROTOCOL

The animals were divided into following groups:

GROUPS	DOSE	DURATION	DAY OF NECROPSY
I	Control (untreated)	21 Days	Sacrificed along with scheduled treated animals
II	Vehicle (Corn oil)	21 Days	22ndpost treatment
III	Permethrin (130mg/kg)	21 Days	22nd post treatment

Gravimetric Parameters

The body weight of control and all treated groups of mice were recorded daily to the nearest milligram on a digital balance (Reptech). Similarly, weights of organs were recorded after the euthanizing, to the nearest milligram on digital balance (Citizen, Japan).

Biochemical Parameters

Protein Estimation

Protein level in liver and kidney of control, vehicle control and treated groups of animals was estimated by the method of Lowry *et al*. 1951**[22]**. Protein containing preparation when treated with phenol reagent of Folin-Ciocalteu, a deep blue colouration develops. This colour development is due to two reactions occurring simultaneously, i.e. the reaction of alkaline copper sulphate solution with peptide bonds and the reduction of phosphomolybdic and phosphotungstic acids by aromatic amino acids present in the protein. The blue colour developed is quantitatively proportional to the total protein, which is measured colorim-etrically at 540 nm.

Cholesterol

The levels of cholesterol in the liver and kidney of control, vehicle control and treated groups of mice were estimated by the method of Zlatkis *et al*., 1953**[23]**.In the presence of concentrated sulphuric acid and glacial acetic acid, cholesterol forms a

coloured complex with ferric chloride ($FeCl_3$) which can be measured on Systronics Digital Spectrophotometer 167 against blank.

Enzymatic assays

Adenosine Triphosphatase (ATPase)

The ATPase activity in liver of control and all treated groups of animals was assayed by the method of Quinn and White, 1968 **[24]**; while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow, 1925 **[25].** Readings were taken at 660 nm on a Systronics Digital Spectrophotometer 167.

Succinate Dehydrogenase (SDH)

SDH activity was measured by the method of Beatty *et al*. 1966 **[26]**.The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor INT which is reduced to red coloured formazan. After extracting it in ethyl acetate the colour intensity was measured at 420 nm against blank. SDH activity was expressed as µg formazan formed/15 minutes/mg tissue weight.

Acid Phosphatase (ACPase)

Activity of ACPase was determined by the method of Bessey *et al.* 1954**[27]**.ACPase catalyzes hydrolysis of p-nitrophenol nitrate at pH 4.8, liberating para-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow colored complex which is measured at 420 nm and is directly proportional to the enzyme activity. Enzyme activity was expressed as µ moles of p-nitrophenol released/30 minutes/mg protein.

Alkaline Phosphatase (ALPase)

Alkaline Phosphatase (ALPase) activity was determined by the method of Bessey *et al*. 1946 **[28]**.The enzyme ALPase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized condition was measured at 410 nm. Enzyme activity was expressed as µ moles p-nitrophenol released/30 minutes/mg protein.

Statistical Analysis

For each parameter, a minimum of 6 replicates were done and the results were expressed as Mean ± Standard Error (S.E.). The data was then statistically analyzed by Analysis of Variance (One way - ANOVA) by Graph-pad Prism 8.0 software. Vehicle control and Permethrin treated groups were compared with control group.

RESULTS

In the present study, Permethrin induced toxicity was evaluated from certain vital organs like Liver and Kidney of Swiss albino mice.

Gravimetric Parameters

Body weight

Non-significant changes in body weights were observed in vehicle control (Group

II), while significant reduction ($p<0.002$) was recorded in treated group (Group III) when compared to control (Group I) **(Table 1)**.

Organ weight

After 21 days duration, non-significant changes were observed in vehicle treated group (Group II) in both liver and kidney tissue weights while Permethrin treated group (Group III) revealed significant decline ($p<0.033$, $p<0.002$ respectively), when compared with control group (Group I) **(Table 2)**.

Biochemical Parameters

Total Protein

Non-significant changes in protein level was seen in vehicle control (Group II), however, significant ($p<0.001$) reduction was recorded in liver and kidney after 21 days of treatment, when compared with control (Group I) **(Table 3)**.

Cholesterol

Non -significant changes in liver and kidney was observed in Group II (vehicle control) and (Group III) Permethrin treated revealed a significant reduction ($p<0.001$) after 21 days duration, when compared to control (Group I) **(Table 4)**.

Adenosine Triphosphatase (ATPase)

ATPase activity in vehicle control (Group II) was seen to alter non-significantly. However, ATPase activity in both liver ($p<0.001$) and kidney ($p<0.033$) reduced significantly after Permethrin administration when compared with control (Group I) for 21 days duration **(Table 5)**.

Succinate Dehydrogenase (SDH)

In vehicle control (Group II) non-significant changes in SDH activity was observed and significant reduction ($p<0.001$) was recorded in both liver and kidney in Permethrin treated (Group III) after 21 days treatment, when compared with Group I (control) **(Table 6)**.

Acid Phosphatase (ACPase)

Both liver and kidney tissues showed non-significant changes in ACPase activity in vehicle control (Group II) and significant elevation in ACPase activity of liver ($p<0.002$) and kidney ($p<0.001$) in Permethrin treated group (Group III) after 21 days duration, when compared with Group I(control) **(Table 7)**.

Alkaline Phosphatase (ALKPase)

Activity of ALKPase was found to be non-significantly changed in vehicle control (Group II). ALKPase activity in liver tissue and kidney revealed a significant reduced ($p<0.033$) in Liver and kidney in Permethrin treated group (Group III) after 21 days duration, when compared with Control (GroupI) **(Table 8)**.

Table 1: Body Weight (gm) in control, vehicle control and treated mice after 21 days

GROUP	DURATION (21 days)
I (Control)	37 ± 0.9661
II (Vehicle control)	36.67 ± 0.8433ns
III (Permethrin treated)	32.17 ± 0.654**

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05level

Table 2: Organ weight (mg) in control, vehicle control and treated mice after 21 days

Group	Liver	Kidney
I (Control)	1163 ± 41.21	298.3 ± 11.26
II (Vehicle control)	1200 ± 33.68^{ns}	295 ± 13.88^{ns}
III (Permethrin treated)	1045 ± 21.94*	235.6 ± 6.54**

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 3: Total protein level (mg/100mg tissue weight) in Liver and Kidney of control, vehicle control and treated mice after 21days

Group	Liver	Kidney
I (Control)	19.00 ± 0.27	15.72 ± 0.17
II (Vehicle control)	19.09 ± 0.15 ^{ns}	15.74 ± 0.05 ^{ns}
III (Permethrin treated)	17.28 ± 0.32***	13.90 ± 0.27***

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 4: Cholesterol level (mg/100 mg tissue weight) in Liver and Kidney of control, vehicle control and treated mice after 21 days

Group	Liver	Kidney
I (Control)	1.31±0.02	1.37±0.04
II (Vehicle control)	1.32±0.02^{ns}	1.36±0.03^{ns}
III (Permethrin treated)	1.08±0.01***	1.06±0.03***

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 5: ATPase activity (µM of inorganic phosphate released / 30 minutes / mg protein) in Liver and Kidney of control, vehicle control and treated mice after 21days

Group	Liver	Kidney
I (Control)	4.27±0.12	5.21±0.21
II (Vehicle control)	4.26±0.11^{ns}	5.24±0.22^{ns}
III (Permethrin treated)	2.50±0.15***	4.38±0.25*

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 6: SDH activity (µg formazan released/ 15 min/ mg protein) in Liver and Kidney of control, vehicle control and treated mice after 21 days

Group	Liver	Kidney
I (Control)	150.3 ± 1.941	234 ± 1.748
II (Vehicle control)	154.4 ± 3.4ns	235.3 ± 1.837ns
III (Permethrin treated)	134.2 ± 1.731***	219 ± 2.028***

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 7: ACPase activity (µM of p-nitro phenol released/ 30 min/ mg protein) in Liver and Kidney of control, Vehicle control and treated mice after 21 days

Group	Liver	Kidney
I (Control)	1.272 ± 0.168	1.043 ± 0.027
II (Vehicle control)	1.297 ± 0.195ns	1.022 ± 0.017ns
III (Permethrin treated)	1.995 ± 0.038**	1.818 ± 0.042***

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

Table 8: ALKPase activity (µM of p - nitro phenol released/ 30 min/ mg protein) in Liver and Kidney of control, vehicle control and treated mice after 21days

Group	Liver	Kidney
I (Control)	0.748 ± 0.041	0.595 ± 0.021
II (Vehicle control)	0.750 ± 0.039	0.603 ± 0.014ns
III (Permethrin treated)	0.622 ± 0.021*	0.518 ± 0.015*

N=6, Values are represented as Mean ± S.E. *p<0.033, **p<0.002, ***p<0.001, ns-non-significant. Analysis of variance at p< 0.05 level

DISCUSSION

The present study was undertaken as Permethrin has been found to affect various non-target organisms at several magnitudes **[29-31]**. As liver and kidney have a central role in metabolism of xenobiotics and detoxification of toxins, they are the frequent targets of drug toxicity. This article thus details their influence upon administration of Permethrin by performing various biochemical assays after 21days.

In the current investigation, Permethrin treated male mice recorded decline in body weight as well as organ weight (Liver and Kidney). Dietary exposure has been suggested the primary route of exposure to pyrethroids and an increase or decrease of body and organ weight than normal could be considered as a preliminary sign of toxicity. Moreover, the Pyrethroid insecticides have poor feed conversion efficiency and therefore may affect the body and organs weights [**33**]. Similar findings were reported by Desai *et al*. 2017 **[34]** which showed decline in body weight due to administration of deltamethrin in mice at both the durations of 14 and 21days **[32-34]**.

Biochemical parameters are sensitive index of the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies **[35]**. In the present study, the level of protein decreased significantly in both liver and kidney upon administration of Permethrin. As protein plays an important role in cellular metabolism and regulates intra and extra cellular interactions **[36]**. Decreased protein content may result from decreased protein synthesis which may reflect the lower consumption of food in treated animals, malabsorption or hepatic dysfunction. Loss of serum protein may also occur due to hemorrhage, and exudative lesions **[37]**. Similar findings were reported by Kalendar *et al.* (2012) where long term exposure to organophosphorus insecticides lead to disturbance in protein and lipid profiles **[38]**. Moreover, in support to the present investigation Prashanth and Neelagund (2008) **[39]** also reported depletion of protein fraction in liver and kidney after administration of cypermethrin for different duration.

Cholesterol is an important body constituent used in the structure of cell

membrane, bile synthesis as well as for the synthesis of steroid hormones and vitamin D which is essential for regulation of calcium and phosphorus metabolism and bone growth **[40, 41]**. The present study revealed decrease in the level of cholesterol after permethrin administration for 21days. Other experimental studies have revealed a similar decline in the cholesterol content in gill, muscle and kidney after monocrotophos administration for varied experimental durations of several hours in *Tilapia mossambica* **[42]**.

Adenosine triphosphatase (ATPase) is an important enzyme in ATP metabolism as it hydrolyses ATP to ADP and it shows energy status of the cell. In the present study the activity of ATPase enzyme decreased in permethrin treated group in both liver and kidney. The probable reasons for the decline in the activity of the enzyme in this study could be due to changes in active sites which affects the phosphorylation and dephosphorylation mechanisms of ATPase system. Reduced aerobic oxidation and ATP generation may also be responsible for the observed reduction in ATPase activity. Similar reduction in ATPase activity was found in gills, muscles and liver of Mrigala at different concentrations after deltamethrin administration **[43]**. Reductions in ATPase activity due to various classes of toxicants have also been documented **[44]**.

SDH is an enzyme complex bound to inner mitochondrial membrane of mammalian mitochondria and membranes of bacterial cells that catalyzes oxidation of succinate to fumarate in TCA cycle and the electron transport chain. In the current study, SDH activity decreased in Liver and Kidney of treated group. Decline in SDH activity might have been caused by the stress induced, which could have changed the metabolic pathway towards anaerobic side to meet the increased and immediate energy demand. Similar decline in SDH activity were also recorded in liver of fish exposed to cypermethrin and alphamethrin for 45 days **[45]**.

Phosphatases are important and critical enzymes in biological processes, they are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function **[46]**.

Acid phosphatases are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign material; hence it has a role in certain detoxification functions. ACPase activity was found to be elevated in both liver and kidney after the administration

of permethrin in the present study. Similar studies have also recorded significant increase in the plasma ACPase activity after 30 days administration of deltamethrin in rats **[47]**. Experimental studies on albino mice have also reported elevated ACPase activity in reproductive tissue treated with cypermethrin for 4 weeks. A change in the enzyme activity is generally related to intensity of cellular damage **[48, 49]**.

Alkaline phosphatase has also been shown to be involved in active transport, glycogen metabolism, protein synthesis, secretary activity and in synthesis of certain enzymes. In the present study the activity of alkaline phosphatase is found to decrease in both Liver and Kidney. In liver inhibition of ALPase activity might be related to breakdown of glycogen for fulfilling energy requirement during stress condition. Similar decrease in the activity of this enzyme was documented by Yousef *et al*. (1998) and El- Demerdash *et al*. (2003) after the use of cypermethrin **[47, 50]**. Moreover, Bhushan *et al*. (2013) also reported reduced ALPase activity in hepatic tissue after Permethrin administration for various durations **[51]**. Membrane damage might also have caused leakage of this enzyme from hepatocytes into the blood stream thus resulting in pathological changes.

CONCLUSION

The present investigation dealt with the Permethrin toxicity on vital organs viz., Liver and Kidney of male albino mice *Mus musculus.* The results obtained show that Permethrin causes gravimetric as well as biochemical alterations in Swiss albino mice thus leading to hepato as well as renal toxicity 21 days of study duration. Therefore, the present study contradicts the so called “Safe pesticide” notation for Permethrin on mammalian tissue. This further suggests need for mitigative studies as well as stringent regulatory measures for use and handling of such pesticides to safe guard human population.

ACKNOWLEDGEMENT

Authors kindly acknowledge Department of Zoology, BMT and Human Genetics, Gujarat University, for providing various research facilities.

CONFLICT OF INTEREST

Conflict of interest declared none.

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