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ORIGINAL ARTICLE **OPEN ACCESS**

Sub-Acute Toxicity Study on the accessory Reproductive Tissues of Male Swiss Albino Mice

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ABSTRACT
Anincessant rise in population has amplified the demand of food supplies which cannot be achieved by the conventional agricultural methods. Therefore, pesticides have been introduced to overcome this shortage which help reduce the damage done by different pests and also enhance the production. However, the effects which these chemical compounds exert on the environment and living organisms still remain unexplored. Therefore, the present study was undertaken to evaluate the effects of a synthetic pyrethroid, namely Permethrin, the popularity of which is on the rise because of its broad range applications and a recent prohibition on the use of organophosphate pesticides by the competent authorities. The experimental model chosen for the study were male Swiss albino mice and Permethrin (130 mg/kg body weight) was administered to them for duration of 21 days via oral route. Gravimetric indices and non-enzymatic parameters like protein, fructose and sialic acid were assessed in the accessory reproductive tissues of mice. It was found that the body weight and organ weight were significantly lowered after the treatment period. Moreover, the protein levels were also significantly dropped which indicated damage to the cells. Furthermore, fructose and sialic acid which are important biomarkers for seminal vesicle and epididymis (caput, cauda) respectively, were also found to be declined significantly. All these indicate the toxic potential of the test compound Permethrin and the lower levels of the assessed parameters clearly point towards the damage done by the pyrethroid pesticide. The study is an important addition to toxicity studies of pesticides.
Keywords: *pesticides, pyrethroid, accessory reproductive tissues, toxicity*

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INTRODUCTION

It is very well-known that the use of pesticides has become an indispensable part of our everyday lives whether it is for household domestic purposes or the outdoor use. However, pesticides represent an important group of environmental pollutants [1].Moreover; it is of grave concern for sustainability of environment and global stability. Their use is not only restricted to agricultural fields, but they are also employed in homes in the form of sprays, poisons and powders for controlling cockroaches, mosquitoes, rats, fleas, ticks and other harmful bugs. Due to these reasons, pesticides are frequently found in our food commodities in addition to their presence in the air [2].

Pesticides have a comprehensive classification based on the target organism, the chemical compound involved and the mode of action. But, this study deals exclusively with pyrethroid class of insecticides which have recently attained popularity due to their wide range application.

These classes of insecticides are extensively employed throughout the world as wide-spectrum products for numerous crops and for indoor pest control in the public health sector and housing [3, 4].The major targets of these pyrethroids are sodium channels and their key property is to interact reversibly with a wide range of ion channels, possibly via their phosphorylation state [5, 6].Pyrethroids are synthesized derivatives of the naturally occurring pyrethrum (Chrysanthemum flowers); and Permethrin is a synthetic version of the same belonging to Type I group [7].It is also used topically in the medical treatment of scabies and diseases associated with lice and mites [8].In addition to that, in the tropics, insecticide nets are often treated with pyrethroids as part of efforts in combating the scourge of Malaria [9].

Although it has been believed that Permethrin shows low mammalian toxicity, an increasing number of studies showing toxicities in animals and humans are now available. Permethrin inhibits natural killer cells which are critical components of the immune system [10], and is both mutagenic and carcinogenic [11]. Moreover; it has been reported to cause neurotoxicity [12],immunotoxicity [13], cardiotoxicity [14], cytotoxicity [15]and haematotoxicity [16].However, it has not been much evaluated on the male reproductive system, which is the main focus of this study.

It has therefore become imperative to bridge this lacuna and hence, this *in vivo* study was carried out where Permethrin was used using oral route in the male Swiss albino mice (*Musmusculus*) for a duration of 21 days and the effect of this pesticide was gauged on the accessory reproductive tissue *viz.* caput epididymis, cauda epididymis, seminal vesicle and vas deferens.

MATERIAL AND METHODS

Animals and Chemicals

Healthy, adult, pathogen free, colony bred male albino mice *Musmusculus* of Swiss strain weighing between 30 and 40 gm, obtained from IAEC recognized supplier were used for the experiments. The experimental protocol and the number of animals used for the experiments were mentioned in a detailed proposal and approval was obtained as per the guidelines of the Institutional Animal Ethics Committee, 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. All the animals were acclimatized for seven days prior to the commencement of experiment. The animals were housed in an air-conditioned animal house at a temperature of 26°C ± 2°C and exposed to10-12 h of day light and relative humidity of40%-50%. Animals were randomized into control and treated groups and were caged separately. Standard chow (obtained from Amrut laboratory, Baroda, India) and water was provided *ad libitum*.

Test chemical Permethrin (technical grade) of 95% purity was procured from Nanjing Essence fine chemicals, China. All the other chemicals used were procured from HiMedia Laboratories, India and Sigma Aldrich (UK). All the chemicals used were of analytical grade.

Experimental design

Permethrin is considered to be readily absorbed when given orally as all pyrethroids are lipophilic; and absorption through gastrointestinal tract is higher than other routes. Hence, oral route of administration was selected for the treatment. Permethrin was administered via oral gavage dissolved in corn oil at a dose level of 130 mg /kg body weight ($1/5^{th}$ of LD_{50}). The doses were determined on the basis of LD_{50} of Permethrin in corn oil i.e. 650 mg/kg body weight [17].

The animals were divided into following groups (6 animals/group):

Group I: Control (given distilled water and food *ad libitum*);

Group II: Vehicle Control (given only corn oil);

Group III: Permethrin (PER) treated (given 130 mg/kg body weight of Permethrin dissolved in corn oil)

All the groups were treated for 21 days and at the end of the experiment, animals were weighed and euthanized using light ether anaesthesia.

Tissue collection

At the termination of the experiment, animals were dissected accessory reproductive tissues *viz*., caput epididymis, cauda epididymis, vas deferens and seminal vesicle were dissected out carefully. Tissues were weighed, processed and homogenates were prepared accordingly.

Parameters Studied

Gravimetric indices (body and organ weight):

The body weight of control, vehicle and treated groups of mice were recorded to the nearest milligram on a digital balance (Reptech). The animals were weighed before and at the end of each week prior to autopsy. Similarly, weights of organs were recorded to the nearest milligram on digital balance (Aczet).

Total protein:

Protein estimation was done using standard protocol of Lowry et al. (1951) [18].

A known weight of tissue was homogenized in a definite volume of glass distilled water. In the sample tube 0.2 ml of tissue homogenate, 0.6 ml of distilled water and 4 ml of alkaline copper sulphate solution was taken. The contents of the tube were vortex mixed. In the blank tube instead of sample, 0.2 ml of distilled water was taken. The tubes were kept for incubation at room temperature for 15 minutes. Then 0.4 ml of FolinCiocalteau phenol (diluted 1:1, Folin-phenol reagent : distilled water) was added to each tube which was thoroughly mixed. The tubes were allowed to stand at room temperature for 30 minutes. The optical density of blue colour developed was read at 540 nm on Systronics Digital Spectrophotometer 167.

Fructose:

Fructose levels were assayed in seminal vesicle by the method of Foreman *et al*. [19].

A known amount of tissue was homogenized in 5 ml of 5% Perchloric acid. 0.2 ml of homogenate was followed by 1.8 ml of 5% Perchloric acid in sample tube while blank tube was run with 2 ml of 5% Perchloric acid. 3 ml of 30% HCL was added to all the tubes and heated in water bath at 80° C for one hour and cooled at room temperature. The colour intensity was read on Systronics Digital Spectrophotometer 167 at 410 nm against blank tube.

Sialic acid:

A periodate resorcinol method by Jourdian *et al.* [20]was used for the quantitative determination of free and glycosidically bound sialic acids.

A known amount of tissue was homogenized in fixed volume of distilled water. To the sample test tube 0.5 ml of homogenate was added whereas in blank 0.5 ml of distilled water was added and in standard tube same volume of standard solution [(0.1 mg/ml) of n-acetyl neuranimic acid was added]. 0.1 ml of 0.04 M periodic acid solution was added, mixed and the tubes were allowed to stand in an ice bath for 5 minutes and the heated at 100ºC in boiling water bath for 15 minutes. The tubes were cooled and 1.25ml of tertiary butyl alcohol was added. The tubes were allowed to stand at room temperature for 3 minutes to stabilize the colour.

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 statistically analyzed by Analysis of Variance (One way ordinary - ANOVA) by Graphpad Prism 8.0 software. Vehicle and Permethrin treated groups were compared with the control group.

RESULTS

Body weight

The present study revealed significant reduction ($p<0.002$) in the body weight of treated animals (Group III) when compared to control (Group I) after 21 days. However, non-significant changes we rerecorded in vehicle group (Group II) when compared to control (Group I) [TABLE-1].

Organ weight

The present study revealed variable changes in the organ weight of accessory tissues. It was found that the weight of caput epididymis was significantly reduced ($p<0.002$) in the treated animals (Group III) when compared to control (Group I) after 21 days. However, the weight of cauda epididymis was found to be non-significant in the treated group (Group III) on comparison to control (Group I). Moreover, the weight of seminal vesicle and vas deferens also showed highly significant reduction ($p<0.001$) in the treatment group (Group III) when compared to control (Group I). Non-significant changes in the accessory tissues were recorded in vehicle group (Group II) when compared to control (Group I) [TABLE-2].

Total protein

The existing study showed highly significant reduction ($p<0.001$) in the protein levels of the seminal vesicle in the treatment group (Group III) when compared to control (Group I). Likewise, protein levels in vas deferens of the treated group (Group III) also showed significant decline ($p<0.002$) on comparison to control (Group I) after 21 days of duration. However, the vehicle group (Group II) showed non-significant changes for both the tissues on comparison to control (Group I)[TABLE-3].

Fructose

In the present investigation, permethrin treated mice (Group III) showed a high significant reduction ($p<0.001$) when compared to control (Group I), however, non-significant changes in fructose levels of seminal vesicle was observed in vehicle group (Group II) after the period of 21 days [TABLE-4].

Sialic acid

After 21 days of Permethrin administration, treated group (Group III) of caput epididymis revealed a highly significant decline ($p<0.001$) and cauda epididymis showed a significant decline ($p<0.002$) when compared to control (Group I), whereas, non-significant reduction in sialic acid concentration in both cauda and caput epididymis were witnessed in vehicle group(Group II) [TABLE-5].

TABLE 1: Body weight (gm) of control, vehicle and treated mice after 21 days.

GROUPS	DURATION (21 DAYS)
	Body Weight
Control (Group I)	37.00 ± 0.9661
Vehicle (Group II)	36.67 ± 0.8433^{ns}
PER treated group (Group III)	32.17 ± 0.6540**

Values are mean ± S.E., *p<0.033, **p<0.002, ***p<0.001, NS=non-significant

TABLE 2: Organ Weights (mg) in control, vehicle and treated mice after 21 days.

GROUPS	DURATION (21 DAYS)			
	Caput Epididymis	Cauda Epididymis	Seminal Vesicle	Vas Deferens
Control (Group I)	45.7 ± 5.021	30.62 ± 1.143	322.1 ± 6.17	41.02 ± 0.621
Vehicle (Group II)	47.28 ± 0.818 ns	30.78 ± 0.671 ns	316.1 ± 6.49 ns	40.76 ± 0.734 ns
PER treated group (Group III)	40.8 ± 0.887 **	28.2 ± 1.283 ns	202.6 ± 14.41 ***	29.34 ± 1.573 ***

Values are mean ± S.E., *p<0.033, **p<0.002, ***p<0.001, NS=non-significant

TABLE 3: Total protein concentration (mg/100 mg tissue weight) seminal vesicle and vas deferens of control, vehicle and treated mice after 21 days.

GROUPS	DURATION (21 DAYS)	
	Seminal Vesicle	Vas deferens
Control (Group I)	24.44 ± 0.121	16.64 ± 0.094
Vehicle (Group II)	23.61 ± 0.388 ns	16.58 ± 0.088 ns
PER treated group (Group III)	11.81 ± 0.322***	11.08 ± 0.732**

Values are mean ± S.E., *p<0.033, **p<0.002, ***p<0.001, NS=non-significant

TABLE 4: Fructose level (µg/ mg tissue weight) in seminal vesicle of control, vehicle and treated mice after 21 days.

GROUPS	DURATION (21 DAYS)
	Seminal Vesicle
Control (Group I)	65.22 ± 0.604
Vehicle (Group II)	65.34 ± 0.916 ns
PER treated group (Group III)	54.96 ± 0.696 ***

Values are mean ± S.E., *p<0.033, **p<0.002, ***p<0.001, NS=non-significant

TABLE 5: Sialic acid concentration (µ/ 100 mg tissue weight) in caput epididymis and cauda epididymis of control, vehicle and treated mice after 21 days.

GROUPS	DURATION (21 DAYS)	
	Caput Epididymis	Cauda Epididymis
Control (Group I)	1.715 ± 0.046	1.353 ± 0.034
Vehicle (Group II)	1.690 ± 0.034 ns	1.277 ± 0.030 ns
PER treated group (Group III)	0.858 ± 0.038 ***	1.040 ± 0.050 **

Values are mean ± S.E., *p<0.033, **p<0.002, ***p<0.001, NS=non-significant

DISCUSSION

Exposure to environmental toxicants including pesticides is a proven factor in impairment of male reproductive system and infertility [21].The present study here focuses on the effect of a chiral pyrethroid pesticide, Permethrin on the accessory tissues viz. caput and cauda epididymis, vas deferens and seminal vesicle of the male reproductive system after the exposure of 21 days.

A gravimetric index is an important criterion for toxicological studies and alteration in body weight can be considered as a sign of toxicity [22]. The present investigation evaluated both the body weight and organ weight of the different groups after the pesticide treatment. The body weight was found to be decreased in the present evaluation which may be due to the properties of Permethrin responsible for poor feed conversion efficiency. Reduced body weight might also be the consequence of direct cytotoxic effect of the pesticide on somatic cells or indirectly through the central nervous system which controls the feed and water intake and regulates the endocrine function [23].Results are in agreement with the previous report which has also noticed reduced body weight in male rats after technical grade formulations of deltamethrin andcypermethrin [24].Mohapatra and Malick [25] also showed similar reduction in body weight when Deltamethrin was fed to Broiler Chicks.

Maina *et al.* [26]have also stated that increase or decrease in organ weight after toxicant treatment is the sign of toxicity of that drug or chemical. The present investigation revealed decline in the weight of

accessory reproductive tissues after Permethrin administration for a period of 21 days. Reduction in the weight of epididymis observed might be attributed to diminished testosterone level, reduced tubular size or decreased number of spermatozoa [27, 28]. Reduction in weight of vas deferens reflected reduced sperm count and androgen level. The results of Desai *et al*. [29] corroborate with our findings where the weight of testis, cauda epididymis and seminal vesicle decreased upon administration of Deltamethrin after 45 days. Similar results were confirmed by Arena *et al.* [30] where testis and epididymis weight were decreased after 30 day exposure to Fenvalerate, a pyrethroid pesticide. Bal *et al*. [31] also reported similar findings where Imidacloprid a (neonicotinoid pesticide) affects the reproductive organ of male rats by decreasing the mass of accessory sex organs.

Proteins of a cell can be considered as an essential parameter to determine the physiological status of animal [32].Moreover, Neeraja and Giridhar [33] also reported that pesticides interfere in the mechanism of protein synthesis and degradation which results into altered dynamic equilibrium. The present study revealed decline in the protein content of the seminal vesicle and vas deferens after permethrin administration for 21 days which might be due to altered activity of antioxidants and impaired protein synthesis[21].Similar results were obtained by Begum [34] where Carbofuran decreased the total protein content in liver and muscle tissues of the fish. Decreased protein level in different organs of fish after pesticide treatment was also noted by El-Sayed *et al*. [35] and Abdelkhalek *et al*. [36] which is in support of this study.

CONCLUSION

The study carried out confirms that permethrin has toxic potentials and causes reproductive toxicity on the accessory tissues of the male reproductive system. This infers that the use of these pyrethroids for a longer duration might also give rise to infertility which has recently witnessed a rise in reports. Moreover, the present investigation throws a striking limelight on the toxicity of pesticides which have got so called "safe" reputation among the chemical compounds. It is therefore imperative to carry out further detailed analysis regarding these chemicals in different experimental models and also search for potent antioxidants to combat the said toxicity. Furthermore, stringent laws and policies should be framed by the competent authorities and the overuse of these hazardous substances should be properly monitored.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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