Original Article

A Morphometric and Histological Study of the Kidney of Mice after Dermal Application of Cypermethrin

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Abstract

Objective: To assess dose related toxic effects of Cypermethrin, a new synthetic pyrethroid insecticide, on the mammalian kidney.

Methods: The study was conducted at Khyber Medical College, Peshawar, in the year 2001, 2002. Adult albino mice (6-8 weeks old) were used for the study. Cypermethrin was administered in doses of 15mg / kg body weight and 30 mg / kg bodyweight as a single daily dermal application for a period of 6 weeks. At the end of the 6th week, all the animals were sacrificed, their kidneys were dissected out and fixed in 10% neutral buffered formalin and processed for histological studies.

Results: There was congestion of vessels and marked lymphocytic infiltration (more than 50 lymphocytes / HPF) in the kidneys of the experimental group receiving 30 mg / kg body weight of Cypermethrin, while no abnormality was observed in the animals receiving 15mg / kg body weight of Cypermethrin and in the animals of the control group.

Conclusions: Cypermethrin is toxic to mammals. It causes marked lymphocytic infiltration and congestion of vessels in the kidneys. The industrial workers exposed to Cypermethrin are also liable to get similar nephrotoxicity, after repeated exposure. However, the magnitude of the injury and its reversibility have not been assessed in the humans. This pilot project has established nephrotoxicity of Cypermethrin after dermal exposure, a route that predisposes industrial workers to toxicity (JPMA 57:587;2007).

Introcuction

The use of present day insecticides began after the World War II. The use of insecticides ushered a new era of increased agricultural production.[1] However, the injudicious use of insecticides has caused serious health problems to non-target organisms including man. The present study was designed as an effort to highlight the toxic hazards of Cypermethrin to mammals. Cypermethrin is a neurotoxin. It acts by increasing the open time of sodium channels leading to prolonged membrane depolarization, enhanced neurotransmitter release and repetitive neuronal activity; eventually resulting in the depletion of the neurotransmitter and block of excitation in the nerve.[2,3] Poisoning is always due to accidental spillage during spray operations. The oral LD 50 for Cypermethrin in mice varies from 82 to 779 mg/kg body weight. The dermal LD50 in rats is 1600 mg/kg and in rabbits is greater than 2000 mg/kg.[4] It is widely claimed that pyrethroids have low toxicity to mammals.[5,6]

Male Swiss albino mice were given 12.5, 25, 50, 100, 200 mg/kg BW of Cypermethrin intraperitoneally, daily for 5 consecutive days. A statistically significant ($p < 0.05$) dose-dependent increase in DNA damage was observed. Brain showed maximum DNA damage followed by spleen > kidney > bone marrow > liver > lymphocytes. It is concluded that cypermethrin induces systemic genotoxicity in mammals as it causes DNA damage in vital organs like brain, liver, kidney, apart from that in the haematopoietic system.[7] Cypermethrin was administered orally to New Zealand white rabbits for a period of fifty days, at a dose of 140 mg/kg bw per day equivalent to 1/10 of the LD_{50}. Clinically, dullness, anorexia, reduced feed and water intake, diarrhoea, hyperirritability, salivation, coarse tremors, movement of the eyeballs, dilation of the pupils, weakness, paralysis of the hindlimbs, gasping and tonic-clonic convulsions were observed. A decrease in body weight was observed in the treatment group.The histopathological findings were vascular congestion, hydrophic degeneration and leukocytic infiltration in the affected organs at the initial stages. At the terminal stage of toxicosis, coagulative necrosis, perivascular/periductal fibrocellular reaction along with mononuclear cellular infiltration in the liver, mucosal eruptions with inflammatory reaction in the gastrointestinal tract and hyalinization of the tubular epithelium of the kidneys were observed. The lymphoid population in the splenic follicles and Peyers patches was depleted. The seminiferous tubules showed degeneration/denudation of the epithelium and reduction in the number of matured spermatids.[8] Wistar male rats were orally fed with laboratory chow combined 60, 150, and 300 mg/kg Kral 250 EC during 28 consecutive days. At the end of the treatment, no significant change was

found in relative liver weights, liver total proteins and cholinesterase enzyme activities of cypermethrin treated rats, when compared with control animals. Histopathological changes such as vacuolar degeneration, enlargement of the sinusoids, degeneration in hepatic cords and hepatocytes, vacuole formations in hepatocytes, pleomorphism in nucleus, and congestion were observed in liver tissues of only 150 and 300 mg/kg cypermethrin treated rats. Mononuclear cell infiltration and an increase in the Kupffer cells in liver parenchymatous tissue were also determined. In all cypermethrin treated groups, the apoptotic index in livers of rats was significantly increased compared to the control group ($p < 0.001$). These results suggest that cypermethrin might cause hazardous effects in different levels to non-target organisms.[9] Cypermethrin was applied dermally to rats for 28 days in two doses, 50 mg/kg and 250-mg/kg-body weight. Changes in the liver occurred only after administration of 250 mg/kg and were confined to increased porosity of the cytoplasm of hepatocytes situated under the capsule of the organ. Changes in the kidney were also observed after administration of 250mg/kg. These included degenerative changes in the epithelial cells of the proximal tubules such as widening of endoplasmic reticulum and swelling of mitochondria. Thickening of the basal lamina of proximal tubules was also observed. In the brain, pyknosis of Purkinje cell nuclei was noted in the cerebellum and some of the purkinje cells disappeared.[10]

Materials and Methods

In this study, 30 adult albino Swiss mice (6-8 weeks old) weighing 27.8-33.5 gm were procured from the Veterinary Research Institute, Lahore and were kept at the animal house of Postgraduate Medical Institute, Lahore. They were fed on commercial diet and water ad libitum. The animals were provided optimal light and temperature.

After two weeks of acclimatisation, animals were randomly divided into three groups; A, B and C, each group comprising of 10 animals. Group A was the experimental group receiving 30 mg of Cypermethrin as a dermal application, group B was the experimental group receiving 15 mg of Cypermethrin as a dermal application while group C was the control group receiving 0.08 ml of 50 % ethanol as a single daily dermal application. The animals were weighed at the start of the experiment and then weekly till the end of the experiment.

At the end of the experimental period i.e. six weeks, all the animals were sacrificed; their kidneys were dissected out, examined macroscopically and weighed. The kidneys of all the animals were washed in normal saline and fixed in 10% neutral buffered formalin. After histological processing the histological sections were stained with haematoxylin, eosin and PAS. The slides were then examined under the light microscope.

Results

Mice in the control group "C" remained healthy and active throughout the experimental period. No morbidity or mortality was seen in this group.

The animals in the experimental groups, 5-10 minutes after application of the drug started scratching and biting at their tails. One of the animals in the group "A" nipped off its tail. Two to three days later, the site of application of Cypermethrin on the tail of mice became red and swollen. Apart from this, the animals of the experimental group "B" remained active and healthy throughout the experimental period. Forty to forty-five minutes after application of the drug, the animals of group "A" became irritable and quarrelsome. After a while, some of the animals showed abnormal movements of the head (side to side movements). The condition of the animals improved over the next two to three hours. In about a week's time the animals of the group "A" became less active and lethargic. No mortality was observed in the experimental groups.

Animals in the control group "C" showed statistically significant weight gain while the animals in the experimental groups also showed weight gain. The weight gain in the experimental group "A" was statistically insignificant while the weight gain in the experimental group "B" was statistically significant.

Kidneys of all the animals of the control as well as the experimental groups were paired structures located on the posterior abdominal wall, one on either side of the midline. The kidneys were reddish brown in colour and were covered by a thin glistening capsule, which was not adherent to the surrounding tissues

All the kidneys were bisected longitudinally and examined with the help of a magnifying glass. Each kidney was unipyramidal i.e. comprising of outer cortex and inner medulla, consisting of a single pyramid. The pelvis was a single cavity with no subdivisions into major and minor calyces.

In the experimental groups there was an increase in the relative kidney weight. It was statistically significant in the experimental group "A" while in the experimental group "B" the change was not significant.

Control group showed normal renal architecture. The morphometric analysis of the different components of the kidneys of this group are given in Table.

No change was observed in the vasculature. There was diffuse lymphocytic infiltration in some of the kidneys of the control group. Experimental groups "A" and

Table. Comparison of the mean values of histological observations of the kidney of mice of the experimental groups with the control group.

Parameters		Experimental Group A	Experimental Group B	Control Group C
Renal Corpuscle	Diameter	X59.1 7.404 µ	X58.1 9.002 µ	X60.1 5.58 µ
	Urinary Space	X10.86 0.92	X 10.02 1.92	X 10.57 1.19
Proximal Convoluted Tubule	Necrosis	Nil	Nil	Nil
	Basal lamina	Normal	Normal	Normal
	Casts	Nil	Nil	Nil
	Diameter	^{x}30.63 4.1	^{x}31.84 3.93	^{x}28.9 5.07
Loop of Henle	Normal /Atrophic	Normal	Normal	Normal
	Necrosis	Nil	Nil	Nil
	Basal lamina	Normal	Normal	Normal
	Casts	Nil	Nil	Nil
	Diameter	^{x}17.15 2.74	^{x}17.5 1.744	^{x} 17.3 1.85
Distal Convoluted Tubule	Normal /Atrophic	Normal	Normal	Normal
	Necrosis	Nil	Nil	Nil
	Basal lamina	Normal	Normal	Normal
	Casts	Nil	Nil	Nil
	Diameter	^{x}32.1 3.65	^{x}34.55 1.542	^{x}34.06 5.98
Collecting Duct	Normal /Atrophic	Normal	Normal	Normal
	Necrosis	Nil	Nil	Nil
	Basal lamina	Normal	Normal	Normal
	Casts	Nil	Nil	Nil
Stroma	Infiltration	Marked	Insignificant	Insignificant
	Haemorrhages	Nil	Nil	Nil
	Congestion	Marked	Insignificant	Insignificant

Values are expressed as mean + standard deviation.
X P > .05 difference insignificant.
* P < .05 difference significant.
** P < .01 difference considerably significant.
*** P < .001 difference very significant.
For statistical significance, experimental groups have been compared to their respective control group.
Marked infiltration More than 50 lymphocytes / HPF
Moderate infiltration 10 - 49 lymphocytes / HPF
Insignificant infiltration Less than 10 lymphocytes / HPF

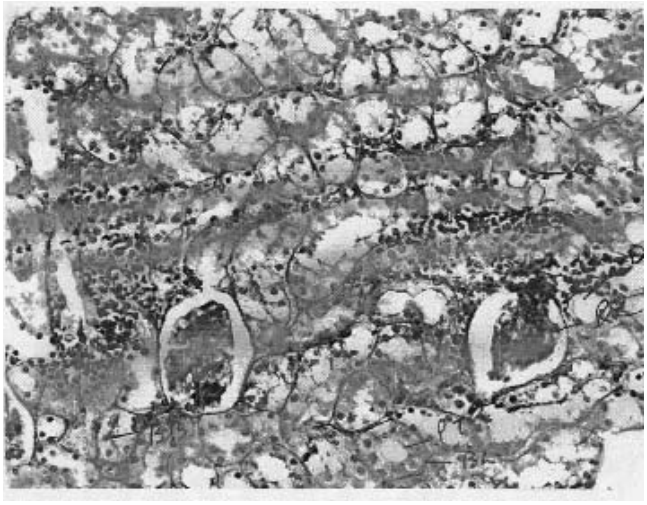

Figure 1. A photomicrograph of the histological section of the mouse kidney of the experimental group A. PAS stain. Magnification 700 X. Showing RC: renal corpuscle, PT: proximal tubule, BB: brush border, DT: distal tubule, L: lymphocytic infiltration, BL: basal lamina

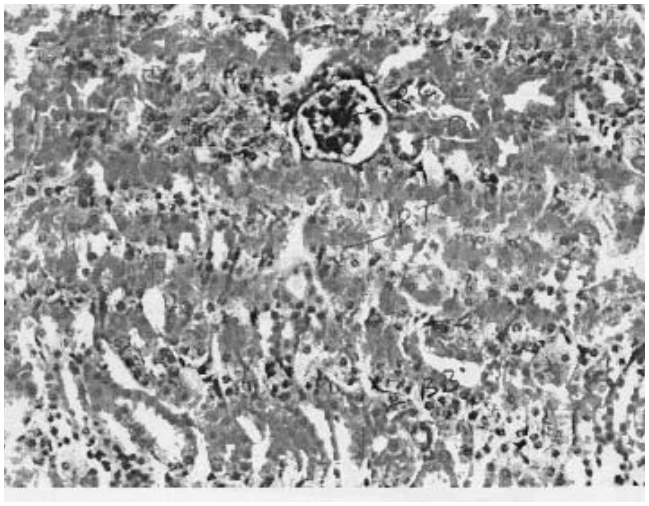

Figure 2. A photomicrograph of the histological section of the mouse kidney of the experimental group B. PAS stain. Magnification 700 X. Showing RC: renal corpuscle, PT: proximal tubule, BB: brush border, DT: distal tubule, BL: basal lamina.

"B"showed normal renal architecture. Mormophetric analysis is given in table. There was congestion of vessels and marked lymphocytic infiltration in the experimental group A. No abnormality was observed in the experimental group B (Table).

Discussion

The animals in both the experimental groups, 5-10 minutes after application of the drug started scratching and biting at their tails. It has been reported that animals after dermal application of pyrethroids responded by licking, rubbing, scratching, or biting at the application site.[11,3] One of the animals in the experimental group A nipped off its tail. Two to three days later, the site of application of Cypermethrin on the tail of mice became red and swollen. In the light of these observations it can be concluded that the biting and scratching behaviour of mice observed in the present study might be due to local irritation of skin caused by Cypermethrin. Apart from this, the animals of the experimental group B remained active and healthy throughout the experimental period. Forty to forty-five minutes after application of the drug, the animals of the experimental group A became irritable and quarrelsome. After a while, some of the animals showed abnormal movements of the head (side to side movements). The condition of the animals improved in the next two to three hours. In about a week's time, the animals of the experimental group A became less active and lethargic. The changes in the behaviour observed can be explained on the basis of toxicity of Cypermethrin.[12] The toxicity signs were observed in the experimental group A while no toxicity was observed in the experimental group B; this indicates that the toxicity of Cypermethrin is dose dependant.[12]

In both the experimental groups, weight gain was observed. However the weight gain was statistically insignificant in the experimental group A, receiving the higher dose. While it was statistically significant in the experimental group B, receiving the lower dose . This indicates that of the two experimental groups, only the animals of the experimental group A were exposed to the toxic levels of the drug. The probable reason for this is that the toxicity of Cypermethrin is dose dependent.[12]

In the present experimental study, there was a statistically significant increase in the kidney weight in the experimental group A. While there was no change in the experimental group B. The increase in the kidney size after exposure to toxins has also been reported by Kumar et al.[13] The probable reason for the increase in the weight of kidneys may be congestion of vessels and lymphocytic infiltration. The absence of changes in the experimental group B may be due to the fact that this group received a lower dose.

The histological changes observed in the kidneys after damage by toxins include changes in the tubules and in the interstitium. There is necrosis predominantly in the proximal tubules. In the interstitium, there is generalised oedema along with inflammatory exudate comprising of polymorphonuclear leukocytes, lymphocytes and plasma cells. In about a weeks time the regeneration process begins and variable degree of tubular necrosis and regeneration can be seen.[13]

The changes observed in the present study were congestion of vessels, diffuse and focal lymphocytic infiltration in the kidneys of the animals of the experimental group A. The changes observed by Tamang et al[14] were more severe. The reason for this may be that a sub-lethal dose of Cypermethrin was administered in the present study. The changes observed in the experimental group B, receiving a lower dose, were not statistically significant. Probably the amount of drug absorbed in this group was not toxic. This may be due to the fact that the absorption of Cypermethrin is less after dermal administration[15] and the toxicity of Cypermethrin is dose dependant.[12]

Conclusion

Cypermethrin is toxic to mammals. It causes marked lymphocytic infiltration and congestion of vessels in the kidneys of mammals. The magnitude of the injury and its reversibility have not been assessed in the humans. This pilot project has however established nephrotoxicity of Cypermethrin in mammals after dermal exposure, a route that predisposes industrial workers to toxicity.

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