Pakistan Vet. J., 2006, 26(4): 193-196.

TOXIC EFFECTS OF CYPERMETHRIN IN FEMALE RABBITS

M. S. ULLAH, M. AHMAD, N. AHMAD, M. Z. KHAN[1] AND I. AHMAD

Department of Animal Reproduction; [1]Department of Veterinary Pathology,
University of Agriculture, Faisalabad, Pakistan

ABSTRACT

The aim of the present study was to explore the fetotoxic effects of cypermethrin (CY) in female rabbits with low and high doses. For this purpose, 32 adult female rabbits were divided in to four equal groups A, B, C and D. Rabbits of groups A, B and C were treated with different levels of CY at the dose rate of 25, 50 and 75 mg/kg body weight intraperitoneally, while the group D served as a control and was given equal volume of normal saline intraperitoneally. The clinical signs exhibited by the rabbits treated with CY included salivation, licking of different body parts, muscular tremors, ataxia and convulsions. There was a significant difference in the numbers of CL and number of fetuses which mean the early embryonic death and post implantation loses at the high dose. There were microscopic changes in the ovaries and uteri of animals treated with CY.

Key words: Toxicity, cypermethrin, rabbits.

INTRODUCTION

Cypermethrin is commonly used to control various pests, including moth pests of cotton, fruit and vegetable crops (Meister, 1992). It is also used for crack, crevice and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses and apartments, greenhouses, laboratories, ships, railcars, buses, trucks and aircrafts. It may also be used in non-food areas in schools, nursing homes, hospitals, restaurants, hotels and food processing plants (Anonymous, 1989). It is being used in veterinary practice against ectoparasites.

Cypermethrin is toxic not only for insects but also for mammals (He, 2000; Barlow *et al*., 2001). The signs like muscular tremors, ataxia, weakness of limbs, convulsions, coma and death from respiratory depression have been reported in animals after ingesting high doses of cypermethrin, while its dermal contact in facial area may cause a subjective sensation of tingling or numbness (Sandhu and Brar, 2000). Cypermethrin is also a skin and eye irritant. Slight to severe skin irritation, decreased food consumption, body weight and absolute and relative gonad weights have been observed in rabbits treated with cypermethrin (Handerson and Parkinson, 1981).

Besides generalized toxic effects of cypermethrin, decreased number of implantation sites, number of viable fetuses and weight gain of fetuses in rabbits treated with cypermethrin have been reported (Elbetieha *et al.,* 2001). Exposure of pregnant laboratory animals to cypermethrin can affect their offspring. Few abnormalities of organs and skeletal muscles have also been observed in offsprings of pregnant rabbits fed cypermethrin (Anonymous, 1989).

Although enough information is available about cypermethrin toxicity from different regions of the world, yet little work has been accomplished on cypermethrin toxicity especially related to fetotoxicity under local conditions. Therefore, to protect the innocent farmers and their animals from the toxic effects of cypermethrin on reproduction, this project was designed with the objectives to investigate clinical signs/symptoms of cypermethrin toxicity and gross and histopathological lesions related to fetotoxicity in rabbits as a model.

MATERIALS AND METHODS

Experimental rabbits

A total of 32 adult female and 8 male rabbits of about the same age and free from any apparent clinical ailment were procured from local market. Their age was approximately one year and body weight was about 1 kg per animal. Female and male rabbits were kept separately in wire cages. Female rabbits were randomly divided into four groups i.e. A, B, C and D with eight rabbits in each group. These rabbits were maintained in cages with equal interval of light and dark i.e. 12 hrs each. The temperature for all these groups was maintained at 30^0C. Fresh grass and green fodder was offered in the morning and evening, whereas fresh drinking water was provided around the clock. Experimental animals were acclimatized for five days.

Treatments

Estrous cycle of female animals was synchronized by injecting prostaglandin (Dalmazine, FATRO Pharma, Italy) intramuscularly 72 hrs before mating. Five days after mating rabbits of groups A, B and C were subjected to different levels of the cypermethrin (CY) @ 25, 50, and 75 mg/kg b. wt intraperitoneally, while the group D served as control to which equal volume of normal saline was injected intraperitoneally. Each group received four injections of respective treatment with 5 days interval i.e. the treatment was given on day 5^{th}, 10^{th}, 15^{th} and 20^{th} after mating. The experiment continued for 24 days.

Post treatment monitoring

The animals in each group were monitored for clinical signs and behavioral alterations twice daily. Body weight of all animals was recorded twice weekly. Four animals from each group were slaughtered on 12^{th} day and the remaining animals were slaughtered on 24^{th} day. After slaughtering of rabbits, the visceral organs, ovaries and uteri were examined for gross lesions. Ovaries of each animal were examined for the presence of corpus luteum (CL). Number of implantation sites and number of viable fetuses in the uterus were recorded. Tissue samples taken from ovaries and uteri were fixed in 10% buffered formalin and processed for the histopathological studies using routine method of dehydration and embedding in paraffin. Section of 5 micro meter thickness were cut, stained with hematoxiline and eosin (Lille and Fulmer, 1976) and examined for histopathological investigations.

Statistical analysis

Mean values (± SE) of various parameters for rabbits of four groups were computed. In order to ascertain the magnitude of difference among different groups, the data were analyzed statistically using two-way analysis of variance (Steel and Torrie, 1980). Duncan's multiple range test was applied for multiple means comparison, where necessary.

RESULTS AND DISCUSSION

Pesticide exposure posses a serious risk to all domestic animals, environment and public health (Oheme and Mannala, 2001). In the present study, skin irritation and reduced feed intake, salivation and semisolid faeces were observed in cypermethrin (CY) treated rabbits in a dose dependent manner. Skin irritation has also been reported earlier (Handerson and Parkinson, 1981).

As observed in the present study, other researchers have reported indicators of digestive system disturbances like reduced feed intake (McDaniel and Moser, 1993; Neuschl *et al.*, 1995), chewing, licking and hypersalivation (Manna *et al.*, 2004), vomiting and diarrhea (Sandhu and Brar, 2000; Jagvinder *et al.*, 2001). In animals of group C, frequent urination was observed which is supported by the findings of the McDaniel and Moser (1993) and Sandhu and Brar (2000).

In dose dependent manner, incoordination, muscular tremors, jerky movements, ataxia, staggering gait and doziness were observed in CY treated rabbits. The nervous signs started about 10 minutes after each CY treatment and persisted mostly for 30 minutes to two hours. Similar nervous signs have been reported by different workers. These included minor tremors, hyper-excitability or depression (Sandhu and Brar, 2000; Manna *et al.*, 2004), grinding of teeth, hyperesthesia, opisthotonus, spastic paralysis (Tamang *et al.*,1991), muscle twitching, staggering gait, sunken eyes and thick eye discharge (Jagvinder *et al.*, 2001).

These nervous signs in CY treated rabbits might be due to disruption of normal functioning of nervous system. Cypermethrin has been shown to delay closing of gates of voltage sensitive sodium channels in mammalian and invertebrate neuronal membranes that produces a protracted sodium influx (Eells, 1992). This results in multiple nerve impulses, causing the nerve to release the neurotransmitter acetylcholine and stimulate other nerves (Eells, 1992). Cypermethrin also inhibits the gamma aminobutyric acid receptor, causing excitability and convulsions (Ramadan *et al.*, 1988). In addition, it inhibits calcium uptake by nerves and also inhibits monoamine oxidase, an enzyme that breaks neurotransmitters (Rao and Rao, 1993).

After the first CY treatment, there was a slight decrease in the weight of animals of group A, B and C, however non significant changes in the weight were observed after 2^{nd}, 3^{rd} or 4^{th} treatment in rabbits of these groups. But in the control group (D), the animals gained weight with the passage of time. This decrease in weight in treatment groups could be due to stress of CY. Lakkawar *et al.* (2004) have also noticed that in addition to typical clinical signs of CY, there was decrease in body weight compared to control group. Non significant difference was observed in number of corpora lutea and number of fetuses in control and all treatment groups on day 12 and 24, except in group C in which the left uterus showed significant ($P<0.05$) decrease in fetuses on day 24 compared to the group A and D (Table 1).

This decrease in the number of the fetuses as compared to CL number clearly indicates the embryonic loss which resulted form the high dose of CY in the present study. These findings are supported

Table 1: Effects of different levels of cypermethrin on number of corpora lutea on the ovaries and number of fetuses in the corresponding uterine horns

Group	No. of CL		No. of fetuses	
	Right ovary	Left ovary	Right horn	Left horn
Day 12				
A	3.33 ± 0.99	5.33 ± 0.36	2.33 ± 0.63	4.00 ± 0.43
B	5.00 ± 0.39	5.00 ± 0.19	1.33 ± 0.46	2.33 ± 0.22
C	4.33 ± 0.88	5.33 ± 0.31	2.00 ± 0.83	2.33 ± 0.33
D	3.00 ± 1.30	4.00 ± 0.36	2.33 ± 0.52	3.33 ± 0.34
Day 24				
A	3.67 ± 0.76	4.33 ± 0.48	2.00 ± 0.33	3.33 ± 0.68a
B	4.67 ± 1.33	5.00 ± 0.48	2.67 ± 1.26	2.67 ± 0.17ab
C	4.33 ± 0.52	4.33 ± 0.20	2.67 ± 0.39	1.67 ± 0.24b
D	4.33 ± 0.82	4.33 ± 0.40	3.67 ± 0.56	3.00 ± 0.36a

Values with different letters within a column differ significantly ($P<0.05$).

by the results of Rustamov and Abbasov (1994), who administered CY at 8 and 34 mg/kg body weight once daily for two months to immature male and female rats and observed that embryonic resorption in these rats was 20% at high dose and normal (10%) at low dose. Biernacki *et al.* (1995) have also reported embryonic resorption in CY treated rabbits. Shukla and Taneja (2002) found high rate of pre and post implantation losses in dose dependent manner in CY treated mice.

In the present study, 8, 22.2 and 41% fetuses were found to be dead in group A, B and C, respectively, where as no foetus was observed to be dead in group D. The possible reason for these embryonic or fetal losses may be the presence of increased connective tissue in the endometrium revealed in the microscopic study of uterine sections of the treated animals, which can interrupt ample blood supply to the fetus, resulting in low body weight gain or increased death rate in the fetuses.

Histologically, changes were observed in ovary and uterine tissue which were more pronounced at higher dose (Group C). Ovaries of rabbits of group C showed connective tissue proliferation in the cortex. There was glandular atrophy, congestion and sloughing of epithelium along with connective tissue proliferation in the uterine tissue, probably due to interference of CY with adinosine triphosphate (ATP) pathway. Inhibition of ATP leads to impaired energy utilization, leading to the damage of cells and reduction in their size, interruption of energy utilization pathway can lead to cell death and sloughing of epithelium (El-Toukhy and Girgis, 1993).

REFERENCES

Anonymous, 1989. US Environmental Protection Agency. Pesticide Fact Sheet Number 199: Cypermethrin. US EPA, Office of Pesticide Programs, Registration Div., Washington, DC, USA.

Barlow, S. M., F. M. Sullivan and J. Lines, 2001. Risk assessment of the use of deltamethrin on bed nets for the prevention of malaria, Food Chem. Toxicol., 39(5): 407-422.

Biernacki, B., B. Wlodarczyk, M. Minta and T. Juszkiewicz, 1995. Influence of cypermethrin on pregnancy and foetal development in rabbits. Medycyna Wete., 51(1): 31-33.

Eells, J. T., 1992. Pyrethroid insecticide induced alterations in mammalian synaptic membrane potential. J. Pharmacol. Exp. Therap., 262: 1173-1181

Elbetieha, A., S. I. Da'as, W. Khamas and H. Darmani, 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch. Environ. Contam. Toxicol., 41(4): 522-528.

El-Toukhy, M. A. and R. S. Girgis, 1993. In vivo and in vitro studies on the effect of larvin and cypermethrin on adenosine triphosphatase activity of male rats. J. Environ. Sci. Hlth., 28: 599-619.

Handerson, H. K. and F. N. Parkinson, 1981. Effect of cypermethrin on haematology, clinical chemistry and gonads of male rabbit. Vet. Med. J. (Giza), 31(1): 32-37.

He, F., 2000. Neurotoxic effects of insecticides-current and future research: a review. Neurotoxicology, 21(5): 829-835.

Jagvinder, K., H. S. Sandhu and J. Kaur, 2001. Subacute oral toxicity of cypermethrin and deltamethrin in buffalo calves. Indian J. Anim. Sci., 71: 1150-1152.

Lakkawar, A. W., S. K. Chattopadhyay and R. Somvanshi, 2004. Experimental cypermethrin toxicity in rabbits. A clinical and patho-anatomical study. Folia Veterinaria, 48(1): 3-8.

Lille, R. D. and H. M. Fullmer, 1976. Histopathologic Techniques and Practical Histochemistry. 4th Ed., McGraw-Hill Inc. New York, USA.

Manna, S., D. Bhattacharyya, D. K. Basak and T. K. Mandal, 2004. Single oral dose toxicity study of alpha-cypermethrin in rats. Indian J. Pharmacol., 36(1): 25-28.

McDaniel, K. L. and V. C. Moser, 1993. Utility of a neurobehavioral screening battery for differentiating the effects of two pyrethroids, permethrin and cypermethrin. Neurotoxicol. Teratol., 15: 71-83.

Meister, R. T., 1992. Farm Chemicals Handbook. Meister Publishing Company, Willoughby, USA.

Neuschl, J., J. Legath, B. Kacmar, E. Kona, V. Konrad and J. Saly, 1995. The effect of supermethrin, an insecticide on health status indicators in sheep during subchronic poisoning. Vet. Med. (Praha), 40: 377-382.

Oheme, W. F. and S. Mannala, 2001. Pesticide use in veterinary medicine. In: Handbook of Pesticide Toxicology. 2nd Ed., Academic Press, New York, USA.

Ramadan, A. A., N. M. Bakry, A. S. M. Marei, A. T. Eldefrawi and M. E. Eldefrawi, 1988. Action of pyrethroids on $GABA_A$ receptor function. Pest. Biochem. Physiol., 32: 97-105.

Rao, G. V. and K. S. J. Rao, 1993. Inhibition of monoamine oxidase-A of rat brain by pyrethroids, an in vitro kinetic study. Mol. Cell. Biochem., 124: 107-114.

Rustamov. Y. M. and T. G. Abbasov, 1994. Gonadotoxic action of cypermethrin in rats given multiple doses. Probl. Vet. Sanit. Ecol., 94: 88-95.

Sandhu, H. S. and R. S. Brar, 2000. Textbook of Veterinary Toxicology. 1st Ed., Kalyani Publ., New Dehli, India, pp: 225-235.

Shukla, Y. and P. Taneja, 2002. Mutagenic potential of cypermethrin in mouse dominant lethal assay. J. Environ. Pathol. Toxicol. Oncol., 21: 259-265.

Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrial Approach. 2nd Ed., McGraw Hill Book Co., New York, USA.

Tamang, R. K., G. I. Jha and K. K. Singh, 1991. Clinicopathology of acute cypermethrin toxicity in goats. Indian J. Anim. Sci., 61(5): 493-494.