Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Toxic Effects of Cypermethrin on the Reproductive Functions of Female Rabbits and Their Amelioration with Vitamin E and Selenium

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ARTICLE HISTORY(14-041)

Received: January 22, 2014
Revised: December 03, 2014
Accepted: January 16, 2015

Key words:
Amelioration
Cypermethrin
Rabbits
Selenium
Toxicity
Vit. E

ABSTRACT

The aim of the present study was to investigate the effects of cypermethrin on the reproductive functions and its amelioration with vitamin E and selenium in rabbits. After 3 days of acclimatization, 30 adult female rabbits were divided into five equal groups A, B, C, D and E. Group A was kept as control, while all other groups received cypermethrin @ 75 mg/kg BW I/P at 5 days interval. Meanwhile animals of group C also received vitamin E @ 150 mg/kg BW and those of group D received selenium @ 0.45 mg/kg BW orally daily, while group E received vitamin E and selenium @ 150 mg/kg + 0.45 mg/kg BW daily. The animals were synchronized on the 5th day, while mating was allowed on the 8th day of experiment. The experimental trial lasted for 32 days. Clinical signs observed in rabbits treated with cypermethrin were muscular tremors, licking of different body parts, depression and reduced feed intake. Non-significant difference was observed in all groups at 12th and 24th day of gestation in number of CLs, while significant ($P<0.05$) decrease was observed in implantation sites and number of recovered fetuses in all treated groups as compared to control group at 12th and 24th day of gestation. At 12th day of gestation, there was significant ($P<0.05$) decrease in serum progesterone concentration in groups B, C and D as compared to groups A and E. In group E, progesterone concentration was lower than group A, but statistically the difference was non-significant. At 24th day of gestation, there was significant ($P<0.05$) decrease in progesterone concentration in groups B, as compared to control. In conclusion, combined use of selenium and vitamin E ameliorated the toxic effects of cypermethrin on reproductive functions in the female rabbits.

To Cite This Article: Sallam MA, M Ahmad, I Ahmad, ST Gul, M Idrees, MI Bashir and M Zubair, 2015. Toxic effects of cypermethrin on the reproductive functions of female rabbits and their amelioration with Vitamin E and selenium. Pak Vet J, 35(2): 193-196.

INTRODUCTION

An increase in pesticides utilization has been noted in animal husbandry and agriculture and particularly pyrethroids are commonly used throughout the world (Sun *et al*., 2007; Auon *et al*., 2014; Ghaffar *et al*., 2014). Cypermethrin, which is a broad spectrum insecticide, is being used extensively in Pakistan (Khan *et al*., 2012). It has low mammalian toxicity and rapid degradation rate (Srivastava *et al*., 2006; Hussain *et al*., 2014).

Studies revealed that cypermethrin had harmful effects on health status of female rabbits and its use is unsafe (Shah *et al*., 2007). It causes early embryonic deaths (Ullah *et al*., 2006) and estrous cycle disruption in rats (Sangha *et al*., 2013). It also induces neurotoxicity by crossing blood brain barrier (Azeez and Al- Hussary, 2012), affects the bone and bone marrow (Suzan *et al*., 2012), exerts adverse effects on female reproductive system in rats (Sangha *et al*., 2013), disrupts the sexual behavior (Solati *et al.,* 2010) and adversely affects the progesterone production in bovines (Gill *et al*., 2011; Ali *et al*., 2014).

These toxic effects of pyrethroids can be prevented by the supplementation of antioxidants like vitamin E (Ahmad *et al*., 2012). Vitamin E has been reported to have ameliorating effect against the reproductive toxicity and plays its role against the oxidative stress induced by cypermethrin exposure (Raina *et al.,* 2009; Yousef, 2010). Simultaneous use of vitamin E and selenium has better ameliorating effect as compared to their individual use against cypermethrin toxicity (Aslam *et al*., 2010; Singh *et al*., 2012; Babi *et al*., 2014).

In Pakistan, a lot of work has been carried out on the effects of cypermethrin in rabbits and other animals. Many studies have been published on, effects of cypermethrin on reproduction, hematological and biochemical parameters of rabbits (Ullah *et al*., 2006; Shah *et al*., 2007; Ahmad *et al*., 2012; Khan *et al*., 2012). However, there is lack of information on amelioration of cypermethrin toxicity, especially on reproductive performance. So, the main objective of present study was to investigate the amelioration effect of vitamin E, selenium and their combination to encounter cypermethrin toxicity on reproductive functions in adult female rabbits.

MATERIALS AND METHODS

Experimental animals: A total of 30 clinically normal and healthy adult female and 8 male rabbits were used in this study. These rabbits were maintained in cages with equal interval of light and dark i.e. 12 hrs each and temperature for all these groups was maintained at 30ºC in a controlled room. 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 three days and experimental trial was continued for 32 days.

Treatments to the animals: The experimental female rabbits were divided into five equal groups A, B, C, D and E, with six animals in each group. Animals of group A were kept untreated as control and equal volume of normal saline was injected intraperitoneally. Groups B, C, D, and E were treated with cypermethrin @ 75 mg/kg BWI/P at five days interval i.e. the treatment was administered on 3rd, 8th, 13th, 18th, 23th and 28th day of experiment. Along with cypermethrin animals in group C received vitamin E @150mg/kg BW., Group D received selenium @0.45mg/kg BW and group E received Vitamin E @150mg/kg BW + selenium @ 0.45mg/kg BW, orally/day, from day 3rd to 32nd of experiment. Estrous cycle of female animals was synchronized by injecting prostaglandin @0.43ug/kg BW (Dalmazine, FATRO Pharma, Italy) intramuscularly on the 5th day, and mating was allowed on 8th day of the trial. Eight male animals were used for the purpose of matting and they did not receive any treatment.

Post treatment monitoring: The animals in each group were monitored for clinical signs and behavioral alterations, twice daily. Three animals from each group were euthanized humanely at 20th day (12th day of gestation) and the remaining animals (three per group) were euthanized at 32nd day (24th day of gestation) of the experiment. Before euthanasia, blood samples were collected without anticoagulant, serum was extracted and stored at -20^{o}C till further analysis. Serum progesterone concentration was determined by using a commercial kit (Progesterone [^{125}I] RIA Kit, Ref: RK-460M, Izotop). Sensitivity of this kit was 0.44±0.12 nmol/l and 100% cross reactivity with progesterone. Ovaries of each animal were examined for the presence of corpuralutea (CL). Number of implantation sites and number of viable fetuses in the uterus were recorded. Rate of early embryonic and fetal deaths were calculated by using following formula (Ahmad, 2010).

Early embryonic death rate = No of CLs – No of implantation sites / No of CLs × 100

Fetal death rate = No of implantation sites – No of fetuses recovered / No of implantation sites ×100

Statistical analysis: Mean±SE of various parameters of five groups were computed. In order to ascertain the magnitude of difference among different groups, data were analyzed statistically using analysis of variance. Duncan's multiple range test was applied for multiple means comparison, where necessary.

RESULTSAND DISCUSSION

In the present study, cypermethrin treated animals developed clinical signs and behavioral alterations in all groups with variable intensities. These signs included irritation, itching, increased urination, restlessness, muscular tremors, in-coordination and decreased feed intake, and were similar to previously reported signs in rabbits (Ullah *et al*., 2006: Shah *et al*., 2007) and male goats (Khan *et al*., 2009). Similarly, paralysis of hind limb, low feed intake, depression and nervous signs in rabbits have been reported by Dahamna *et al*. (2009), and in rats by Manna *et al*. (2004) and Nair *et al*. (2011). It has been shown that uncontrolled use ofcypermethrin can lead to unwanted results in non-target species. It induces neurotoxicity by crossing blood brain barrier and causes hyper excitation of CNS by prolonging the opening of sodium channels. It also modulates the level of neurotransmitters (Singh *et al.,* 2012; ul Hassan *et al*., 2014).

Regarding the number of CLs on the ovaries, non-significant difference was observed in all treated groups compared to control group at both days 12 and 24 of gestation (Table 1), which is similar to findings of Ullah *et al*. (2006) in rabbits. In the present study, significant ($P<0.05$) decrease was observed in number of implantation sites in cypermethrin treated group B as compared to groups A and E but non significantly with groups C and D on 12th day of gestation. The implantation sites were also observed to be significantly decreased in groups B, C and D compared to groups A and E on 24th day of gestation (Table 1). However, groups B, C and D differed non significantly from one another on the same day of gestation. The group E also significantly differed for the number of implantation sites as compared to group A on 24th day of gestation. These results indicated that vitamin E and selenium in combination substantially ameliorated the toxic effects of cypermethrin. Pyrethroids induce changes both in endoplasmic reticulum and mitochondrion of ovarian corpus luteal cells (He *et al*., 2006), which may result in disruption of endocrine functions and pyrethroids also induce glandular atrophy of uterus (Ahmad, 2010), causing early embryonic death before implantation subsequently decreasing implantation sites.

In the present study, number of recovered fetuses (Table 1) were significantly ($P<0.05$) lower in cypermethrin treated groups B, C, D and E at day 12th compared to control group A. However, groups B, C and D at 24th day of gestation showed significantly ($P<0.05$)

Table 1: Mean values of reproductive parameters of various groups at 12th and 24th day of gestation

Groups	12th day of gestation			24th Day of gestation		
	Corpora lutea	Implantation sites	Recovered fetus	Corpora lutea	Implantation sites	Recovered fetus
A	4.67±1.15^{A}	4.33±0.57^{A}	3.67±0.57^{A}	4.63±0.57^{A}	4.33±1.52^{A}	3.67±1.52^{A}
B	3.67±1.52^{A}	1.33±1.15^{C}	0.67±0.57^{C}	3.33±1.52^{A}	1.33±0.57^{C}	0.67±1.15^{C}
C	3.33±0.58^{A}	2.00±1.0^{BC}	1.33±0.6^{BC}	3.00±0.0^{A}	1.66±1.52^{C}	1.11±1.1^{BC}
D	3.33±1.15^{A}	1.67±1.52^{BC}	1.00±1.0^{C}	3.00±1.00^{A}	2.33±0.57^{C}	1.33±1.0^{BC}
E	4.00±1.00^{A}	3.33±0.57^{AB}	2.93±0.57^{B}	3.66±0.57^{A}	3.00±0.0^{B}	2.67±0.6^{AB}

Mean±SEvalues with different letters in a column differ significantly (P<0.05).

Table 2: Percentages of early embryonic death (EED) and fetal death in rabbits of various groups at 12th and 24th day of gestation

Groups	12th Day of gestation		24th Day of gestation	
	EED	Fetal death	EED	Fetal death
A	7.28	15.24	6.47	15.24
B	63.76	49.62	60.06	49.62
C	39.93	33.50	44.66	33.13
D	49.84	40.11	22.33	42.91
E	16.75	12.00	18.03	11.00

Table 3: Serum progesterone concentrations in rabbits of various groups at 12th and 24th day of gestation

Group	Progesterone (ng/ml)	
	12th day	24th day
A	7.32±0.096^{a}	5.65±0.132^{a}
B	4.09±0.096^{c}	4.76±0.034^{d}
C	5.75±0.1^{b}	5.03±0.06^{cd}
D	5.72±0.04^{b}	5.26±0.052^{bc}
E	5.97±0.065^{a}	5.58±0.62^{ab}

Mean±SE with different letters in a column differ significantly (P<0.05).

lower number of fetuses than control group A. Similar results were obtained at 24th day of gestation by Ullah *et al*. (2006) in rabbits given 50 mg/kg BW cypermethrin at 5 days interval. However, at 12th day of gestation, opposite results were observed by these authors. The possible reason for significant (P<0.05) decrease in number of recovered fetuses at 12th day of gestation could be that in present study animals received 4 injections of 75 mg/kg BW cypermethrin, while in the study of Ullah *et al.* (2006), animals received 2 injections during this period. Perhaps more number of exposures resulted in low number of recovered fetuses in cypermethrin treated groups as compared to control group.

In the present study, early embryonic and fetal death rates were maximum in group B and minimum in group A, meanwhile other three groups were higher from group A but lower than group B at 12th and 24th day of gestation (Table 2). No dead fetus was recovered in the uterus. The possible reason for these embryonic or fetal losses may be the presence of increased connective tissue in the endometrium 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 (Ullah *et al*., 2006).

Results of the present study also indicated that supplementation of either vitamin E or selenium alone did not ameliorate toxic effects of cypermethrin as far as number of implantation sites and number of recovered fetuses is concerned. However, their combined use ameliorated the toxic effects of cypermethrin significantly (P<0.05), which may be due to the fact that simultaneous use of vitamin E and selenium is more effective against cypermethrin toxicity than when these are used alone (Atessahin *et al.,* 2005).

In the present study, significant (P<0.05) decrease was observed in serum progesterone concentration at 12th and 24th day of gestation in cypermethrin treated group as compared to control group (Table 3). Gill *et al*. (2011) observed that the exposure of bovines to the cypermethrin decreased the progesterone concentrations which support our findings. The reason for this decrease may be that pyrethroids damage the ovarian CL cells by inducing changes in the endoplasmic reticulum and mitochondrion of CL (He *et al*., 2006), which results in the disruption of endocrine functions i.e. decrease in the progesterone concentration.

Conclusion: Cypermethrin toxicity induces clinical signs and behavioral alterations in treated rabbits like irritation, itching, increased urination, restlessness, muscular tremors, incoordination and low feed intake. Its toxicity also causes significant decrease in number of implantation sites, increase in early embryonic death and decrease in progesterone concentrations. However, toxic effects can be ameliorated with Vit. E and Se, which reduce the severity of the clinical signs and early embryonic death rates in rabbits.

Authors' Contribution: Authors played an active role in the conduction of this trial, parameters and data analysis and manuscript write up and revision.

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