Punjab Univ. J. Zool., Vol. 29 (2), pp.85-90, 2014

ISSN 1016-1597 (Print)
ISSN 2313-8556 (online)

Original Article

Developmental defects induced by lambda cyhalothrin in mice

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(**Article history:** Received: October 01, 2014; Revised: December 12, 2014)

Abstract

Lambda cyhalothrin was tested for its adverse effects in developing fetuses of mice. Different dose concentrations of the insecticide (5.00µg/g, 10.00µg/g and 15.00µg/g BW) were given orally to the pregnant female mice on day 6, 7 and 8 of gestation. A vehicle control was maintained by giving only 0.1ml corn oil to the mothers. Fetuses were recovered on day "18"of gestation. Developmental defects like micromelia, dysplasia and short tail were observed in the treated groups. The morphometric parameters included body weight, crown rump length, brain size, length and width of eye. Morphometric values were subjected to analysis of variance to observe significance difference against control. The present study indicates that administration of lambda cyhalothrin exerts potentially adverse effects on the development of mice fetuses.

Key words: Lambda cyhalothrin, Developing fetuses, Developmental defects.

To cite this article: KHAN, M.K.A., PERVEEN, M., ANDLEEB, S., ARA, C., SHAHZAD, K. AND ASMATULLAH., 2014. Developmental defects induced by lambda cyhalothrin in mice. *Punjab Univ. J. Zool.,* **29**(2): 85-90.

INTRODUCTION

The pyrethroid insecticides are used in agriculture and home products. They cover almost one fourth of the world insecticide markets (Casida and Quistad, 1998). These insecticides are used in public health programme, and annually 520 tons active ingredients of them are used in vector control programme (Zaim and Jambulingam, 2004). How much insecticides are used in world is not clear because no sufficient and reliable data is available in the survey of FAO (Curti, 1994). The Enviromental Protection Agency estimates that domestic users in the America spend 8.5 billion US dollars for 1.1 billion pounds of pesticide active ingredients per annum (Dalaker and Naifeh, 1998). Major pesticides companies have focused on rapidly growing insecticide in market of Asia to enhance their business. The Asian people produce 90% of the total world rice, while use almost 14% of the world's pesticide (Dinham, 1994). These insecticides are considered as the safest group because of their rapid knock down effects against insects in a minimal dose and low mammalian toxicity (Katsuda *et al.,* 1999). These insecticides are classified into two large groups. Type I pyrethroids (allethrin, permethrin and resmthrin) do not contain an alpha-cyano group while type II (deltamethrin fenvelarate and cyhalothrin) contain an alpha-cyanogroup in their molecules. In mammals T-syndrome is produced by type I pyrethroids, while type II induce CS Syndrome (Gammon *et al.,* 1981; Verschoyle and Aldrige, 1980**,** 1990; Gassner *et al.,* 1997 and Miyamoto *et al.,* 1995). Pimpao (2007) investigated that pyrethroids affect the metabolizing system and immunologic system of *Ancistrus multispinis* (Pisces) a condition that can be worsened in the presence of inflammation or infection. In male salmond fish reproductive pheromones are greatly affected (Alia *et al*., 2006). The pyrethroid insecticides are embryo toxic in avian embryos (Anwar, 2003).

Exposure of pesticides to pregnant women creates many hazards because these are designed to affect on the nervous system. This is understood when we realize that all of us are exposed to pesticides every second of day (Robert, 1987). They increase the risk of sperm abnormality, decreased fertility and fetal growth

0079-8045/14/0085-0089 $ 03.00/0

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retardation in humans (Frazier, 2007). Pyrethroid induces adverse effects on the morphology of mouse sperm (Kumar et al., 2004).

Objective of this experiment was to estimate the potential impacts of lambda cyhalothrin on prenatal development of mice with a view of possible extrapolation of the findings to human.

MATERIALS AND METHODS

Swiss Websters variety of *Mus musculus* of 30g weight was used for this study. Mature male and females mice in the estrus phase were taken and caged together. Breeding stock was kept in controlled environmental conditions in the form of 12 hours' light/dark cycles and temperature of 25±1°C and relative humidity from 40 to 55%. Presence of sperm plug confirmed the mating and was considered as day "0" of gestation. Different dose groups were prepared like 5µg/g BW, 10µg/g BW and 15µg/g BW of insecticide, respectively. These doses were prepared by dissolving the insecticide in corn oil in such a way that 0.1 ml of solution contained required concentration.

These doses were given at days 6, 7 and 8 of gestation one time in a day. The treated mice were kept singly in different cages till day 18 of gestation. In case of vehicle, the animals were without any treatment. On 18th day of gestation, the pregnant female mice were weighed and then anaesthetized with ether. The fetuses were counted and dissected out of the uterus and fixed in Bouin's fixative for the period of 48 hours. The fetuses, which were preserved in Bouin's fluid, were then preserved in 70% ethanol.

Morphometric studies involved recording of wet weight as well as crown-rump length measurements of each fetus. These fetuses were macro photographed with the help of a close-up lens fitted camera.

Statistical data were analyzed by using one-way ANOVA through SPSS software

RESULTS

The morphometric observations and calculations showed a significant differences (P<0.001) between fetuses of experimental (05.00 µg/g BW, 10.00 µg/g BW and 15.00 µg/g BW) and control groups in parameters like wet body weights, crown rump lengths, brain sizes, lengths and widths of eyes, lengths of hind and forelimbs and lengths of tail; excluding eye widths of fetuses belonging to lowest dose group (05.00 µg/g BW) which were not significantly different from control (Table I-II). The differences increased gradually with increase of dose. While the morphological comparison between experimental and control groups showed significant differences. Fetuses having abnormalities of limbs (micromelia, club shaped manus and pes, dysplasia), brain (malformed), eye (open eyelids) and tail (short tail) (Table II, Fig. 1) were observed.

Table I: Lambda cyhalothrin effects on the development of day 18 old fetuses obtained from pregnant female mice, given orally with different doses of lambda cyhalothrin on days 6-8 of gestation.

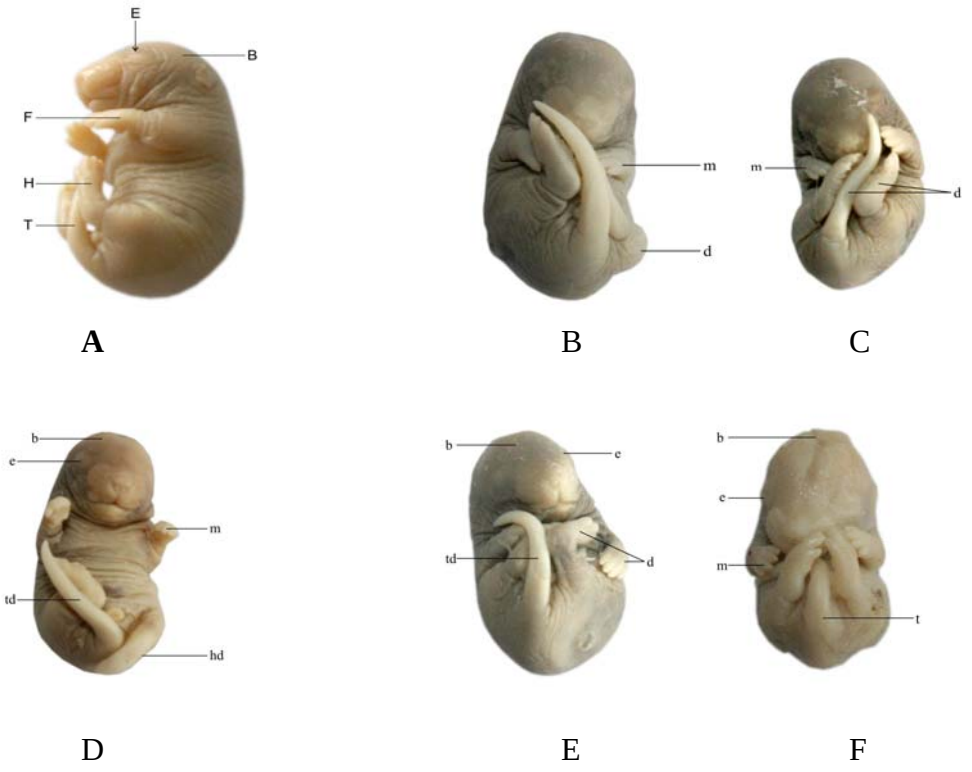
Dose Groups	Body Weight (mg±S.E.)	CR Length (mm±S.E.)	Eye Length (mm±S.E.)	Eye Width (mm±S.E.)	Brain Size (mm±S.E.)	Forelimb Size (mm±S.E.)	Hindlimb Size (mm±S.E.)	Tail Length (mm±S.E.)
Control	1704.24± 64.58a	22.67± 0.14a	03.40± 0.05a	02.80± 0.07a	08.37± 0.06a	08.37± 0.06a	08.87± 0.06a	11.93± 0.23a
(05.00)	960.45± 22.25b***	18.33± 0.33b***	03.06± 0.04b***	02.76± 0.06a	07.2± 0.06b***	07.4± 0.11b***	07.53± 0.12b***	10.63± 0.15b***
(10.00)	643.06± 22.56c***	16.03± 0.20c***	02.33± 0.06c***	02.26± 0.06b***	07.2± 0.06c***	06.6± 0.13c***	06.94± 0.09c***	09.10± 0.12c***
(15.00)	423.84± 29.01d***	14.03± 0.29d***	02.13± 0.05d***	02.13± 0.05b***	05.4± 0.08d***	05.8± 0.09d***	06.30± 0.14d***	07.84± 0.18d***

DMRT Comparison of values within a group; any two values within a same group do not have common small alphabet differ significantly from each other. A comparison based on ANOVA (single factor) Asterisks show significant difference against control, * = p <0.05, **= p<0.01, ***= p<0.001

Table II: Developmental abnormalities induced by Lambda Cyhalothrin, on 18-day old fetuses obtained from pregnant female mice and given orally on days 6 - 8 of gestation.

Dose	No. of Fetuses Observed	Axis Defects (%)	Eye Defects (%)	Brain Defects (%)	Limb Defects (%)	Tail Defects (%)
Control	30	(00.00)	(00.00)	(00.00)	(00.00)	(00.00)
(05.00)	30	(40)	(00.00)	(00.00)	Dysplasia Micromelia (20)	(00.00)
(10.00)	30	(46.66)	Open eyelids Malformed eyes (6.66)	Malformed (13.33)	Dysplasia Micromelia (26.66)	Short (6.66)
(15.00)	30	(53.33)	Open eyelids (26.66)	Malformed (20)	Clubshaped Dysplasia manus & Pes (53.33)	Short (I3.33)

no. of fetus observed: 30



A B C

D E F

Figure 1: Morphological observations: A, Control showing the normal development B: normal brain, E: normal eye, T: normal tail, H: normal hind limb, F: normal forelimb; B and C: fetus of mothers given a dose of 5.00 µg/g B.W Fetuses axis distortion, m: micromelia, d: dysplasia of hind limb; D and E: fetus of mothers given a dose of 10.00 µg/g B.W showing; hd hind limb defects, td, tail defects, m, micromelia, e, eye defects, b, malformed brain,d dysplased tail; F: fetus of mothers given a dose of 15.00 µg/g B.W b, brain defects, e, eye defects, m, micromelia, t, tail defects.

DISCUSSION

In the present study teratogenic effects of lambda cyhalothrin had been worked out in the mice fetuses. Different concentrations of lambda cyhalothrin were administrated on days 6-8 of gestation. Morphometric and morphological studies were carried out to see the results. It was revealed that lambda cyhalothrin exposure during gestation period is harmful. It produces axis distortion, open eyelids, malformed brain and eyes; dysplasia of limbs, micromelia and club shaped manus and pes with short and wrinkled tail.

Although particularly from the standpoint of teratology it is difficult to draw discrete conclusions on the basis of available literature. Kennedy *et al*. (2005) studied affected women and observed that the exposure of pyrethroids during pregnancy appears to be relatively safe. The administration of pesticides deltamethrin and endosulfan don't produce reproductive toxicity to pregnant female rats exposed during critical periods of development. (Kenia *et al.,* 2004) Whereas in another study the estrogenic potential of pyrethroid products in MCF-7 human breast carcinoma cell line indicated that pyrethroids could alter estrogen homeostasis in human **(**Vera *et al.,* 1999). The evaluation of maternal residual exposure to pesticides on birth weight among women in an agricultural district in Central Poland, concluded that mother*s* who were engaged in field work had similar pregnancy period but delivered fetuses with a significantly higher birth weight than mothers not reporting such activities in the critical period of pregnancy (Wojciech *et al.,* 2003). The in utero and lactational exposures to pyrethroids produce changes in reproductive system and physiology of baby rats (Anderson *et al.,* 2002). The occupational exposure is associated with an increase in sperm DNA damage in factory workers (Bian *et al.,* 2003).

The pyrethroids act as hormone disruptors and can also alter estrogen homeostasis (Vera *et al.,* 1999**).** They are also moderate antiandrogenic chemicals **(**Jun *et al.,* 2008) Their low concentrations have negative effects on the reproductive parameters in male rats **(**Kilian *et al.,* 2007**)** The prenatal exposure to pyrethroids alters the expression of xenobiotic metabolizing cytochrome P450. These enzymes are not only involved in the neurobehavioral toxicity of deltamethrin but also have a role in regulating the levels of ligands that modulate growth, differentiation, and neuroendocrine functions (Ashu *et al.,* 2006). The sensitivity of neonate rat to pyrethroid is due to incomplete development of enzymes, that catalyze the metabolism of these insecticides in the liver (Franco, 1993).

Lambda cyhalothrin induces oxidative damage in tissues and erythrocytes. It also modifies biochemical parameters and histological aspects of liver (Fatma, 2007; Hamadi *et al.,* 2008**).** They also induce the chromosomal and lymphocytes aberrations in mammals (Georgieva, 2006**).** In vivo mammals are susceptible to the genetic toxicity and cytotoxic potential of lambda cyhalothrin and other pyrethroids. They also damage the DNA in vital organs like brain,liver and kidney (Ayla *et al.,* 2003; Sushila *et al.,* 2007; Hasan *et al.,* 2008).

The ICON (trade name of lambda-cyhalothrin) had no effect on fertility, but sexual competence was seriously impaired (Ratnasooriya *et al.,* 2002). The potential effect of lambda cyholthrin on rats during mid pregnancy (days 8-14) pose a considerable threat to pregnancy (Ratnasooriya *et al,* 2004). The ICON exposure during early gestation may also threat to pregnancy (Ratnayake *et al.,* 2003). The prenatal exposure to cyhalothrin can induce alterations in the development of certain physical characteristics of rats that are not correlated with functional deficiencies in the animals' later life (Gomes *et al.,* 1991) which is in comparison with present study.

Conclusion

From this study, it has been concluded that pre and peri- implantation embryonic resorptions increase as indicated by the reduce number of implantation sites and average litter size depending upon the dose and frequency of exposure to the insecticide. The rates of anomalies are also increased with the increase of dose.

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