Pakistan J. Zool., vol. 45(3), pp. 857-864, 2013

Teratogenic Effect of Sublethal Doses of Deltamethrin in Mice

Muhammad Khalil Ahmad Khan* and Asmatullah
Developmental Biology Lab, Department of Zoology, University of the Punjab, Lahore

Abstract.- Present study was conducted to evaluate the teratogenic effects of sublethal concentrations *viz.*, 19.36, 9.7, and 4.8µg/g BW of deltamethrin, which was administered orally to the pregnant mice on day 6 of gestation. The fetuses were recovered on day 18 of gestation. The morphological studies revealed abnormalities including sacral hygromae, microcephally, micromelia, open eyelids, microphthalmia, exophthalmia, cryptophthalmia, anophthalmia, droopingwrist, kyphosis, and short tail. Fetal resorptions increased with the increasing dose. Morphometric analysis showed an overall significant ($P<0.001$) reduction in bodyweight, crown rump length, brain and eyes circumferences, lengths of hind and forelimbs and tail size. The histological studies showed malformations including defective nasal pouch, nasal septum with atrophied inferior cochlea, missing of eye ball, pericranial hydrocephaly, cleft palate, degeneration of jaw muscles. Tissue necrosis of brain, liver and intestine were also observed as compared to vehicle control.

Key words: Deltamethrin, teratogenic , micromelia, embryotoxic, synthetic pyrethroid.

INTRODUCTION

Pyrethroid insecticides are considered to be the safest group of insecticdes because of their rapid knockdown effects against insects in a minimal dose and low mammalian toxicity (Katsuda, 1999). Pyrethroid induces lethal effects both in invertebrates (Gowland *et al*., 2002) and in vertebrates (Das and Mukherjee, 2003). Pimpao *et al***.** (2007) investigated that pyrethroids affect metabolizing and immunologic systems of *Ancistrus multispinis* (Pisces). They affect the responses of male salmonids to reproductive pheromones (Jaensson *et al*., 2007). The pyrethroid insecticide is highly toxic in developing chick embryo (Anwar, 2003). Deltamethrin is considered to be of relatively low toxicity in mammals compared to other insecticides such as the organophosphates.

Toxic potentials of these pesticides on male rats clearly demonstrate the adverse effects on fertility and reproduction (Elbetieha *et al*., 2001). In human, reproductive toxicology includes the effect on sperm count, male fertility and semen quality (Bian *et al*., 2004; Xia *et al*., 2008; Patel *et al*., 2007). The prenatal exposures to pyrethroids alter the expression of xenobiotic metabolizing cytochrome P450 (CYPs). During development, significance of these CYP enzymes is 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 (Johri *et al*., 2006). The neonates are more sensitive to the exposure of pyrerhroid insecticide than adults. The sensitivity of neonate rat to pyrethroid toxicity is due to incomplete development of enzymes, which catalyze the metabolism of pyrethroids in the liver of young animals (Cantalamessa, 1993; Sheets, 2000; Scheuplein *et al*., 2002). Genotoxic effects of pyrethroids have also been reported (Celik *et al*., 2003; Patel *et al*., 2007).

There are few studies reported on teratogenic effects of deltamethrin which is a commonly used pyrethroid for pest control in the agricultural fields. The present study was aimed at determining the developmental toxicity of deltamethrin.

MATERIALS AND METHODS

Swiss Webster variety of *Mus musculus* having average weight of 30 g were used for this study. Females in the estrus phase were taken and caged with male. Breeding stock was kept in controlled environmental conditions in the form of 12 hour light/dark cycles and temperature of 25 ± 1^{o}C and humidity 40-55%. Presence of sperm plug confirmed mating and it was considered as day "0" of gestation.

* Corresponding author. kkzoologist@yahoo.com
0030-9923/2013/0003-0857 $ 8.00/0

Experimental design

LD50 was calculated by probit analysis for which a total of 60 pregnant females were divided into six groups each of 10, one for control and five for treatment. Five dose groups were maintained like 80, 60, 40, 20, and 10 µg/g BW. Deltamethrin dissolved in corn oil was orally administered. The females were monitored for respiratory, central nervous system and behavioral disorder. LD_{50} was determined.

Different dose groups were maintained like 19.36, 9.7, and 4.8 µg/g BW of insecticide as sublethal concentrations. These doses were prepared by dissolving the insecticide in corn oil in such a way that 0.1 ml of solution contain desired concentration. These doses were given on day 6 of gestation, once a day. The treated mice were kept singly in different cages till day 18^{th} of gestation. In case of Vehicle (VC), the animals were maintained by giving only 0.1 ml corn oil. On 18^{th} day of gestation, the mothers were weighed and then anaesthetized with ether. The fetuses were counted and were dissected out of the uterus. Then these fetuses were fixed in Bouin's fixative for 48 hours. The fetuses were then preserved in 70% alcohol. Morphological and morphometric studies involve wet weights as well as crown-rump (CR) length, brain and eyes circumferences, fore and hind limbs size and tail length measurements of each fetus. For histological preparations, selected fetuses from all groups were processed for paraffin sections which were stained with hematoxylin and eosin and anomalies of brain, heart, liver and lungs were recorded.

Statistical analysis

Data were analyzed by using probit analysis and one-way ANOVA through MINITAB-16 and SPSS-20 software.

RESULTS AND DISCUSSION

The LD_{50} for deltamethrin was calculated as 38.71 µg/g BW (30.61-47.46), while LD95 was 61.89 µg/g BW with confidence interval of 51.92-84.97 (Fig.1). The fetuses observed from vehicle control group were well developed. All the organs were quite normal. The morphometric observations

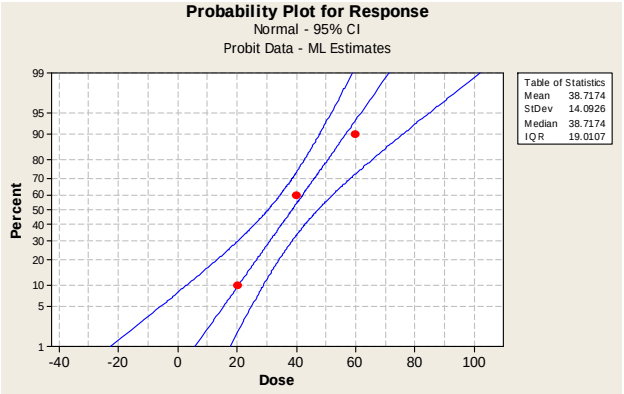

Fig. 1. A probability plot for response showing LD50 value of pregnant female exposed to different concentrations of deltamethrin on day 6 of gestation and surviving for 96 hours.

of wet body weights, crown rump length, brain and eyes circumferences, length of hind and forelimbs and length of tail, showed significant difference ($P<0.001$) between fetuses of experimental (19.36, 9.7, and 4.8 µg/g BW) and vehicle controls parameters in a dose-dependent manner (Table I). The litter size also decreased with the increase of dose. The fetuses study from dose group 19.36 µg/g BW showed some morphological anomalies including open eyelids, macromelia, micro-phthalmia, exophthalmia, cryptophthalmia palpebral coloboma , dysplasia of limbs and short tail (Table II, Fig. 2) while fetuses belonging to dose group 9.7 µg/g BW showed deformities including skin hemorrhage, micromelia, open eyelids, anophthalmia, microphthalmia, bulging eyes, microcephally, club shape pes, hyperplastic umbilical card, sacral hygroma, and dysplasia of limbs (Table II, Fig. 2) and in dose group 4.8 µg/g BW the deformities observed included imbalanced axis, sacral hygroma, open eyelids, cryptophthalmia, anophthalmia, kinky and hemorrhagic tail and runt size fetuses. So the morphological comparison between treated groups and vehicle controls showed significance difference (Table II, Fig. 2).

The histological studies for vehicle control showed normal development in head (Fig. 3A-B) and abdominal region (Fig. 4A-B), while in experimental group for dose 19.36 µg/g BW showed malformations including defective nasal pouch, nasal septum with atrophied inferior cochlea,

Table I.- Effects of deltamethrin on the development of 18 day old fetuses recovered from pregnant mice, administrated orally with different concentrations of deltamethrin on day 6 of gestation.

Dose group (µg/g B.W.)	Body weight	CR length (mm)	Eye circumference (mm)	Head circumference (mm)	Forelimb size (mm)	Hindlimb size (mm)	Tail length (mm)
19.36 (n=62)	1.14±0.08***	19.44±0.12***	4.98±0.17***	19.44±0.17***	5.2±0.01***	7.22±0.18***	8.53±0.13***
9.70 (n=80)	1.29±0.06***	20.39±0.10***	5.11±0.005***	20.29±0.06***	6.94±.19***	7.44±0.23***	9.92±0.15***
4.8 (n=90)	1.44±0.04***	21.17±0.11***	5.15±0.01***	20.75±0.17***	7.57±0.06***	7.37±0.07***	10.49±0.51***
V. Control (n=100)	1.58±0.04	22.50±0.02	6.13±0.008	22.57±0.18	08.07±0..03	8.81±.02	1089±0.04

n, no. of fetuses observed; V, Vehicle control.
A comparison based on ANOVA (single factor) Asterisks show significant difference against control; Mean±SE, Student's `t' test, * = p <0.05, ** = p<0.01, *** = p<0.001

Table II.- Developmental anomalies induced by deltamethrin in 18-day old fetuses recovered from pregnant mice administrated orally with insecticide on day 6 of gestation.

Parameters and dose group µg/g BW	No. of malformed fetuses (%)	Eye defects (%)	Head defects (%)	Limb defects (%)	Tail defects (%)
19.36 (n=38)	72.65	Open eyelids microphthalmia exophthalmia palpebralcoloboma (42.66)	Malformed microcephaly (03.33)	Club shaped pes, Dysplasia macromelia, Micromelia, drooping wrist (24.33)	Short tail (2.33)
9.80 (n=46)	59.31	Openeyelids bulging eyes annophthalmia (32.66)	Malformed (13.33)	Dysplasia, Micromelia (6.66)	Short tail (6.66)
4.80 (n=64)	38.43	Cryptophthalmia anophthalmia (8.44)	Hemorrhagic (13.66)	Dysplasia Micromelia (9.33)	dysplased and kinky tail 7.00
V. Control (n=76)	00.00	(00.00)	(00.00)	(00.00)	(00.00)

n, no. of fetuses observed; V, vehicle control.

degenerated eye ball, pericranial hydrocephaly, cleft palate, degeneration of jaw muscles (Fig. 3C). The selected sections of dose groups 9.7 and 4.8 µg/g BW indicate multiple histological defects including pericranial hydrocephaly, missing of eyes ball, necrosis in cerebral hemisphere as well as other parts of brain (Fig. 3D-E). Tissue necrosis of liver and intestine were also observed in serial sections of this dose group as compared to vehicle control (Fig. 4C).The necrosis of heart muscle, ventricle thrombosis, degeneration of trabecular zone of heart, lung emphysema and enlarged meningospinocoele were also observed with the comparison of vehicle control (Fig. 4D-E).

Our results revealed that deltamethrin is highly toxic during pregnancy. In the present investigation, different doses of deltamethrin were given at day 6 of gestation because sensitivity of mice embryo starts from that day. However, these doses may also be given to mice at different post-pregnant days depending upon the critical period of development in mice which may have different results as compared to results observed during the present investigation.

Kennedy *et al.* (2005) observed that the exposure of pyrethroid products on women during pregnancy appears to be relatively safe because there was no increase in the rates of major

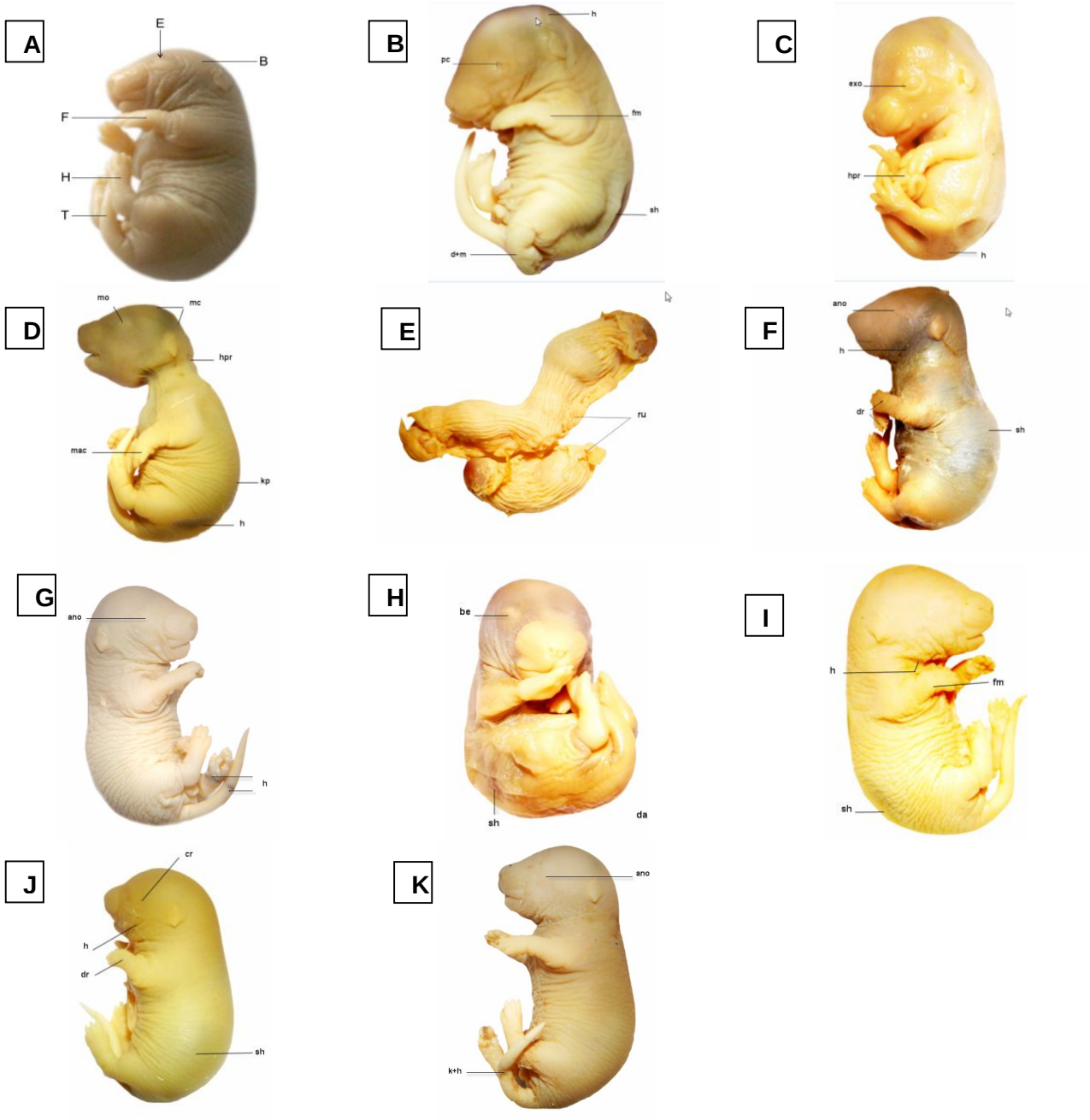

Fig. 2. Morphology of mice Embryo. **A,** vehicle Control group showing normal development of brain (B), eye (E), forelimb (F), hind limb (H) and tail (T). **B-E**, fetus from 19.36µg/g body weight (BW) exposure group showing microphthalmia (mo), exophthalmia (exo), palpebral coloboma (pc), hyperplastic umbilical card (hpr), macromelia (mac), forelimb micromelia (fm), sacral hygroma (sh), hemorrhage (h), microcephaly (mc), kyphosis (kp), dysplasia and micromelia (d+m) and resorbed uterus. **F-H**, fetus from 9.66µg/g BW exposure group showing hemorrhagic hind limb and tail (h), open eyelids (op), anophthalmia (ano), bulging eyes (be), sacral hygroma (sh); kyphosis; short tail (st) and dysplasia (da). **I-K**, foetus from 4.33µg/g BW exposure group showing micromelia (fm) and dysplasia (d),drooping wrist (dr), hemorrhage (h), sacral hygroma (sh), cryptophthalmia (cr), anophthalmia (ano), kinky and hemorrhagic tail (k+h).

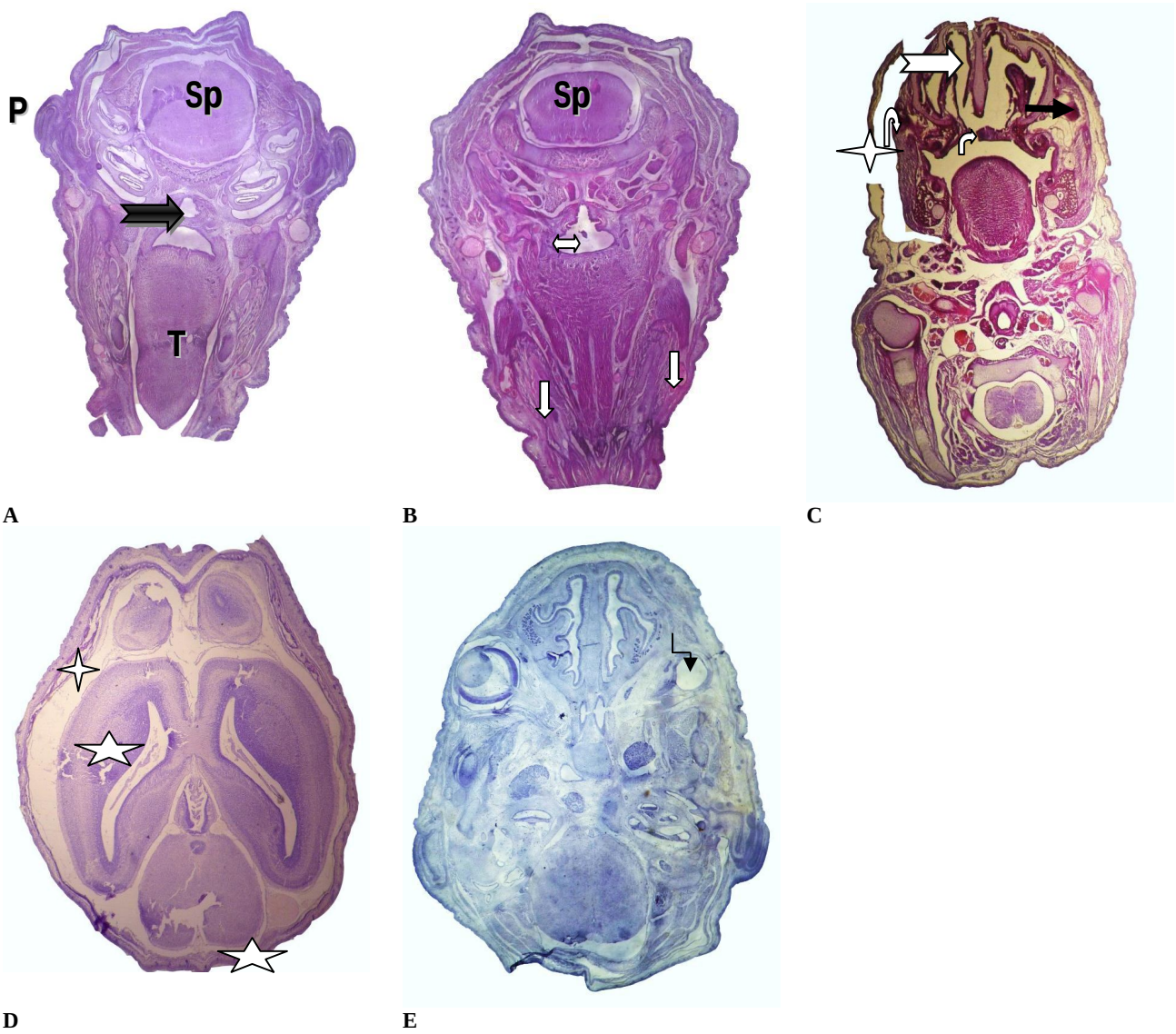

Fig. 3. Histological sections of 18 days old mouse fetuses from head regions. A and B, section from vehicle control. C, D and E sections from deltamethrin treated fetuses. Pinna (P), spinal cord (sp), tongue (T), jaw muscles, left and right white arrow; oronasalphyrinx, white arrow; orophyrinx, black arrow; defective nasal septum with atrophied inferior cochlea, broad white arrow; missing of eyes ball, elbow arrow connector; degenerated eye ball, broad black arrow; cleft palate, bent arrow; degeneration of jaw muscles, u-turn arrow; pericranial hydrocephaly, four point star; necrosis in brain, five point star.

malformations. The administration of pesticides, deltamethrin and endosulfan do not pose a reproductive hazard to female rats exposed during critical periods of development, indicating that the combination does not exert estrogen-like effects *in vivo* or is not delivered to target organs (Presibella *et al*., 2005). While in another study, the estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line was observed, it is stated that pyrethroids could alter estrogen homeostasis in human **(**Go *et al*., 1999). They are also moderate antiandrogenic chemicals (Zhang *et al*., 2008**).** Their low concentrations have negative effects on the reproductive parameters in male rats (Kilian *et al*., 2007**).**

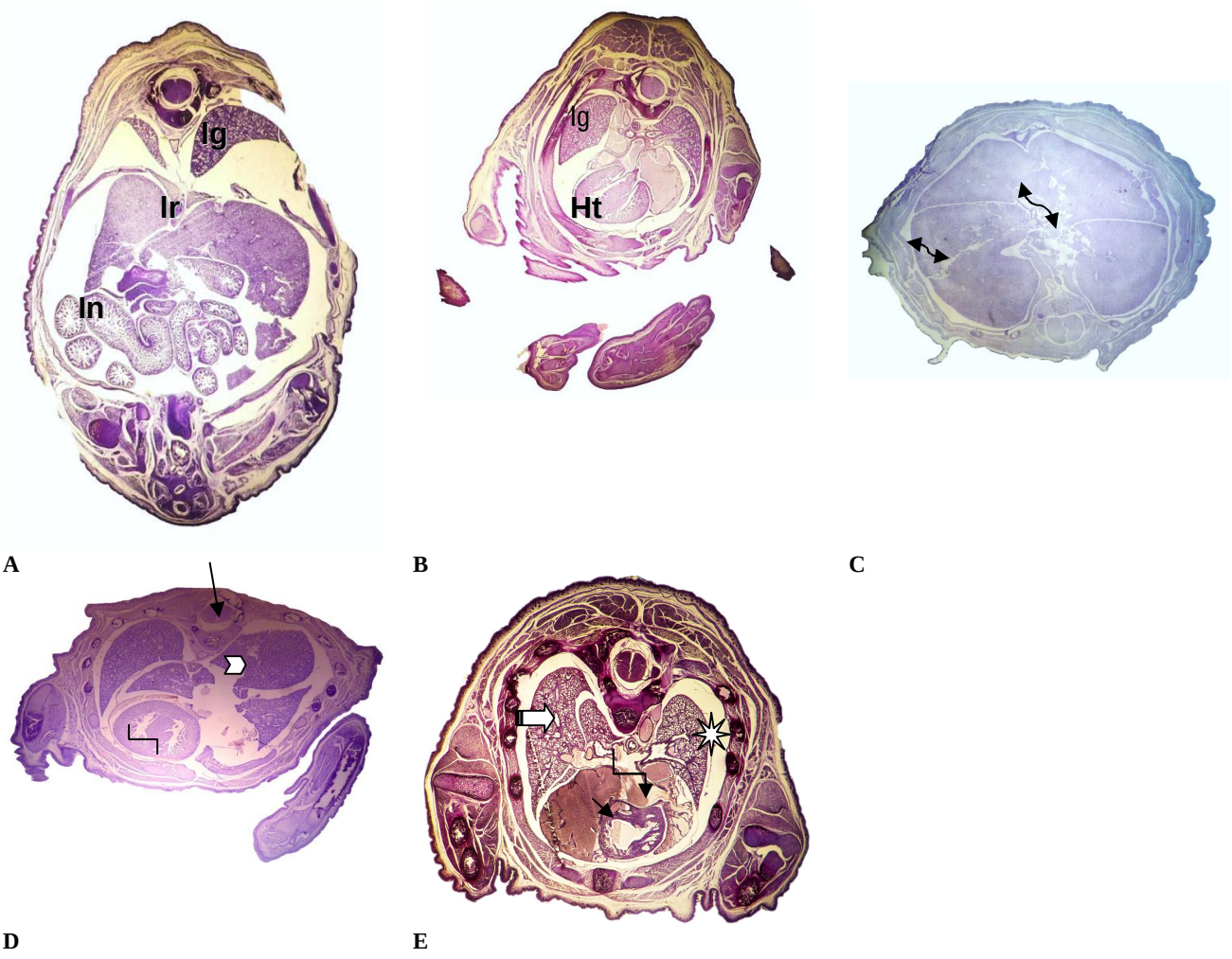

Fig. 4. Histological sections of 18 days old mouse fetuses from cardiac and abdominal region. A and B sections of vehicle control fetus and C, D and E sections of deltamethrin treated fetuses. Lungs, (lg); intetine, (In), heart (Ht), liver (lr), Necrosis of heart muscles, elbow conector; ventricle thrombosis, elbow arrow connector; degeneration of trabecular zone of heart, narrow arrow; lung emphysema, cheveron; degenerated tissue of lung, black tailed white arrow; enlarged meningospinocoele, narrow black arrow; hygroma, eight point star; Tissue necrosis of liver and intestine, elbow double arrow connector.

During the evaluation of maternal residual exposure to pesticides on birth weight among women in an agricultural district in Central Poland, it was observed that mothers who reported involvement in field work had similar pregnancy duration but delivered infants with a statistically higher birth weight than mothers not reporting such activities in the first or second trimester of pregnancy (Hanke *et al*., 2003). In utero and lactational exposure to deltamethrin may induce subtle changes in reproductive behavior and physiology of male offspring rats (Andrade *et al*., 2002). Pyrethroids penetrate through the placental barriers and produce a cardiolesioning effects on the progeny of rats, coincides with the present study (Badaeva and Nedorenko, 1991). 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).The deltamethrin causes retardation of growth, hypoplasia of the lungs, dilatation of the renal pelvis and increase in placental weight (Abdul-khalik *et al*., 1993). The pyrethroid insecticides also cause developmental abnormalities in pinna, down appearance, eye opening and reductions of body weight at higher dose groups which are in agreement with the present study (Farag *et al*., 2007). In addition the exposure of pyrethroids during early gestation poses threat to pregnancy of rats which supports our results (Ratnasooriya *et al*., 2003).

The present study indicates that deltamethrin has potentially embryotoxic properties. On the basis of these findings, it is concluded that oral exposure to deltamethrin is embryotoxic at dose levels below than that cause perceptible toxicological symptoms in Dams, particularly if such exposure occurs at the beginning of organogenesis. Thus pyrethroid insecticide should be used with great care to avoid undesirable effects on human (Eil and Nisula, 1990).

ACKNOWLEDGEMENTS

Authors are thankful to the Higher education commission of Pakistan and Department of Zoology, University of Punjab, Lahore for providing facilities to carry out this research work.

REFERENCES

ABDEL-KHALIK, M. M., HANAFY, M. S. AND ABDEL-AZIZ, M.I., 1993. Studies on the teratogenic effects of deltamethrin in rats. *Dtsch.Tierarztl.Wochenschr*., **100**: 142-143.

ANDRADE, A. J., ARAUJO, S., SANTANA, G. M., OHI, M. AND DALSENTER, P. R., 2002. Reproductive effects of deltamethrin on male offspring of rats exposed during pregnancy and lactation. *Regul. Toxicol. Pharm*., **36**: 310-317.

ANWAR, K., 2003. Toxic effects of cypermeyhrin on the biochemistry and morphology of 11th day of chick embryo (*Gallus domesticus*). *Pakistan J. appl. Sci*., **3**: 432-445.

BARLOW, S. M., SULLIVAN, F. M. AND LINES, J., 2001. Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. *Fd. Chem. Toxicol*., **39**: 407-422.

BADAEVA, L. N. AND NEDORENKO, N. I., 1991. Placental histogenesis and the cardiotoxic effect in rat progeny under the influence of the synthetic pyrethroid Decis. *Vrach. delo.*, 68-71.

BIAN, Q., XU, L. C., WANG, S. L., XIA, Y. K., TAN, L. F., CHEN, J. F., SONG, L., CHANG, H. C. AND WANG, X. R., 2004. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. *Occup. environ. Med.*, **61**: 999-1005.

CANTALAMESSA, F., 1993. Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. *Arch. Toxicol.*, **67**: 510-513.

CASIDA, J. E., 1980. Pyrethrum flowers and pyrethroid insecticides. *Environ. Hlth. Perspect.*, **34:** 189–202.

CASIDA, J.E. AND QUISTAD, G.B., 1998. Golden age of insecticide research: past, present, or future? *Annu. Rev. Ent.*, **43**: 1–16.

CELIK, A., MAZMANCI, B., CAMLICA, Y., ASKIN, A. AND COMELEKOGLU, U., 2003. Cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow. *Mutat. Res.*, **539**: 91-97.

CROFTON, K. M., KEHN, L. S. AND GILBERT, M. E., 1995, Vehicle and route dependent effects of a pyrethroid insecticide, deltamethrin, on motor activity in the rat. *Neurotoxicol. Teratol*., **17**: 489-495.

EIL, C. AND NISULA, B.C., 1990. Binding properties of pyrethroids to human skin fibroblast androgen receptors and to sex hormone binding globulin. *J. Steroid Biochem.*, **35**: 409-414.

ELBETIEHA, A., DA'AS, S. I., KHAMAS, W. AND DARMANI, H., 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. *Arch. environ. Contam. Toxicol.*, **41**: 522-528.

ELLIOTT, M.,1976. Properties and applications of pyrethroids. *Environ. Hlth. Perspect.*,**14**:1-13.

FARAG, A. T., GODA, N. F., SHAABAN, N. A. AND MANSEE, A. H., 2007. Effects of oral exposure of synthetic pyrethroid, cypermethrin on the behavior of F1-progeny in mice. *Reprod. Toxicol.*, **23**: 560-567.

GO, V., GAREY, J., WOLFF, M. S. AND POGO, B. G., 1999. Estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line. *Environ. Hlth. Perspect*., **107**: 173-177.

GOMES M. S., BERNARDI, M. M. AND SPINOSA H. S., 1991. Effects of prenatal pyrethroid insecticide exposure on the sexual development of rats. *Vet. Hum. Toxicol*., **33**: 427-428.

GOWLAND, B. T. G., MOFFAT, C. F., STAGG, R. M., HOULIHAN, D. F. AND DAVIES, I. M., 2002. Cypermethrin induces glutathione S-transferase activity in the shore crab, *Carcinus maenas*. *Mar. Environ. Res.*, **54**: 169-177.

HANKE, W., ROMITTI, P., FUORTES, L., SOBALA, W. AND MIKULSKI, M., 2003. The use of pesticides in a

Polish rural population and its effect on birth weight. *Int. Arch. Occup. environ. Hlth.*, **76**: 614-620.

JAENSSON, A., SCOTT, A. P., MOORE, A., KYLIN, H. AND OLSEN, K. H., 2007. Effects of a pyrethroid pesticide on endocrine responses to female odours and reproductive behaviour in male parr of brown trout (*Salmo trutta* L). *Aquat. Toxicol.*, **81**:1-9.

JOHRI, A., DHAWAN, A., LAKHAN S. R. AND PARMAR, D., 2006. Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic metabolizing cytochrome P450s in the brain and liver of offsprings. *Toxicol. appl. Pharm.*, **214**: 279-289.

KATSUDA, Y., 1999. Development of and future prospects for pyrethroid chemistry. *Pestic. Sci*., **55**: 775–782.

KENNEDY, D., HURST, V., KONRADSDOTTIR, E. AND EINARSON, A., 2005. Pregnancy outcome following exposure to permethrin and use of teratogen information .*Am. J. Perinatol*., **22**: 87-90.

KILIAN, E., DELPORT, R., BORNMAN, M. S. AND JAGER, C.D., 2007. Simultaneous exposure to low concentrations of dichlorodiphenyltrichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Spraque-Dawley rats. *Andrologia*, **39**: 128-135.

PATEL, S., BAJPAYEE, M., PANDEY, A. K., PARMAR, D. AND DHAWAN, A., 2007. In vitro induction of cytotoxicity and DNA strand breaks in CHO cells exposed to cypermethrin, pendimethalin and dichlorvos. *Toxicol. In Vitro,* **21**: 1409-1418.

PIMPAO, C. T., ZAMPRONIO, A. R. AND SILVA DE ASSIS, H. C., 2007. Effects of deltamethrin on hematological parameters and enzymatic activity in *Ancistrus multispinis* (Pisces, Teleostei). *Pestic. Biochem. Physiol*.**, 88**: 122-127.

PRESIBELLA, K. M., KITA, D. H., CARNEIRO, C. B., ANDRADE, A. J. AND DALSENTER, P.R., 2005. Reproductive evaluation of two pesticides combined (deltamethrin and endosulfan) in female rats. *Reprod. Toxicol*., **20**: 95-101.

RASHID, A., ASMATULLAH, ZARA, N. AND ARA, C., 2012. Testicular toxicity induced by Deltamethrin in albino mice. *Pakistan J. Zool*., **44:**1349-1353.

RATNASOORIYA, W.D., RATNAYAKE, S.S. AND JAYATUNGA, Y.N., 2004. Effects of icon, a pyrethroid insecticide on early pregnancy of rats. *Hum. exp. Toxicol*., **22**: 523-533.

SHEETS, L. P., 2000. A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroids insecticides. *Neurotoxicology,* **21**: 57-63.

SCHEUPLEIN, R., CHARNLEY, G. AND DOURSON, M., 2002. Differential sensitivity of children and adults to chemical toxicity: i. biological basis. *Regul. Toxicol. Pharm.*, **35**: 429-447.

SODERLUND, D.M., CLARK, J.M., SHEETS, L.P., MULLIN, L.S., PICCIRILLO, Y.J., SARGENT, D., STEVENS, J.T. AND WEINER, M.L., 2002. Mechanisms of pyrethroid neurotoxicity:implications for cumulative risk assessment. *Toxicology,* **171:** 3-59.

TOS-LUTY, S., HARATYM-MAJ,A., ALATUSZYŃSKA, J., OBUCHOWSKA-PRZEBIROWSKA, D., TOKARSKA-RODAK, M., 2001. Oral toxicity of deltamethrin and fenvalerate in Swiss mice. *Ann. Agric environ. Med.*, **8**:245-54.

XIA, Y., HAN, Y., WU, B., WANG, S., GU, A., LU, N., BO, J., SONG, L., JIN, N. AND WANG, X., 2008. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. *Fertil. Steril*., **89**: 1743-1750.

YAO, K.W. AND WANG, J.D., 2008. Progress in the studies of the male reproductive toxicity of pyrethroid insecticides. *Nat. J. Androl*., **14**:268-271.

ZHANG, J., ZHU, W., ZHENG, Y., YANG, J. AND ZHU, X., 2008. The antiandrogenic activity of pyrethroid pesticides cyfluthrin and beta-cyfluthrin. *Reprod. Toxicol*., **25**: 491-496.

(Received 4 April 2013; revised 24 April 2013)