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Original Article

Developmental anomalies induced by permethrin in *Gallus domesticus*

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Abstract
In present study different doses (0.3125, 0.625, 1.25 and 2.5µg of permethrin/0.1ml/egg) were evaluated for their strength of teratogenicity in developing chicks. The doses were prepared in corn oil and injected into eggs on 4th day of incubation. Following recovery on day 7, the embryos were analyzed morphologically and morphometrically. Morphological studies showed different abnormalities such as spina bifida, gastroschisis, exencephaly, hydrocephaly, micromelia, micrognathia, ectopia cordis, meningocephalocoel and microphthalmia. The morphometric analysis showed significant decrease in body weight and crown- rump length. These results suggest that even lower doses of permethrin are potentially toxic to developing chick.
Key words: Permethrin, teratogenicity, embryotoxicity, *Gallus domesticus*.

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INTRODUCTION

Permethrin is a pyrethroid, with wide use in agriculture, household (Bradberry *et al.*, 2005; Khan *et al.*, 2012)and health (Yoon *et al.*, 2003). Pyrethroids induce bone retardation (Garge*t al*.,2004), morphological as well as skeletal malformations (Bhaskar *et al*., 2014), microcephaly, micromelia, microphthalmia, exophthalmia, cryptophthalmia, anophthalmia, drooping wrist, kyphosis, short tail, defective nasal pouch, missing of eye ball, pericranial hydrocephaly and cleft palate in addition to histological disorders, like degeneration of jaw muscles. Tissue necrosis of brain, liver and intestine have been reported to be caused by deltamethrin (Khan, 2013). It is also known to cause growth retardation, hematomas, subcutaneous hemorrhage, ectopic viscera, anencephaly, exencephaly, skeletal malformations and significant decrease in wet body weight in chick (Bhaskar *et al*., 2014).It has been suggested that some pyrethroids also cause developmental neurotoxicity and tumors (Rehman *et al.*, 2014).

Beyond advantages, permethrin is harmful against non-targeted organisms too (El-Magd *et al.*, 2011; Koureas *et al*., 2012). It can be absorbed through inhalation, oral and dermal routes in human body (Saieva *et al*., 2004; Nakamura *et al*., 2007),and causes oxidative stress, changes in the immune system, heart and liver cells in rats (Falcioni *et al.*, 2010; Gabbianelli *et al.*, 2013; Nasuti *et al.*, 2007; Vadhana *et al.*, 2011). Both permethrin and cypermethrin are capable to reduce sperm motility (Yuan *et al.*, 2010), and arrest of spermatogenesis (Issam *et al.*, 2011).

MATERIALS AND MATHODS

Seventy two fresh fertilized eggs of White leg horn (*Gallus domesticus)* were divided into six groups, each with twelve eggs. Among these groups, four were injected with different concentrations of permethrin *i.e.,* 0.3125, 0.625, 1.25 and 2.5 µg/egg. Group C was intact control, whilst C (vehicle) was experimental control. The eggs were incubated at 37±0.5 $^{\circ}$C prior to injection over the layer of cotton under ventilation and moisture. These eggs were rotated twice a day. Thereafter, 0.1 ml of each prepared concentration was injected with the help of sterilized micro applicator by making window in egg shell. These windows were blocked immediately with molten paraffin wax

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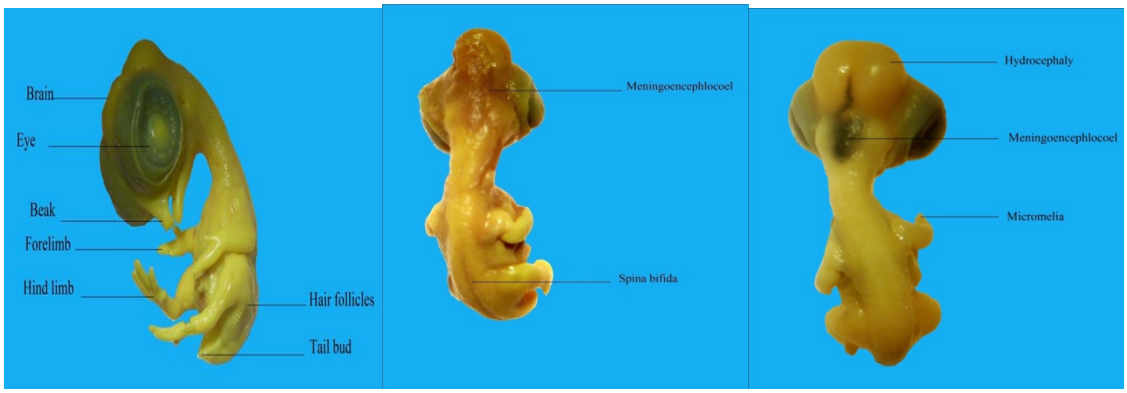
and eggs were incubated again at 37±0.5 °C. The embryos were recovered on the 8th day of incubation and fixed in Bouin's fluid. Various developmental parameters like wet body weight, crown rump lengths and malformations in chick embryos were noted. The data are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA)-Tukey's multiple comparison test using Prism Graph pad 5 Software (San Diego, CA) to establish significant differences (P<0.05) among groups.

RESULTS

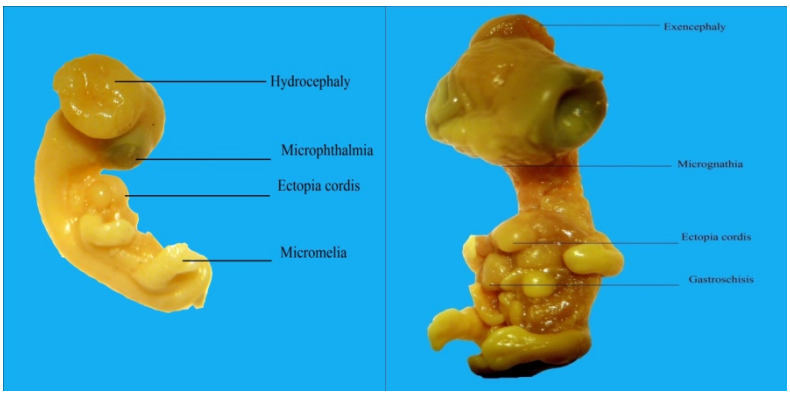
Significant (P<0.05) decrease in the body weight among all groups was observed; when compared with intact controls while CR length of treated groups differ significantly only from intact as well as vehicle control, but not from each other (Table 1).

Embryos recovered from intact control and vehicle control group (showing micromelia and microphthalmia in very few cases) were well developed, while those treated with various concentrations of permethrin appeared with meningoencephlocoel, spinabifida, hydrocephaly, micromelia, microophthalmia, ectopiacordis, micrognathia, gastroschisis and exencephaly (Fig 1-2). Dose dependent percentage of these anomalies was observed. Among these anomalies ectopiacordis, micromelia, microophthalmia and micrognathia were observed in most embryos (Table II).



(a) (b) (c)

Figure 1 Macrophotographs of chick embryos recovered on day 8 of incubation from (a) Control and dose groups (b)0.3125µg of permethurin/egg showing meningoencephlocoel and spina bifida, and (c) 0.625 µg of permethurin /egg showing hydrocephaly and micromelia.



(a) (b)

Figure 2 Macrophotographs of chick embryos, recovered on day 8 of incubation from dose groups (a) 1.25µg of permethurin /egg showing hydrocephaly, microphthalmia, ectopiacordis, and micromelia and (b)2.5 µg of permethurin /egg showing ectopiacordis, micrognathia, gastroschisis andexencephaly.

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Table I: Effect of different doses permethrin on Body weight and CR length of chick embryos injected on 4th and recovered on 8th day of incubation

	C	C(Vehicle)	0.3125µg/egg	0.625µg/egg	1.25µg/egg	2.5µg/egg
Body weight (mg)	940.8± 6.037[a]	903.3± 3.650[b]	705.6± 2.607[c]	578.2± 2.437[d]	526.9± 2.119[e]	477.6± 3.646[f]
Crown rump length (mm)	23.26± 0.3825[a]	19.55± 0.3114[b]	17.64± 0.1882[c]	17.28± 0.0883[c]	16.79± 0.0369[c]	16.27± 0.307[c]

Values are Mean±SEM of 12 replicates. Values not sharing common alphabet indicate significant difference (P<0.05) with each other [One way ANOVA-Tukey's multiple comparison test].

Table II: Morphological abnormalities (%age) produced by permethrin exposure to developing chick embryos on day 8th of incubation.

	C	C(Vehicle)	0.3125µg/egg	0.625µg/egg	1.25µg/egg	2.5µg/egg
Spina bifida	0.00	0.00	8.33	16.67	16.67	8.33
Gastroschisis	0.00	0.00	0.00	8.33	33.33	33.33
Exencephaly	0.00	0.00	0.00	0.00	0.00	16.67
Hydrocephaly	0.00	0.00	16.67	16.67	33.33	25
Micromelia	0.00	16.67	16.67	25	25	83.33
Micrognathia	0.00	0.00	16.67	25	33.33	83.33
Ectopiacordis	0.00	0.00	0.00	25	50	50
Meningocephlocoel	0.00	0.00	16.67	16.67	0.00	33.33
Microphthalmia	0.00	8.33	16.67	16.67	25	50

DISCUSSION

Although permethrin does not accumulate in liver and heart (Santos *et al.*, 2011), but still it is known to be cardio toxic (Vadhana, 2012; Vadhana *et al.*, 2010) and a potential necrotic as well as apoptotic agent (Guvenc *et al.*, 2013). In chick embryo, higher doses of cypermethrin have been reported to reduce the crown rump length, size of eye ball, micrognathia, agnathia(Anwar, 2003b) as reported in present study in case of permethrin , and severe damage to endothelial layer of Bowman's capsule as well as epithelial layer of glomeruli by permethrin(Anwar, 2003a). Permethrin causes reduction in DNA content as well as uric acid (Anwar *et al.*, 2004a). Its prenatal exposure mayaffect brain development and standing ability in mice(Imanishi *et al.*, 2013). It leads to neurobehavioral deficits (Abou-Donia *et al.*, 2001).

Observations demonstrate that in combined form it may promote epigenetic transgenerational inheritance (Manikkam *et al.*, 2012). Permethrin appears to be inducer of histopathological changes in liver including increased sinusoidal spaces in hepatic parenchyma, cytoplasmic vacuolations in hepatocytes, hepatocytic nuclear condensation, fatty degeneration, hydropic degeneration and necrosis of hepatocytes, tubular necrosis of glomeruli in developing chick and serious testicular damage in mice(Anwar *et al.*, 2004b; Anwar, 2003a; Anwar, 2003c; Jin *et al.*, 2012; Patrick-Iwuanyanwu and Charles, 2014).

From these prospects of previous studies as well as current dose dependent embryotoxic strength, it may be concluded that permethrin is attributed to teratogenicity. These findings suggestthe use of insecticide with immense care, following recommended doses.

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