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Research Article

Effect of deltamethrin containing formulation on developing chick embryo: morphological and skeletal changes

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ABSTRACT

The teratogenicity of a commercial formulation of the insecticide deltamethrin (Decis®) in chick embryos was evaluated. Fertilized eggs of *Gallus domesticus* were immersed in aqueous emulsions of deltamethrin at concentrations of 12.5 mg L^{-1}, 25 mg L^{-1} and 50 mg L^{-1} for 60 min at 37°C on 4th day of incubation. Two control groups of eggs were used: One group was immersed in distilled water (vehicle) and the second group was kept as untreated to study background toxicity. On embryonic day 16; recovered embryos were evaluated for mortality rate, wet body weight, gross morphological and skeletal malformations. The result revealed that embryonic mortality markedly increased after administration of deltamethrin. The significant decrease ($p \leq 0.05$) in wet body weight and significant increase ($p \leq 0.05$) in percentage of abnormal survivors was observed in dose dependent manner. A spectrum of external and skeletal malformations was exhibited by deltamethrin treated embryos. These finding suggests that deltamethrin exhibits embryotoxic and teratogenic effects in the developing chick embryos.

Keywords: synthetic pyrethroid, teratology, embryotoxic, morphology, skeletal anomalies.

INTRODUCTION

A key contributor to human health and diseases is our environment. Exposure to many environmental stressors such as pesticides have detrimental effects on health and are considered to contribute substantially to most diseases of major public health significance[1]. Pesticides use is increasing globally, particularly in third world countries[2]. In India, there has been a steady growth in production of technical grade pesticides, from 5,000metric tons in 1958 to 102,240 metric tons in 1998[3]. Now, India is second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. The pattern of pesticide usage in India is different from that for the world in general. In India 76% of the pesticide used is insecticide, as against 44% globally[4]. Despite government restrictions, these insecticides are preferred by many small farmers because of their low cost, easy availability and a wide spectrum of bioactivity[2].

Chicken is not only a major global food source, but also a unique and very important animal model for toxicology and biomedical research. It has been increasingly appreciated for the reasons such as small size, known embryonic development and lack of placenta, which may reveal the extent of maternal protective factors, minimal expenditure of time and money, possibility of experimenting on large scale for statistically valid results [5,6]. Easy accessibility and manipulation of chicken embryo from incubated eggs has traditionally been one of the greatest advantages of this animal model.

Deltamethrin, [(S)-a-cyano-3-phenoxybenzyl-(1R)-cis-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylate], which is the active ingredient of Decis®, belongs to type II synthetic pyrethroid. Synthetic pyrethroids, including deltamethrin, are manmade analogues of naturally occurring pyrethrins found in the flowers of *Chrysanthemum cinerariaefolium.* Deltamethrin was synthesized in 1974, and since then, it is widely used in agriculture, public health preservation programs for farm animals and pets. However, it is generally assumed, that pyrethroids affect neuroactivity by delaying the closing of sodium channels. This affects action potentials and often results in repetitive activity (type I) or blockage of nerve conduction (type II). Deltamethrin, a pyrethroid containing a cyano group, predominantly produces type II effects. Deltamethrin is reported to have a fast metabolism in living organisms, and a low level of residues in the environment; these may vary depending on the environmental conditions. Though this chemical is broken down via UV and sun lights, it is quite tolerant to storage and can preserve its activity for 6 months at 40ºC and pose risks to mammals and ecosystem as whole[7].

The embryotoxicity and teratogenicity of deltamethrin on mammals [8] and fishes [9, 10,11,12] have been reported. However, a very little data is available concerning the effect of deltamethrin on developing chick embryos. Therefore, present study was designed to analyze the possible embryotoxic and teratogenic effect of deltamethrin on morphological and skeletal characteristics of developing chick embryo.

MATERIALS AND METHODS

Use of the chick embryos was in conformity with the policies of Institutional Animal Care and Use Committee (IACUC, 2008). All procedures performed on the animals were approved by the Animal Ethical Committee of Institute.

Toxicant - The pesticide used in the present study was deltamethrin 2.8% EC (Emulsifiable Concentrate) with

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commercial name Decis®, manufactured by Bayer CropScience Limited, Gujarat, India.

Test animals – Fertilized eggs of *Gallus domesticus* (BV 300 breed) were obtained from a commercial hatchery (Kewalramani Hatcheries, Ajmer, India). All eggs were cleaned and placed in an incubator with capabilities of maintaining and monitoring temperature, humidity and turning the eggs periodically. The temperature in the incubator was maintained at 38 ±0.5° C and relative humidity was kept between 70-80%.

Experimental Design -After 4 days of incubation (stage 24, Hamburger and Hamilton 1951[13]), all the eggs were candled .Those which were infertile or contained dead embryos were discarded. The remaining eggs were divided into three groups (20 eggs per treatment group). Aqueous emulsions of deltamethrin formulation were prepared in distilled water in 12.5 mg L^{-1}, 25 mg L^{-1} and 50 mg L^{-1} concentrations based on the recommended dose (25 mg L^{-1}) of insecticide used for crop protection. Experimental eggs of treated group were immersed in these three suitable non lethal doses of deltamethrin for 60 min at 37°C temperature. The other two groups were the control groups (vehicle control and control).Vehicle control and control, were respectively immersed in distilled water and no treatment. The incubation was terminated on embryonic day 16 (stage 42, Hamburger and Hamilton, 1951[13]) and embryos were recovered for analysis of deltamethrin toxicity in sacrificed chick embryos. All the embryos were examined for any external malformations first and then embryos from treated and control groups were randomly selected for study of skeletal deformities. For visualizing skeletal abnormalities, selected embryos were deskinned, eviscerated and stained with Alcian blue and Alizarin red S [14].

STATISTICAL ANALYSIS

All the obtained values of wet body weight were presented as mean ± Standard error (S.E) and statistical significance was analyzed using student "t" test. Mortality rate and number of malformed embryos were compared by Mann- Whitney "U" test. Differences were considered significant when $p \leq 0.05$.

RESULTS

Table 1. Toxicity of deltamethrin in the chick embryo on embryonic day 16

Treatment	Number of eggs/ Treatment	Mortality (%)	Number of Surviving embryos	Abnormal Survivors (%)	(N)	Wet Body weight (g)
Control I(Untreated)	20	0	20	10	2	16.53±0.94
Control II (Vehicle treated)	20	5	19	16	3	15.58±0.64
12.5 mg L^{-1}	20	25	15	47	7	12.72±1.34*
25 mg L^{-1}	20	40#	12	67 #	8	11.50±1.38*
50 mg L^{-1}	20	35	13	85 #	11	11.44±1.07*

*Each value of wet body weight represents Mean±Standard error. * Significantly different from controls at p≤0.05 using student "t" test. # Significantly different from controls at p≤0.05 using Mann- Whitney "U" test.*

Embryotoxicity of the insecticide deltamethrin was investigated by comparing the percent mortality, wet body weight, and number of abnormal survivors with that of untreated control and vehicle treated control. Embryos exposed to 12.5 mg $L^{-1,}$ 25 mg L^{-1} and 50 mg L^{-1} of deltamethrin had mortality percentage of 25, 40 ($p \leq 0.05$) and 35 respectively which was markedly higher than that of control I and Control II (5%). The significant ($p \leq 0.05$) decrease in embryonic body weight, with a clear correlation with different concentrations of deltamethrin doses were observed in treated chick embryos as compared to control embryos (Table 1). A dose dependent increase in embryolethality and abnormal survivors were observed at all the three doses of deltamethrin. At 25 mg L^{-1} and 50 mg L^{-1} of deltamethrin, the percentage of malformed embryos was significant ($p \leq 0.05$) as compared to its lower dose 12.5 mg L^{-1} and controls.

The spectrum of embryonic malformations observed in deltamethrin treated embryo comprises the following:

External malformations: *Head region:* small size of brain (microcephaly), exposure of brain through the skull (exencephaly), absence of large part of brain (anencephaly), *Eye:* small eye (microthalamus), eyes entirely missing (anophthalmus), swelling and edema of eye, bulging eyes (exophthalmus), *Neck:* narrow neck, twisted neck, *Beak:* defects in development of beak, parrot beak, cleft beak, blood patches on the body (hematomas) ,internal organ abnormally exposed (ectopia viscera/gastroschisis), general growth retardation, *Leg:* short and twisted legs/digits, (Table 2 and Fig 1).

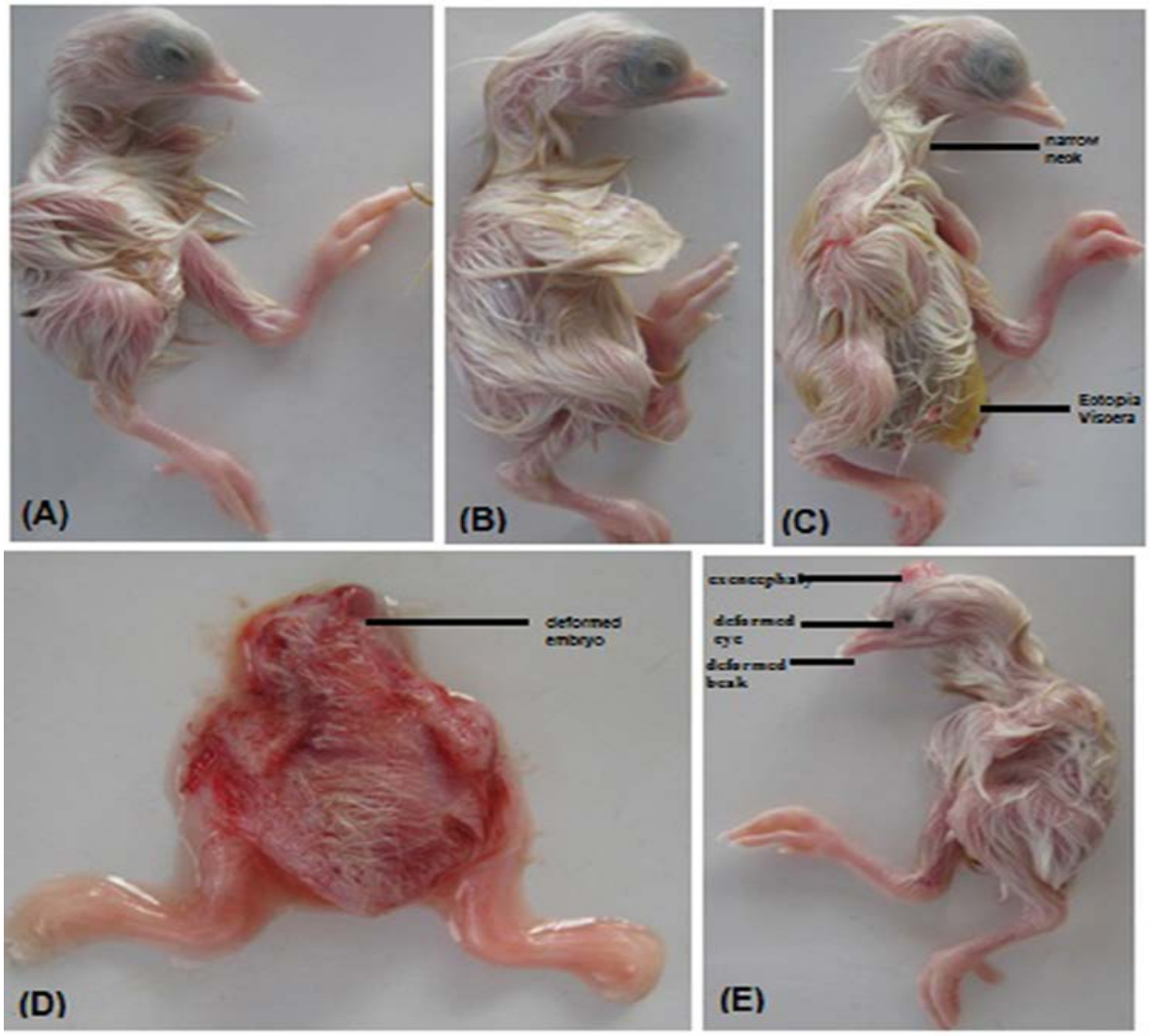
Skeletal malformations: *Skull:* short maxilla or mandible, congenital absence of cranium either partial or complete (acrania), *Vertebrae:* displaced or fused, lateral curvature of spine (scoliosis), anterior curvature of spine (lordiosis), posterior curvature of spine (kyphosis), reduction in size of caudal part of skeleton or complete absence of tail (Caudal Regression Syndrome), *Ribs:* wavy or flying, union of separate bone by osseous tissue (synostosis), *Sternum:* dumb bell shaped, synostosis, congenital absence of sternum(asternia), *Upper limb and lower limb:* shortened or bent (humerus, radius, ulna, femur, tibia, fibula) Any other change (less or poor ossification

Table 2. Incidence of external malformations in surviving chick embryo treated with deltamethrin

Treatment	No of Abnormal Survivors	Incidence of external malformation* (N)							
		Growth retardation	Head	Eye	Beak	Neck	Hematomas	Gastroschisis/ Ectopia viscera	Leg
Control I(Untreated)	2	0	0	0	0	1	0	1	0
Control II (Vehicle treated)	3	0	0	0	0	0	1	2	0
12.5 mg L^{-1}	7	2	2	1	2	2	2	3	1
25 mg L^{-1}	8	4	3	2	3	3	2	4	3
50 mg L^{-1}	11	3	3	3	3	4	4	6	4

***Head region: small size of brain (microcephaly), exposure of brain through the skull (exencephaly), absence of large part of brain (anencephaly), Eye: small eye (microthalamus), eyes entirely missing (anophthalmus), swelling and edema of eye, bulging eyes (exophthalmus), Neck: narrow neck, twisted neck, Beak: defects in development of beak, parrot beak, cleft beak, blood patches on the body (hematomas) ,internal organ abnormally exposed (ectopia viscera/gastroschisis), general growth retardation, Leg: Short legs or digits, twisted legs*

Figure 1. Photographs of 16 day old control and treated embryos, (A) Control I, (B) Control II, (C) 12.5 mg L^{-1} of deltamethrin treated embryo shows narrow neck and exposed internal organs(ectopia viscera), (D) an embryo treated with 25 mg L^{-1} of deltamethrin shows missing anterior part of body, and hematomas on body, (E) 50 mg L^{-1} of deltamethrin treated embryo shows exposure of brain through the skull, deformed eye, twisted cleft beak.



of bone, absence of bones, lack or reduction of cartilage and bone formation) in the axial and appendicular skeleton were also examined (Table 3 and Fig 2).

DISCUSSION

The present study is an attempt to evaluate the embryotoxic effects of the insecticide deltamethrin. This is first detailed analysis of deltamethrin toxicity in the chick embryo for which there are no detailed data available in literature to the best of our knowledge. In present study, fertilized eggs were exposed to deltamethrin by immersion technique since this way of application of insecticides imitates exposure associated with agricultural practice and allows us to assess the potential hazard posed by chemicals [15]. Insecticides often interfere with the fundamental developmental mechanisms and physiological functions of animal [16]. The data presented in this report indicate that, exposure to deltamethrin produce a higher percentage of mortality in chick embryos than that of observed controls. The incidence of higher embryonic mortality may be either due to intervention of deltamethrin in metabolic process [17] or due to damage and dysfunction of vital organs [18] during critical phase of embryogenesis. Köprücü and Aydin[10] in their study with *Cyprinus carpio*, reported that even the lowest concentration of deltamethrin (0.005µg/L) produced a significant increase in embryomortality and have adverse effect on the developing carp. Similarly, several other investigators also reported mortality in early stages of other experimental models such as *Clarias gariepinus*[9] ,*Silurus glanis* [11] and rats [8] due to exposure of deltamethrin. Furthermore, present finding are in accordance with those of Rachid et al.[17], Sahu and Ghatak[18], Kumar and Devi [19], Beverly and Leslie[20], Pourmirza[21], Petrovova et al.[22] , Waghet et al.[23] ,Mobarak and Al-Asmari [24] and Nitu et al.[25] , who reported high level of embryonic mortality in chick embryo exposed to various other pesticides.

The prominent effect of the toxicant in the present study was observed in the wet body weight of chick embryo exposed to deltamethrin. Dehydration of cells and intracellular space is perhaps a factor in the significant reduction of body weight of chick embryo exposed to deltamethrin. The results strengthen findings from other investigations that correspondingly indicated decrease in body weight of chick embryo exposed to Malathion or Endosulfan [21], Bendiocarbamate[22] and Endosulfan[24].

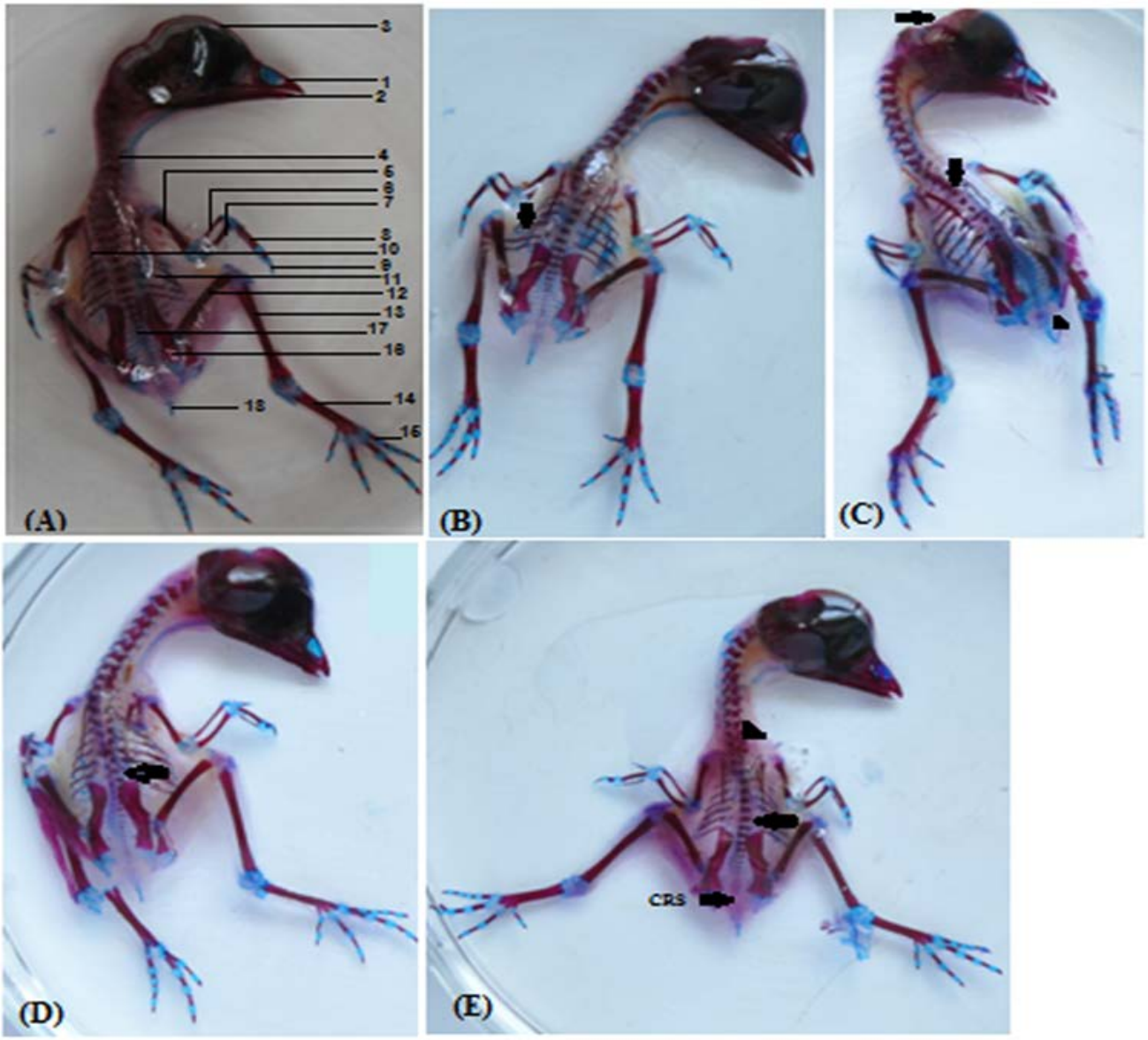
Presently, a significantly higher percentage of abnormal chick embryos were resulted from application of deltamethrin. The frequency of external and skeletal malformations in abnormal survivors was in dose dependent manner that is, greater the dose of deltamethrin the embryo was exposed to, the greater the likelihood it developed one or more deformities. Incidence of external malformations observed in present study such as microcephaly, exencephaly, anophthalmus, narrow neck or twisted neck, parrot beak, hematomas ,ectopia viscera/gastroschisis, general growth retardation, short legs or twisted legs are quite similar to earlier observations reported in chick embryo exposed to chloropyrifos and cypermethrin[16] ,dimecron[18], formocresol[20], lufenuron[23], dicofol[25], propotoxM[26], primicid[27] , bendiocarb[28], and. Similarly, pesticide (endosulfan) induced growth retardation were also observed by Mobarak and Al-Asmari[24] in chick embryo. According to them, malformations or abnormal development could be due to a consequence of gene mutation induced by insecticide which is a potent inhibitor of cell proliferation, development and differentiation and induces DNA fragmentation in developing chick embryo. According to Anwar[29], microcephaly in chick embryo reflects the reduction in size of brain that occurs as result of degenerative changes in neurons which might be due to pesticide induced

Table 3. Incidence of skeleton abnormalities in surviving chick embryo treated with deltamethrin

Treatment	No of embryo examined	No of embryos with skeleton abnormalities		Incidence of skeleton abnormalities* (N)					
				Axial				Appendicular	
		(N)	(%)	Skull	Vertebrae	Ribs	Sternum	Upper Limb	Lower Limb
Control I(Untreated)	15	2	13	0	1	1	0	0	0
Control II (Vehicle treated)	13	1	8	0	1	0	0	0	0
12.5 mg L^{-1}	10	4	40	1	2	2	0	1	1
25 mg L^{-1}	9	5	56	2	3	3	1	1	0
50 mg L^{-1}	11	6	55	2	4	3	1	2	1

**Skull: short maxilla or mandible, congenital absence of cranium either partial or complete (acrania), Vertebrae: displaced or fused, lateral curvature of spine (scoliosis), anterior curvature of spine (lordiosis), posterior curvature of spine (kyphosis), reduction in size of caudal part of skeleton or complete absence of tail (Caudal Regression Syndrome), Ribs: wavy or flying, union of separate bone by osseous tissue (synostosis), Sternum: dumb bell shaped, synostosis, congenital absence of sternum(asternia), Upper limb and lower limb: short or bent (humerus, radius, ulna, femur, tibia, fibula) ,less or poor ossification of bone, absence of bones, lack or reduction of cartilage and bone formation in axial and appendicular part of skeleton*

Figure 2. Photographs of 16 day old control and treated embryos, (A) Control I, (B) Control II, (C) 12.5 mg L^{-1} of deltamethrin treated embryo shows narrow neck and exposed internal organs(ectopia viscera), (D) an embryo treated with 25 mg L^{-1} of deltamethrin shows missing anterior part of body, and hematomas on body, (E) 50 mg L^{-1} of deltamethrin treated embryo shows exposure of brain through the skull, deformed eye, twisted cleft beak.



apoptosis. Furthermore, formation and development of the eye could be affected by injury of roof plate of the neural tube [18] and formation of hematomas could be suspected as possible cause of craniofacial malformation especially facial cleft [30]. Moscioni et al.[31] reported that insecticide (deltamethrin in present study) inhibition of kynurenine formamidase, which impairs conversion of tryptophan to essential pyridine nucleotide cofactors in the yolk sac membrane and possibly in the embryonic liver at later stage can be a possible mechanism for certain teratogenic effects in chick embryo.

In present study, various teratogenic anomalies were observed in axial and appendicular skeleton of chick embryo which cannot be compared with the results of Abdel-Khalik et al.[8] who reported no skeletal changes in fetuses recovered from deltamethrin treated female rats. Deformities such as incomplete or poor ossification of bone observed in chick embryo can result from interference of any environmental xenobiotics (deltamethrin) in endochondral and intramembranous pathways which are major ossification pathways of skeletal development in living vertebrates. Similarly, axial skeletal defects such as scoliosis, lordosis and fusion of cervical vertebrae were also reported by Hoffman and Eastin[32] in mallard embryo exposed to malathion, diazinon, and parathion and they concluded that these vertebral defects results from disruption of cholinergic system. Pyrethroids are known to effect the neurotransmission and according to Uggini et al. [16], such hindrance in neural activity and acetylcholinesterase activity can attribute to various vertebral malformations in chick embryo. Furthermore, our results are in

conformity with the findings of Kumar and Devi [19], Wagh et al.[23] and Mobarak and Al-Asmari[24] who reported several types of skeletal deformities induced by other pesticides in developing chick embryo.

CONCLUSION

From the results of the present study, it is quite clear that treatment of eggs with deltamethrin induced significant effects in the developing chick embryos. It was also noted that treated embryos exhibited a number of external and skeletal malformations. In the light of present investigation, it can be concluded that the deltamethrin is a potential teratogenic compound and therefore its use should be limited.

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