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Toxic Effects of Chlorpyrifos on 12th Day Desi Chick Embryo (*Gallus gallusdomesticus*)

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ABSTRACT

The toxicity and lethal effects, on the survival and developmental stages of Gallus gallusdomesticus, of widely used insecticides on the ticks and mites (Arachnida) was investigated. Fertilized eggs of Desihen (Gallus gallusdomesticus) at day 0 of incubation were treated with commercially available Chlorpyrifos insecticide at constant temperature 37 ± 0.5 ^{0}C.Five different concentrations of this insecticide (0, 0.12, 0.25, 0.50, and 1% per 100 µl/egg) were used in this experiment. Data on embryos survival and abnormalities were used to observe hazardous effects on developing embryos after pesticide exposure. The embryo mortality, ratio of abnormal embryos were statistically significantly ($p < 0.05$) different in treated groups compared to untreated. Moreover, toxicity increased with concentration in all experimental groups. The application of Chlorpyrifos (organophosphate) insecticide maybe potentially harmful to embryonic development of birds and other animals.

1. Introduction

Poultry is a rich source of cheap, palatable, and nutritious protein, hence an important constituent of our daily food in the form of eggs and white meat. Chicken are excellent table birds and people all over the world consume them in large quantities [1-2]. The poultry sector is one of the most important agricultural sectors and generates employment directly or indirectly for about 1.5 million people. Moreover almost 25.8% of the total meat production is contributed by poultry meat and the current investment in poultry industry is about Rs. 200 billion in Pakistan. Its contribution in agriculture and livestock is 6.4 %and 11.5 %, respectively. The poultry sector has shown a growth rate of 8 to 10 %annually [3]. Poultry meat is termed as non-fattening and is thought to prevent hardening of arteries. This is because as compared to red meat (beef and mutton) it contains low amount of fat [4].

In Pakistan, rural poultry farming has contributed significantly in household food security and in reducing poverty. Commercial and rural poultry (Desi breed) is playing a vital role in filling the gap between supply and demand of animal protein for the ever increasing human population [5]. However, Desi breed production has greatly decreased compared to market demand with poor productive potential and lack of genetically improved indigenous breeds being the major factors responsible for this low production [6-7]. Furthermore, insecticides, metals and fungicides cause morphological and histo-pathological abnormalities, biochemical changes, organ dysfunction and mortality in young embryos [8-9].

Pesticides are of great concern, because they are largely applied in agricultural fields, homes, offices, lawns and other public places to eliminate pests and parasites [10]. Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$), an organophosphate insecticide is widely used to control domestic and agricultural insect pests. It directly or indirectly affects the poultry sector, especially the Desi breed (*Gallus gallusdomesticus*) is more vulnerable to insecticides as compared to broiler poultry breed. This is due to the fact that pesticides are largely applied to eliminate pests in the rural areas. Irrespective of this, Chlorpyrifos affects human and animal health even at exposure at low levels over a long period of time. In organisms, it affects neurotransmitters, enzymes and cell signaling, thereby damage their nervous system processes and also causes developmental abnormalities. Furthermore pest resistance and resurgence has also been induced by the indiscriminate use of pesticides [11-12].

The hazardous impact of insecticides on broiler (*Gallus gallusdomesticus*) has been well studied [2, 13, 14]. However few studies have been conducted regarding the impact of insecticides on Desi breed *Gallus gallusdomesticus* [15] (Alhifi 2011). Furthermore the impact of Chlorpyrifos on Desi (non-descript native breed) chick embryo has still not been studied in Pakistan. Therefore, the present study was designed to evaluate the

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teratological effects of Chlorpyrifos on the mortality, morphology, crown-rump length, head, eye, beak, neck, fore and hind limbs' length and weight of the developing chick embryos of *Gallus gallusdomesticus*.

2. Materials and Methods

Teratogenicity of Chlorpyrifos40 EC in Desi chick embryos was evaluated. The experiment was conducted on fertilized eggs of *Gallus gallusdomestics*, in the Developmental Biology Laboratory, Department of Zoology, University of Gujrat, Gujrat, Pakistan. A total of150 eggs were purchased directly from different homes/farmers of Chak Sada, District Gujrat. Further information was collected from the farmers regarding the age of eggs to make sure uniformity of purchased eggs. Only same age eggs were selected for experiments. The eggs were randomly divided into five groups (n= 30 each group) irrespective of their size and colour. These groups were treated with five different concentrations viz., 0, 0.12, 0.25, 0.5, 1% /100µl/egg of Chlorpyrifos respectively. These concentrations were obtained by serial dilution of freshly prepared stock solution (4%) with corn oil. Chlorpyrifos (40% EC, Zarco Chemicals Pvt. (LTD.), 162-B Small Industrial Estate Sahiwal) was purchased from a local pesticide vendor.

A small hole was made in the shell of each egg with the help of a needle, avoiding the membrane. An aspirator was used to remove particles of egg shell. Around 100 µl of each concentration was injected horizontally into the yolk sac of the eggs of respective groups with micro applicator (needle, 1 inch long no. 27). The hole in the shell was sealed with adhesive tape in order to avoid contamination.

Incubation of eggs was carried in an incubator (Memmert 100-800) having capacity of 100-150 eggs. The shelves of the incubator were covered with cotton to avoid direct contact of eggs with shelf surface and the eggs were incubated with their broad ends up. The eggs were rotated after every 8 hours, maintained at an optimum temperature of 37 ± 0.5 ^{0}C, relative humidity of 60% and the ventilator was left open. Eggs were candled daily and dead embryos were removed.

Recoveries were made by cracking egg shell at 12th day of incubation. The remaining portion of the albumin was removed with the help of blunt forceps and embryos were separated in Petri dish containing water. The embryos were weighed (in grams) on an analytical balance and different measurements viz., crown-rump length, anterior-posterior head diameter, eye diameter, beak length, neck length and forelimbs (humerus, radius and ulna and metacarpus) and hind limbs (femur, fibula and metatarsus) lengths (in centimeters) were made.

Qualitative anomalies including microcephaly, hydrocephaly, ex-encephaly, swelling and hematomas formation, anophthalmia, microphthalmia, short upper beak, agnathia, micromelia, Amelia, omphalocele and ectopiacardis were recorded. The organs were studied with the help of a magnifying lens and the naked eye depending upon the size of the embryo.

2.1 Data Analysis

The data obtained of different morphometric parameters are presented as mean ± standard error (S.E). One way ANOVA was applied to test statistical significance using statistical package of Statistix (Version 8.1). Differences were considered significant when $p \leq 0.05$.

3. Results and Discussion

Table 1 shows the percent mortality of Desi chick embryos with the administration of five different concentrations of commercially used Chlorpyrifos (40% EC). The highest mortality rate (80%) was recorded with 1% concentration, while lowest mortality (26.6%) was found with 0.12% concentration. These results are in accordance with the findings of Asmatullah et al. [16], they reported that Chlorpyrifos has adverse effects on the development of avian embryos, even at very low concentrations. Similarly Tian [17] observed that Chlorpyrifosis teratogenic and embryo toxic in mice at doses below those that caused significant maternal toxicity.

Table 1: Embryos Mortalities Resulting from Injection of Chlorpyrifos into Yolk Sac of Chicken Eggs (n= 30 each group)

Treatment	Dose/egg (%)	Dead embryos (n)	Mortality (%)
Control	-	3	10
Chlorpyrifos	0.12	8	26.6
	0.25	15	50
	0.5	21	70
	1	24	80

Table 2 shows morphometric measurements of both control and all treated groups. Many authors have empirically confirmed that the impact of pesticides viz., Enrofloxacin, Chlorpyrifos and Cypermethrinon the development of embryos such as micropthalmia in chick embryos [18-19]. Both wet body weight and crown-rump length decrease significantly ($p<0.05$) in treated embryos compared to un-treated. The results obtained are in line with the findings of Whyatt [20], who described a decrease in fetal body weight dependent upon the level of Chlorpyrifos dose. However, Farag [21] and Ahmad [13] assessed the toxicity of Chlorpyrifos in developing mice and documented a decrease in head and body weight. In addition Asmatullah [22] examined the impact of Malathion on development of chick embryo and noted

Table 2: Morphometric parameters (mean±se) of 12-day-old chick embryos treated with five different concentrations of chlorpyrifos

Eggs Groups Parameters	Control Mean±SE	Treated groups			
		0.12% Mean±SE	0.25% Mean±SE	0.5% Mean±SE	1% Mean±SE
Wet body weight (g)	5.66±0.09^{a}	4.63±0.09^{b}	3.67±0.09^{c}	3.13±0.09^{d}	2.37±0.09^{e}
Crown Rump length (cm)	5.18±0.07^{a}	4.62±0.07^{b}	4.28±0.07^{c}	3.66±0.07^{d}	3.36±0.07^{e}
A.P head diameter (cm)	1.58±0.03^{a}	1.40±0.03^{b}	1.37±0.03^{b}	1.25±0.03^{c}	1.06±0.03^{d}
Eye diameter (cm)	1.00±0.02^{a}	0.97±0.02^{ab}	0.94±0.02^{b}	0.88±0.02^{c}	0.74±0.02^{d}
Beak length (cm)	1.32±0.04^{a}	1.32±0.04^{a}	1.21±0.04^{b}	1.02±0.04^{c}	1.03±0.04^{c}
Neck length (cm)	0.96±0.02^{a}	0.91±0.02^{ab}	0.82±0.02^{b}	0.70±0.02^{c}	0.58±0.02^{d}
Humerus length (cm)	0.91±0.03^{a}	0.89±0.03^{ab}	0.82±0.03^{bc}	0.78±0.03^{c}	0.60±0.03^{d}
Radius and ulna length (cm)	0.92±0.03^{a}	0.88±0.02^{a}	0.78±0.03^{b}	0.68±0.03^{c}	0.60±0.03^{d}
Metacarpus length (cm)	0.85±0.02^{a}	0.84±0.02^{a}	0.75±0.02^{b}	0.61±0.02^{c}	0.59±0.02^{c}
Femur length (cm)	1.35±0.04^{a}	1.28±0.04^{a}	1.24±0.04^{a}	1.08±0.04^{b}	0.84±0.04^{c}
Fibula length (cm)	0.86±0.04^{a}	0.85±0.04^{ab}	0.73±0.04^{bc}	0.68±0.04^{c}	0.59±0.04^{c}
Metatarsus length (cm)	0.99±0.03^{a}	0.86±0.03^{b}	0.84±0.03^{b}	0.76±0.03^{b}	0.61±0.03^{c}

Means with different superscripts differ in sign in a row^{a,b,c,d,e} marked on different rows refer to the significant difference to control and with each other

reduction in body weight and crown rump length. Furthermore, there was a significant (p<0.05) decrease in anterior-posterior head diameter of treated groups as compared to the control and among treated groups except between 0.12% and 0.25% concentrations. Pinakin [2] documented concentration dependent impact of Lufenuron on developing chick embryos. Moreover, Ahmad [23] reported head and skeletal abnormalities such as hydrocephaly, microcephaly, micromelia, agnathia, and hind limb twist in pregnant mice fetuses treated with Chlorpyrifos.

The average eye diameter (1.00±0.02), beak and neck of control group embryos showed a significant (p<0.05) difference as compared to all treated groups except 0.12% concentration. However, beak length between 0.5% and 1% treated group revealed insignificant (p>0.05) difference. These results coincides with the results of Mobarak [24], who documented that the beak and neck length of chick embryos treated with low concentrations of endosulfan showed insignificant difference compared to control. Moreover, Walker [25] reported abnormal beak development in chick embryos treated with Maloxon. While Schom [26] reported chronic toxicity of Chlorpyrifos in wild birds such as adult chickens and observed malformations like twisted necks and short or indented backs.

The average length of forelimbs (humerus, radius and ulna, metacarpus) of embryos showed significant (p<0.05) difference between treated and un-treated groups except at 0.12% concentration. However, the humerus was found insignificantly (p>0.05) different between 0.12% and 0.25% and also between 0.25% and 0.5% concentrations. Mobarak [24] have documented similar results associated with limb deformities in

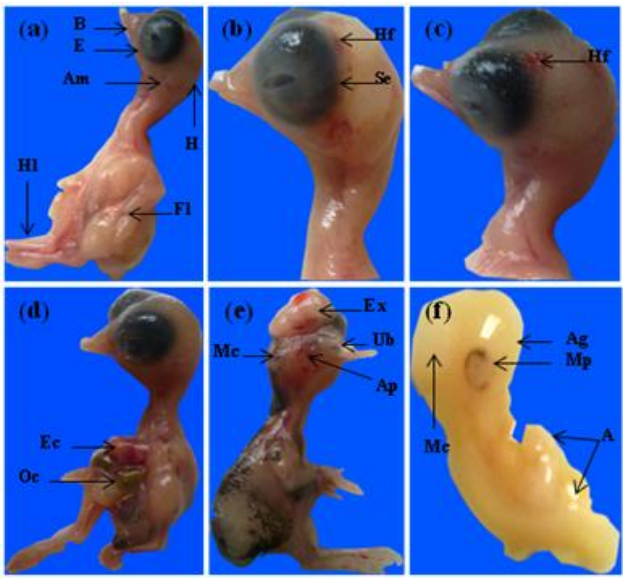

Fig.1: Photographs of 12-day-old chick embryos (a) control group having normal body parts with well-developed external auditory meatus (arrow head). (b,c) 0.12 % treated group showing hematomas formation on head and swelling around the eye. (d) 0.25% treated group showing ectopiacardis and omphalocele (poorly developed abdominal wall), (e) 0.5% treated group showing ex-encephaly, microcephaly, underdeveloped upper beak and anophthalmia (complete absence of one eye). (f) 1% treated group showing microcephaly, micropthalmia, agnathia, amelia

endosulfan treated chick embryos. The average length of hind limbs (femur, fibula, metatarsus) of treated groups of developing chick embryos showed significant (p<0.05) difference from control at high concentration. However,

an insignificant (p>0.05) difference was recorded at low concentrations. These findings are supported by studies of Asmatullah [22], who documented that high concentrations (125, 250 and 500mg/g) of malathion showed a significant lag in the development of main body parts such as limbs, accompanied by an significant increase in uncovered area of eyeball.

Table 3 shows frequency percentage of different anomalies observed in Chlorpyrifos treated embryos at different concentrations. Higher % of anomalies was recorded at1% concentration. However, short beak was observed in all treated groups. Microcephaly, anophthalmia, micromelia, omphalocele and ectopiacardis anomalies were recorded in 0.25, 0.5 and 1% concentrations. Fig.1a, shows a normal embryo of control group with normal body parts and well developed external auditory meatus**.** Fig. 1 (b, c) indicates swelling and hematomas formations on the head and around the eyes of the embryos treated with 0.12% concentration. Nancy [27-28] documented the various sub-lethal effects of pesticides (organochlorines and organophosphates) which include reproductive and developmental toxicity and endocrine disruption, abnormal ovulation and egg-shell formation in chicken.

Table 3: Frequency percentage of different anomalies observed in Chlorpyrifos treated 12-day-old Desichick embryos

Anomalies	Control	Treated Embryos			
		0.12%	0.25%	0.50%	1.00%
Short beak	-	4.54	6.06	11.11	33.33
Microcephaly	-	-	13.33	11.11	16.66
Swelling around eye	-	13.63	-	-	-
Microphthalmia	-	9.09	0	33.33	50
Anophthalmia	-	-	6.66	22.22	33.33
Hematoma	-	9.09	-	-	-
Amelia	-	-	-	-	33.33
Micromelia	-	0	13.33	11.11	16.66
Ex-encephaly	-	-	6.66	11.11	-
Agnathia	-	4.54	-	-	50
Omphalocele	-	-	13.33	22.22	16.66
Ectopiacardis	-	-	6.66	11.11	16.66

The embryos treated with 0.25% concentration of Chlorpyrifos showed ectopiacardis (heart outside) and poorly developed abdominal wall (omphalocele) due to which the abdominal organs including liver, stomach and gut extrude ventrally within abdominal sac (Figure, 1d). Anwar et al. [29] outlined severe teratological abnormalities at 100, 200, and 400 µl of Cypermethrin treatment in chick embryos Malformations viz., microcephaly, hydrocephaly, ex-encephaly, under developed upper beak and anophthalmia were observed with 0.5% concentration (Fig. 1e). It is clear from the previous investigations that organophosphorus insecticides are highly toxic to mammalian embryonic system even at lower concentrations [30-32].

Embryos treated with 1% concentration showed malformations including ex-encephaly, microphthalmia, anophthalmia, agnathia, micromelia, amelia and also resorbed embryos (Fig. 1f). Anwar [8] reported teratogenic changes such as micropthalmia, anophthalmia, agnathia and reduction in size of head such as microcephaly in cypermethrin treated chick embryos. However, Pinakin [2] documented macrocephaly and macrophthalmia in Lufenuron treated chick embryos in contrast to our results in which micropthalmia and microcephaly was observed.

4. Conclusions

It is evident from the results obtained from the present study that Chlorpyrifos (organophosphate) is potentially dangerous to embryonic development and may be hazardous to other animals including humans. Therefore, further research is needed to evaluate the impact of Chlorpyrifoson other animals including humans.

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