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Cypermethrin, a Pyrethroid Insecticide Induces Teratological and Biochemical Changes in Young Chick Embryos

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Abstract: Present study was designed to investigate the toxic effects of 50, 100, 200 and 400 ppm cypermethrin administered as a single dose at '0' day of incubation, on the development of chick at day 7 of incubation. The study included the investigation of teratological and biochemical changes in the developing embryos. Among biochemical constituents, activities of a few enzymes and some biochemical contents of the whole embryo were investigated. Activities of the enzymes amylase and AkP were affected with cypermethrin treatment. Activity of amylase increased whereas the activity of AkP decreased. Amylase activity was elevated at 400 ppm by 762%. In contrast, the activity of AkP was inhibited at 100, 200 and 400 ppm by 32, 85 and 53%, respectively. However, the activities of AcP, ALT, AST and LDH remained unaltered. Of the biochemical components, glycogen, free amino acids, total lipids, cholesterol, DNA and RNA contents were seriously affected. Total protein, soluble protein, uric acid and urea contents also remained unaffected with cypermethrin treatment. Cypermethrin treatment had no effect on embryonic glucose content, whereas, it affected glycogen content of the whole embryo in non-consistent manner. Glycogen content was increased at 50, 200 and 400 ppm and decreased at 100 ppm. Free amino acid content was decreased at 100, 200 and 400 ppm. Cholesterol content showed a significant increase at 200 ppm. Total lipid content was increased at 200 ppm and decreased at 400 ppm. DNA was increased at 200 ppm and decreased at 400 ppm, while RNA showed change only at the dose of 50 ppm. Teratological changes observed in the present study included the reduction in crown rump length, the size of brain and the size of eyeballs, incomplete development of eyes, beak and wing buds, micromelia, exocardiogenesis. In some treatment groups, eyes and in some groups beaks were totally absent.

Key words: Cypermethrin, chick embryo, teratology, biochemistry

INTRODUCTION

Pyrethroid insecticides, including cypermethrin are widely used against pests all over the world to increase the production of food grains and other agricultural-products. These insecticides produce reverse effects on the non-target organisms including both vertebrates and invertebrates living in the exposed area (Sibley and Kaushik, 1991; Ghosh, 1989; Majmunder *et al*., 1994 and Akhtar *et al*., 1992) are seriously affected by the insecticides.

Cypermethrin, (RS)-α-cyano-3phenoxy benzyl (IRS)-cis,trans-3-(2,2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate, belongs to type II pyrethroid and possess α-cyano group. Its degradation products are 3-(2,3-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylic acid(cis + trans isomer) and 3-phenoxy benzoic acid. It is photostable and possesses high insecticidal activity. Cypermethrin is widely used against pests all over the world (Usmani and Knowles, 2001) and there is increased risk of food being contaminated with the insecticide. This contaminated food may harm humans and the domesticated animals. It produces reverse effects on the non-target organisms including both invertebrates (Gowland *et al*., 2002) and vertebrates (Das and Mukherjee, 2003). For example, in amphibians, it induces apoptosis in the telencephalon of Physalaemus biligonigerus tadpoles (Anura: Leptodactylidae) (Izaguirre *et al*., 2000). In fishes, cypermethrin inhibits trypsin, lipase and carboxipeptidase A activity in Carp and causes a slight increase in alpha chymotrypsin activity (Simon *et al*., 1999). It is known to cause decrease in glycogen, pyruvate and lactate dehydrogenase and phosphorylase b activity and increase in lactate level, phosphorylase a and aldolase activities (Reddy and Yellamma, 1991). Sheela and Muniandi, (1992) noticed decreases in protein, RNA and glycogen in muscle and liver of fish following cypermethrin treatment. Das and Mukherjee (2003) observed cypermethrin-induced changes in various biochemical parameters like, DNA, RNA and enzyme activities i.e. LDH, SDH and ATPase in muscle, liver, brain and kidney of the Indian major carp, Labeo rohita. Cypermethrin also induces NADPH-cytochrome c reductase and cytochrome b5 in chicks (Kapoor *et al*., 1988).

In mammals, cypermethrin toxicity has been evaluated to a greater extent. Cypermethrin inhibits Na, K and Mg dependent ATPase activity in liver of albino rats (El- Toukhy and Girgis, 1993). Cypermethrin also affects the blood and immune system. Santoni *et al.* (1997) cypermethrin-induced increases in peripheral natural killer cell and antibody dependent cytotoxic activity in rats. Institoris *et al.* (1999) found that cypermethrin decreases delayed type hypersensitivity reaction, increases the number of numerical chromosome aberrations of the bone marrow cells, decreases mean cell volume of the RBCs and Ht value and reduces the white blood cell count in the peripheral blood of male Wistar rats. Haratym-Maj (2002) observed the increase in the number of leukocytes in peripheral blood, inhibition and mobilization of hemopoietic system in female mice following cypermethrin administration. In addition, cypermethrin is also known to reduce fertility in male rats through affecting of testosterone, follicle-stimulating hormone and luteinizing hormone and the number of cell layers of the seminiferous tubules as well as to cause congestion and haemorrhage in testes (Elbetieha *et al.*, 2001). Cypermethrin also effects electrically evoked contractions in muscles of guinea pig (Tonini *et al.*, 1990). It affects the nervous system through the release of acetyl cholinesterase from rat brain synaptosomes and also affects the voltage-sensitive sodium channel (Eells *et al.*, 1992). Eells *et al.* (1993) observed that cypermethrin causes depolarizing responses in rat and trout synaptosomes. Sheets, (2000) observed that young rats are more sensitive to pyrethroids than old rats and this greater susceptibility of the neonates to cypermethrin appears to be due to limited metabolic capacity.

Although a lot of work has been done on the toxicity of cypermethrin on fishes and mammals, but a little work has been done on chicks (Kapoor *et al.*, 1988), which are widely used as a source of protein all over the word. Any harmful substance like insecticides, metals, fungicides, gases and their residues can affect growth and development in chicks. Insecticides from insecticides-contaminated feed can be transported to young embryos through eggs and thus can cause teratological abnormalities, biochemical changes, organ dysfunction and mortality in the young embryos. A little information is available on the toxicity of cypermethrin in chick embryos. So the present study was designed to investigate the toxic effects of a single dose of cypermethrin on the development of chick in terms of biochemical and teratological changes. As cypermethrin is commercially used in large quantity, the studies of the secondary affects of this insecticide in developing chicks are of great toxicological importance.

MATERIALS AND METHODS

Fertilised eggs were administered with different doses of cypermethrin insecticide. Dilutions were prepared in acetone. LD_{50} was obtained using probit analysis and was found to be 800ppm. A single sub lethal dose (0.05 ml) of the insecticide of each concentration (50, 100, 200 and 400 ppm) was administered through injection to 4 groups (6 eggs in each), respectively, into the yolk of each egg at vegetal pole by disposable tuberculin syringes at day '0' of incubation. Equal volume of acetone was injected into the controls. The eggs were incubated at 38 ± 0.5°C in incubators with a relative humidity of 70% with proper ventilation. The eggs were rotated every two hrs to avoid the sticking of the embryo to the shell membranes.

At day '7' of incubation, embryos were taken out from the eggs, weighed, minced and mixed and then divided into two parts. One part was used for making saline homogenate, while the other part was used for the extraction of lipid, cholesterol and nucleic acid contents. The saline homogenate was used for the estimation of various enzyme activities and some biochemical components.

Estimation of enzyme activities: The activities of alkaline phosphatase (AkP, orthophosphoric monoester phosphohydrolase, alkaline optimum, EC: 3:1:3:1) and acid phosphatase (AcP, orthophosphoric monoester phosphohydrolase, acid optimum, EC: 3:1:3:2) were estimated according to the method of Kind and King (1954). Lactate dehydrogenase (LDH, L, lactate: NAD oxidoreductase (EC 1:1:1:27) activity was estimated by a method based on Cabaud and Wroblewski (1958). The activities of aspartate aminotransferase (ASAT; L, aspartate: 2 oxoglutarate aminotransferase, EC 2:6:1:1) and alanine amino transferase (ALAT; L, alanine: 2 oxoglutarate aminotransferase (EC 2:6:1:2) by the method of Reitman and Frankel (1957). The amylase (1, 4 a-D glucanhydrolase, EC 3:2:1:1) activity was estimated according to the procedure described in Wootton (1964).

Soluble proteins were determined from saline tissue extract, while same saline extract was digested in 0.5N NaOH for 24 h and used for the estimation of total proteins. Both total and soluble proteins were estimated according to Lowry *et al.* (1951).

Glucose content was estimated by O-toluidine method of Hartel *et al.* (1969). Glycogen content in the supernatant left after centrifugation of saline homogenate was precipitated with ethanol and then dissolved in distilled water and estimated by the Anthrone method of Consolazio and Lacono (1963). Amino acid contents were estimated according to the Ninhydrin method of Moore

and Stein (1954). Estimation of urea was performed according to the DAM method as described by Natelson *et al.* (1951). Uric acid content was determined according to the method described by Carraway (1963).

For the extraction of total lipid and cholesterol, the tissue was ground in hot ethanol (60°C) and kept for extraction overnight. After centrifugation at 5,000 rpm for 10 min, the supernatant was obtained and used for the estimation of total lipid by Vanillin reagent (Zollner and Kirsch, 1962) and cholesterol content according to Liebermann and Burchardt Reaction (Henry and Henry, 1974). Nucleic acids were extracted according to the method described by Shakoori and Ahmed (1973). The pellet left during lipid extraction was used for preparation of DNA and RNA extracts. DNA was extracted in hot PCA and estimated according to diphenylamine method, while RNA extract was prepared in cold PCA and estimated according to the orcinol method. Both these estimations follow the procedure as described in Schneider (1957).

Instruments: Teflon Glass homogeniser (TRI-R STIR-R, Model S63C USA), UV Spectrophotometer (Model M 302, Camspec, England), Spectrophotometer (Sequola-Turner, Model 340, USA), Refrigerated Centrifuge (Sigma, Germany), Centrifuge (PHG Hermle Z 230, West Germany), Water Bath (LCB 800 NEDTEX Co Taiwan), Incubator (Memmert, West Germany) and Analytical Balance (Sartorius, West Germany).

Place of work: All the work was done in Biochemistry and Toxicology Laboratory, Zoology Department, Azad Jammu and Kashmir University Muzaffarabad, Azad Kashmir.

RESULTS

Biochemical changes

Enzyme activities: Table 1 and 2 show the effect of various concentrations (50 ppm, 100 ppm, 200 ppm and 400 ppm) of cypermethrin administered at '0' day of incubation on the activities of amylase, AkP, AcP, ALT, AST and LDH of 7-day-old whole chick embryo. Amylase and AkP activities were affected with cypermethrin treatment. Activity of amylase increased whereas the activity of AkP decreased. Amylase activity was elevated at 400 ppm by 762%. The activity of AkP was inhibited at 100, 200 and 400 ppm by 32, 85 and 53%, respectively. The activities of AcP, ALT, AST and LDH remained unaltered.

Biochemical components: Results are presented in Table 3 and 4. Among carbohydrates, cypermethrin treatment had no effect on embryonic glucose content, whereas, it affected glycogen content of the whole embryo in non-consistent manner. The embryonic glycogen content was increased by 98, 103 and 27% at 50, 200 and 400 ppm respectively, whereas it was decreased at 100 ppm by 53%. Both toatal proteis and soluble proteins remained unaffected with cypermethrin treatment. Free amino acid content was decreased by 44, 38 and 54% at 100, 200 and 400 ppm, respectively. Cholesterol content showed increase of 144% at 200 ppm whereas it remained unaffected at remaining doses. Total lipid content was increased at 200 and decreased at 400 ppm by 40 and 45%, respectively. Nitrogenous wastes, the urea and uric acid contents also remained unaltered with cypermethrin treatment. Among nucleic acids, DNA was increased at 200 ppm by 52% and decreased at 400 ppm by 45%, while RNA showed increase at the dose of 50 ppm.

Teratological changes

Control embryo: At day '7' of chick development maturation of organ system starts as the differentiation of various organ rudiments are established by the end of 4th day of incubation. In the present study, the stage in 7-day-old chick embryo correlates with the stage classified by Hamburger and Hamilton (1951). In 7th day chick embryo crown rump length is 14mm. Brain is well developed, brain bulges are prominent especially prosencephalon and mesencephalan are well developed. Eyeball is at advance stage of development with a diameter of 3.8mm. Beak rudiment looks just emerged. Limbs are differentiated and flapper-like structures are formed indicating future digit formation. Heart is enclosed in body cavity. Wing buds are prominent.

Vehicle-treated control embryos: Results are shown in Fig. 1. In vehicle-treated control embryos, the crown rump length was normal. Brain bulges were prominent, especially fore and mid brain structure was quite well developed. Eyeballs were at advance stage of development, beak was prominent and limbs were differentiated with flapper like structure indicating future digit formation. Wing buds were also present.

Treated embryos: Results are shown in Fig. 1. No malformations were seen in embryos treated with 50 ppm of cypermethrin when compared with those of controls. Teratological abnormalities were observed in embryos treated with 100 and 200 and 400 ppm of cypermethrin and these changes were not in dose dependent manner. Exposure of embryos to 100, 200 and 400 ppm of cypermethrin has resulted in the marked reduction in crown rump length. At 100 ppm, eye was not developed, owever, At 200 and 400 ppm, the eyes were developed but

Table 1: Effect of Cypermethrin on some of the enzyme activities of 7-day-old chick embryos developed from eggs injected at '0' day of incubation with a single sublethal dose of cypermethrin of various concentrations (50, 100, 200 and 400 ppm)

	Control (n=6)	50 ppm (n=6)	100 ppm (n=6)	200 ppm (n=6)	400 ppm (n=6)
Amylase So U/g	18.3±3.29	33.48±9.09	20.5±2.43	35.45±8.14	157.78±52.14*
AkP KAU/g	0.34±0.04	0.33±0.11	0.23±0.02*	0.05±0.02***	0.16±0.05*
AcP KAU/g	0.27±0.07	0.25±0.04	0.26±0.05	0.19±0.05	0.25±0.08
AST IU/g	13.88±0.91	10.93±1.81	10.83±1.53	17.85±3.06	15.46±2.62
ALT IU/g	1.59±0.54	0.75±0.34	1.22±0.36	1.64±0.75	1.75±0.57
LDH IU/g	30.33±7.7	39.05±7.91	22.5±3.59	69.07±21.79	36.99±7.41

Table 1 shows the toxic effects of cypermethrin on amylase, AkP, AcP, ALT, AST and LDH activities '7' day old whole chick embryo. Cypermethrin of various concentrations (50, 100, 200 and 400 ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Embryos were taken out from the eggs at 7th day of incubation and analysed for the enzyme activities. Only statistically significant changes were considered.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Abbreviations used

AkP, Alkaline Phosphatase; AcP, Acid Phosphatase; ALT, Alanine aminotransferase; AST. Aspartate aminotransferase; LDH, Lactate dehydrogenase;

IU: International unit, the amount of enzyme which under defined assay conditions will catalyse the conversion of 1 micro mole of substrate per minute;

SoU: Somogyi Unit: The amount of enzyme that catalyses digestion of 5 mg of starch under the experimental condition;

KAU: King Armstrong Unit: The amount of enzyme that transforms 1 mg of phenol in 15 min.

Table 2: Percent change in the Enzymes Activities of 7-day-old chick embryos developed from eggs injected at '0' day of incubation with a single sublethal dose of cypermethrin of various concentrations (50, 100, 200 and 400 ppm)

Parameters	50 ppm (n=6)	100 ppm (n=6)	200 ppm (n=6)	400 ppm (n=6)
Amylase So U/g	-	-	-	+762
AkP KAU/g	-	-32	-85	-53
AcP KAU/g	-	-	-	-
AST IU/g	-	-	-	-
ALT IU/g	-	-	-	-
LDH IU/g	-	-	-	-

Table 2 shows the percent change in the activities of amylase, AkP of 7-day-old whole chick embryo. Cypermethrin of various concentrations (50, 100, 200 and 400 ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Embryos were taken out from the eggs at 7th day of incubation and analysed for the enzyme activities. Only statistically significant changes were considered

Table 3: Effects of various concentrations of Cypermethrin on some Biochemical Components of '7' day old Chick Embryo

	Control (n=6)	50 ppm (n=6)	100 ppm (n=6)	200 ppm (n=6)	400 ppm (n=6)
Glucose mg g^{-1}	1.43±0.47	0.93±0.09	0.56±0.1	1.12±0.45	1.29±0.34
Glycogen mg g^{-1}	7.16±0.45	14.23±2.33*	3.39±1.02**	14.54±2.56*	9.1±0.75*
Total Protein mg g^{-1}	73.4±15.47	94.13±11.04	54.12±4.49	125.71±15.18	97.02±25.93
Soluble Protein mg g^{-1}	14.29±0.95	15.62±2.21	15.23±1.32	14.52±3.04	18.72±3.03
Free Amino acids mg g^{-1}	1.97±0.28	1.86±0.38	1.19±0.12*	1.33±0.18*	0.99±0.21**
Cholesterol mg g^{-1}	4.62±1.1	7.47±0.77	5.75±1.01	11.29±2.45*	2.66±0.27
Total Lipids mg g^{-1}	47.38±7.04	57.91±4.09	41.03±11.64	66.24±3.42*	26.24±2.67*
Urea mg g^{-1}	0.80±0.43	0.68±0.14	0.54±0.06	0.77±0.18	0.61±0.17
Uric Acid mg g^{-1}	0.88±0.18	0.94±0.12	0.6±0.13	0.75±0.18	0.42±0.07
DNA mg g^{-1}	1.72±0.21	2.82±0.47	2.52±0.54	2.61±0.16**	0.94±0.07**
RNA mg g^{-1}	8.75±0.67	14.52±1.16**	8.96±0.87	10.99±0.85	8.94±1.04

Table 3 shows the toxic effects of cypermethrin on some biochemical components of 7-day-old whole chick embryo. Cypermethrin of various concentrations (50, 100, 200 and 400 ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Embryos were taken out from the eggs at 7th day of incubation and analysed for some biochemical components. Only statistically significant changes were considered.

* $P < 0.05$; ** $P < 0.01$

Table 4: Percent change in the Biochemical Components of 7-day-old chick embryos developed from eggs injected with a single sub lethal dose of cypermethrin of various concentrations (50, 100, 200 and 400 ppm) at day '0' of incubation

Parameters	50 ppm (n=6)	100 ppm (n=6)	200 ppm (n=6)	400 ppm (n=6)
Glucose mg g^{-1}	-	-	-	
Glycogen mg g^{-1}	+98	-53	+103	+27
Total Protein mg g^{-1}	-	-	-	-
Soluble Protein mg g^{-1}	-	-	-	-
Free Amino acids mg g^{-1}	-	-44	-38	-54
Total Lipids mg g^{-1}	-	-	+40	-45
Cholesterol mg g^{-1}	-	-	+144	-
Urea mg g^{-1}	-	-	-	-
Uric Acid mg g^{-1}	-	-	-	-
DNA mg g^{-1}	-	-	+52	-45
RNA mg g^{-1}	+66	-	-	-

Table 4 shows the percent change in glycogen, free amino acids, total lipids, cholesterol, DNA and RNA contents of the '7' day old whole chick embryo developed from eggs administered with (0.05ml) of cypermethrin of various concentrations (50, 100 and 200 ppm). Cypermethrin was dissolved in acetone. Control eggs received acetone only. Embryos were taken out from the eggs at 7th day of incubation and analysed for the biochemical components. Only statistically significant changes were considered

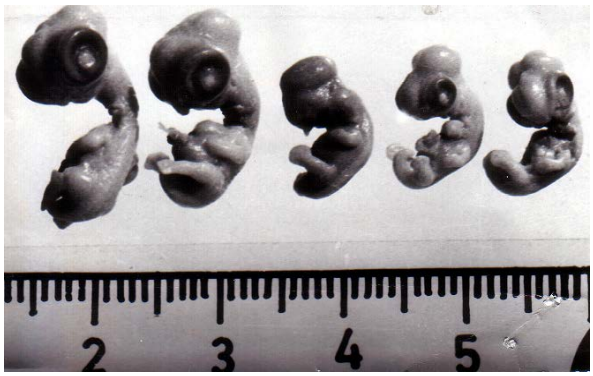

Fig. 1: Shows the comparative gross morphological changes in young chick embryos treated with a single sublethal dose of various concentrations of cypermethrin. Right embryo is vehicle treated control embryo Next to it are 50, 100, 200 and 400ppm treated embryos. 50 ppm of cypermethrin treated embryo is not affected whereas, the remaining three embryos treated with 100, 200 and 400ppm are severly affected with the insecticide. Embryo # 3 from left to right is 100ppm treated embryo. Incomplete beak development and the absence of eye is visibe in this embryo. In the last two embyos treated with 200 and 400 ppm, beak is totally absent and eyeballs are significantly reduced. Limb differentiation is seen totally absent in 400 ppm treated embryo (last embryo)

the size of eyeball was also significantly reduced. Exocardiogenesis was observed at 200 ppm. Beak was incompletely developed in 100 ppm-treated embryos, whereas, at 200 and 400 ppm, it was totally absent. Micromelia was observed in embryos treated with 100 and 200 ppm of the pesticide. Limbs were not differentiated in the embryos at the dose of 400 ppm. Reduction in the size of brain and the poor development of wing buds was observed at 100, 200 and 400 ppm.

DISCUSSION

Biochemical components: In the present study, glycogen was decreased at 100 ppm and increased at all the remaining doses of 50, 200 and 400 ppm. Decrease in glycogen content might have occurred as a result of its utilization to detoxify the cypermethrin or its metabolite through the process of glucuronidation (a process by which toxic metabolites combine with glucose phosphate and are excreted via bile). Decrease in glycogen content has been observed in muscle and liver of fish following cypermethrin treatment (Sheela and Muniandi, 1992). Phosphorylase a and phosphorylase b enzymes are involved in glycogen break down. Reddy and Yellamma, (1991) observed the increase in phosphorylase a and decrease in phosphorylase b activity with cypermethrin treatment, although both the enzymes degrade glycogen. Their activation can increase the glycogen breakdown and their inhibition can decrease the breakdown of glycogen and thus increase its synthesis. In the present study decrease in glycogen content at 100 ppm and increase in it at the other doses (50, 200 and 400 ppm) might have occurred through the activation and inhibition of phosphorylase a and phosphorylase b enzyme activities. Other possibility for increased glycogen content could be the reduced metabolic activity caused by cypermethrin. Increase in lactate and decrease in pyruvate level by cypermethrin as observed by Reddy and Yellamma, (1991) also indicate slow carbohydrate metabolism. Decrease in the free amino acid content may indicate the utilization of the amino acids either for the oxidation to provide energy or used for the synthesis of protein to repair the injured tissues. Increase in cholesterol content 200 ppm may indicate slow metabolism that resulted in total lipid contents. Total lipid content also showed a significant increase at this dose. Increase in cholesterol and total lipids content at this dose correlates well with the increase in glycogen content, which altogether show the decreased metabolic rate. However, at 400 ppm, total lipid contents were significantly reduced as compared to controls. Similar changes were observed in DNA content, which showed increase at 200 ppm and decrease at 400 ppm. Decrease in embryonic total lipid and DNA content may indicate tissue damage with high dose of the toxin. The high dose of 400 ppm of cypermethrin seems to have caused cell death (necrosis or apoptosis or both) in the embryonic tissue and thus has resulted in the decrease of DNA. RNA was significantly increased at the low dose of 50 ppm while at higher doses no change in RNA content was observed. This increase in RNA content might have occurred to synthesize proteins under cypermethrin-induced stress conditions.

Enzyme activities: From amongst enzymes, only the activities of amylase and AkP were affected, whereas, the remaining enzymes were unaltered with cypermethrin treatment. Increase in amylase activity indicates the direct interference of cypermethrin with carbohydrate metabolism. Activity of AkP was decreased at 100, 200 and 400 ppm of cypermethrin treatment. AkP is membrane bound enzyme, it is found on all cell membranes where active transport occurs and is hydrolase and transphosphorylase in function. This decrease in AkP

activity is taken as an index of parenchymal damage (Onikienko, 1963). In the present study, decrease in AkP activity may indicate damage to embryonic tissues as revealed by the reduction in crown rump length and other abnormalities in chick embryos caused by cypermethrin insecticide.

Teratological changes: High doses (100, 200 and 400 ppm) of cypermethrin caused severe teratological changes in the chick embryo. These changes included the reduction in crown lump length, size of head and size of eyecup, incomplete development and in some cases totally absence of beak. The decrease in crown rump length was also observed in the chick embryo following treatment with CCl_4 at the doses of 25 ppm, 35 ppm and 75 ppm (Clemedson *et al*., 1989). Mufti and Nasim (1987) observed these teratological changes in 7th day chick embryos with the dose of 2mg/egg of dimicron (an O P insecticide). Furthermore, Misawa *et al*. (1981) observed such kind of teratological abnormalities in the chick embryo induced with diazinon an OP insecticide. Van Steenis and Van Loghten (1971) observed muscular atrophy, shortening of the extremities and peripheral neuropathy in 7-day-old chick embryo exposed to dithiocarbamate tecoram. Walker, (1971) observed abnormal beak development in chick embryos treated with 30 imoles of maloxon. He also observed inhibition of plasma cholinesterase enzyme with maloxon. Eells *et al*. (1992) have already shown the release of acetyl cholinesterase from rat brain synaptosomes following cypermethrin administration. Recently, Das and Mukherjee (2003) observed the decreased acetyl cholinesterase activity in the brain of the carp Labeo rohita treated with cypermethrin. So in the present study deformities observed in the muscular and nervous system of cypermethrin treated chick embryos seem to be due to the interference of cypermethrin with neurotransmitter acetyl cholinesterase activity.

Reduction in the size of head was observed by at 100 and 200 and 400 ppm. Reduction in the size of head reflects the reduction in the size of brain that occurs as a result of degenerative changes in neurons. Degenerative changes in neurons might have occurred as a result of cypermethrin-induced apoptosis. Izaguirre *et al*. (2000) observed cypermethrin-induced apoptosis (a programmed cell death) in the telencephalon of Physalaemus biligonigerus tadpoles (Anura: Leptodactylidae), which is also mediated by reactive oxygen species (ROS) and lipid hydroperoxide radicals (Sarafian and Bredesen, 1994). Generation of free radicals and reactive oxygen species (ROS) as a result of increased cerebral lipid peroxidation have also been observed in rat brain by cypermethrin intoxication (Giray *et al*., 2001). Cypermethrin-induced free radicals have been observed in plasma, liver, brain and testes of rabbits (El-Demerdash *et al*., 2003). ROS, free radicals and reactive metabolites are generated during the metabolism of toxins via mixed oxidase system including cytochrome P450s. El-tawil and Abdel-Rahman (2001) found the involvement of cytochrome P450 in cypermethrin metabolism. Induction of Cytochrome P450 CYP2B1 with cypermethrin was also observed in rat hepatocyte cultures (Heder *et al*., 2001). Cypermethrin also induces NADPH-cytochrome c reductase and cytochrome b5 in chicks (Kapoor *et al*., 1988). These ROS, free radicals and reactive metabolite are either detoxified through conjugating with glutathione or react with tissue macromolecules to initiate the damage. The activity of glutathione S-transferase (GST), a detoxification enzyme is induced with cypermethrin in shore crab (Gowland *et al*., 2002). Toxic effects of cypermethrin on chick embryo at higher doses and no effect at lower doses indicate that cypermethrin is metabolised to its toxic metabolites, which at lower doses can be detoxified by conjugating with glutathione but at higher doses are unable to be detoxified as the glutathione level in the tissue depletes. This damage can lead to teratological abnormalities in growing embryos. In addition, the other evidence, which may support the teratogenic effects of cypermethrin, comes from the findings of Giri *et al*. (2003) who observed the genotoxic effects of cypermethrin. He observed that cypermethrin induces sister chromatid exchanges in bone marrow cells in a murine test system *in vivo*. Present finding, the decrease in DNA at high dose of cypermethrin (Tables 3 and 4) also indicates the damage to DNA with cypermethrin. Since the growing embryo needs a lot of energy as the cells multiply rapidly, the decreased energy supply as evidenced by the accumulation of glycogen and total lipid contents of whole embryo as observed in the present study may affect the embryonic growth. Increase in DNA contents at 200 ppm and decrease at 400 ppm indicates that dose of 200 ppm of cypermethrin might have caused some mutations, which resulted in the teratological abnormalities. Amer and Aboul-ela (1985) have already shown the mutagenic potential of cypermethrin as evidenced by the induction of micronuclei in mouse bone marrow. Later, similar observations were made by Surralles *et al*. (1995) who noticed the increase in the number of micronuclei and micronucleated cells in whole blood lymphocyte cultures in humans as a result of cypermethrin toxicity.

Changes in the biochemical components observed in the present study seem to contribute towards the development of teratological changes in the young chick embryos. In the light of these observations it is recommended that cypermethrin should be used with caution, as it could be hazardous to domestic animals and human beings as well.

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