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Toxic Effects of Cypermethrin on the Development of Muscle in Chick Embryo of *Gallus domesticus*

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ABSTRACT

Present study was aimed at investigating the toxic effects on the muscle of 16 day-old-chick embryo of different concentrations (50, 100, 200 and 400 ppm) of cypermethrin insecticide administered as a single sublethal dose (0.05 ml) into the eggs at day '0' of incubation. Toxicity was evaluated in terms of biochemical changes in the muscle of developing embryo. Among biochemical constituents, activities of a few enzymes like, amylase, alkaline phosphatase (AkP), acid phosphatase (AcP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) and some biochemical constituents like, glucose, glycogen, total protein, soluble protein, free amino acids, total lipids, cholesterol, urea, uric acid, DNA and RNA were investigated. Of the enzymes the activity of only ALT was affected with cypermethrin treatment and that was its increase at all the doses. Cypermethrin treatment resulted in the increase in glycogen, total protein, soluble protein and free amino acids contents and decrease in the total lipid, cholesterol, urea and RNA contents of the muscle. Among carbohydrates, glycogen was increased at all the doses, whereas glucose content remained unaltered. Among proteins, total protein was increased at 100, 200 and 400 ppm, whereas soluble protein increased only at 100 ppm. Total free amino acids content also showed increase at 100 ppm. Among lipids, total lipid content was decreased at 200 and 400 ppm, whereas cholesterol content was decreased at all the doses. Urea content was decreased only at 400 ppm. RNA was decreased at 200 and 400 ppm. Uric acid and DNA showed mixed response, uric acid content was increased at 100 and 200 ppm and decreased at 400 ppm while, DNA was increased at 100 ppm and decreased at 50 and 200 ppm. These biochemical changes induced with cypermethrin treatment reflect the muscular damage.

Key Words: Cypermethrin; Chick Embryo; Biochemistry; Muscle

INTRODUCTION

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 to increase the production of food grains and other agricultural-products (Usmani & Knowles, 2001) and there is increased risk of food being contaminated with the insecticide, which may harm humans and domesticated animals. Insecticides from insecticides-contaminated feed can be transported to young embryos through eggs and thus can cause teratological abnormalities, organ dysfunction and mortality in the young embryos hence affects the growth.

Cypermethrin is metabolised through cytochrome P450 (El-tawil & Abdel-Rahman, 2001) and also induces cytochrome P450 CYP2B1 in rat hepatocyte cultures (Heder *et al*., 2001). In chicks, it induces NADPH-cytochrome c reductase and cytochrome b5 (Kapoor *et al*., 1988).

Cypermethrin produces drastic effects on both the invertebrates (Gowlan *et al*., 2002) and vertebrates (Das & Mukherjee, 2003). In vertebrates, 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). Cypermethrin 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 & Yellamma, 1991a). 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 DNA, RNA, LDH, SDH and ATPase of muscle, liver, brain and kidney of Indian major carp, *Labeo rohita*.

Sheets (2000) observed that young rats are more sensitive to the toxic effects of cypermethrin than old rats because of their less developed metabolic capacity. Cypermethrin is known to affect the blood and immune system, e.g. Santoni *et al*. (1997) noted the cypermethrin-induced increases in peripheral natural killer cell and antibody dependent cytotoxic activity in rats and 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 hematocrit value and reduces the white blood cell count in the peripheral blood of male Wistar rats. Recently, Haratym-Maj (2002) observed an increase in the number of leukocytes in peripheral blood and inhibition and mobilization of hemopoietic system in female mice following cypermethrin administration.

Toxicity of cypermethrin has also been evaluated in muscular and nervous system of fish and mammals. Tonini *et al.* (1990) observed that cypermethrin affects the electrically evoked contractions in the muscles of guinea pig. Neurotoxic effects of cypermethrin have been studied in rats by Eells *et al*. (1992) who observed the release of acetyl cholinesterase from rat brain synaptosomes with cypermethrin and its effects on the voltage-sensitive sodium channel. Later on, these authors noted the cypermethrin-induced depolarizing responses in rat and trout synaptosomes (Eells *et al*., 1993).

Although a lot of work has been done on the toxicity of cypermethrin on fishes and mammals, but a little work has been carried out on chicks (Kapoor *et al*., 1988). Muscle in the chicks provides a bulk of protein to the consumers and its development reflects the growth of the animal. No information is available on the toxicity of cypermethrin on the development of muscles in chick embryos. Therefore, the present study has been planned to investigate the toxic effects of cypermethrin on the development of muscles in chick embryos. The study included the investigation of biochemical changes in muscles. As cypermethrin is commercially used in large quantity, the studies of its secondary affects in developing chicks are of great toxicological importance and the information gained in the study regarding embryotoxicity will be equally applicable to human beings.

MATERIALS AND METHODS

This study was carried out in Biochemistry and Toxicology Laboratory, Zoology Department, Azad Jammu and Kashmir University Muzaffarabad, Azad Kashmir.

Thirty fertilized eggs obtained from Government Poultry Farm were used in the experiment. These eggs were administered with different doses of cypermethrin insecticide. Dilutions were made in acetone. LD50 of cypermethrin was obtained using probit analysis and was found to be 800 ppm. A single sublethal dose (0.05 ml) of the insecticide of each concentration (50, 100, 200 and 400 ppm) was administered through injection to four 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 control eggs. The eggs were incubated at 38 ± 0.5°C in incubators (Memmert, West Germany) with a relative humidity of 70% and with proper ventilation. The eggs were rotated every two hours to avoid the sticking of the embryo to the shell membranes.

At day '16' of incubation, muscles from different parts of the embryos were taken out, minced and mixed and then divided into two parts. One part was used for making saline homogenate using Teflon Glass homogeniser (TRI-R STIR-R, Model S63C USA), while the other part was used for the extraction of lipid, cholesterol and nucleic acid contents. The saline homogenate was also centrifuged at 8000-10000 rpm in Refrigerated Centrifuge (Sigma, Germany) to get extract. The saline homogenate and the extract were used for the estimation of various enzyme activities and some biochemical components. For the quantitative estimation of the biochemical constituents, the optical densities of their respective reaction mixtures were obtained using UV Spectrophotometer (Model M 302, Camspec, England), and simple spectrophotometer (Sequola-Turner, Model 340, USA). For the estimation of enzyme activities the reaction mixtures were incubated in water Bath (LCB 800 NEDTEX Co Taiwan). Water bath was also used for the development of colour in the reaction mixture to be read by the spectrophotometer. Weighing of chemicals and tissues was done on analytical balance (Sartorius, West Germany).

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 by Wootton (1964).

Soluble proteins were determined from saline tissue extract, while same saline extract was digested in 0.5N NaOH for 24 hours 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 (1957). 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 minutes in centrifuge (PHG Hermle Z 230, West Germany), the supernatant was obtained and used for the estimation of total lipid by Vanillin reagent (Zollner & Kirsch, 1962) and cholesterol content according to Liebermann and Burchardt Reaction (Henry & 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).

RESULTS

Enzyme activities. Tables I and II show the changes in the activities of amylase, AkP, AcP, ALT, AST and LDH of the muscle of 16th-day-old chick embryo developed from eggs treated with a single sublethal dose of various concentrations (50 ppm, 100 ppm, 200 ppm and 400 ppm) of cypermethrin. Activity of ALT was seriously affected with cypermethrin treatment, whereas, the activities of remaining enzymes remained unaltered. A marked increase in ALT activity was noted at all the doses. It was increased by 8 fold at 50 ppm, 20 folds at 100 and 200 ppm and by 12 folds at 400 ppm.

Biochemical components. Results are presented in the tables III and IV. Among carbohydrates, glycogen content of the muscle was increased at all the dose levels, it was increased by 53, 125, 70 and 115% at 50, 100, 200 and 400 ppm, respectively. In contrast, the embryonic muscular glucose content was not affected with this insecticide. Among proteins, total protein content was elevated at 100, 200 and 400 ppm by 80, 47 and 27%, respectively. However, soluble protein content showed elevation only at 100 ppm and this elevation was 59%. Free amino acid content showed the same pattern of change as soluble protein content did. It was increased by 179% at 100 ppm. Total lipid contents were decreased by 31% at 200 ppm and by 38% at 400 ppm. Like total lipids, cholesterol content was also decreased, however, decrease was observed at all the dose levels. Decrease in cholesterol content was 58% at 50 ppm, 51% at 100 ppm, 54% at 200 ppm and 50% at 400 ppm. Of the nitrogenous wastes, urea content was significantly reduced (32%) at 400 ppm. In contrast uric acid content showed a mixed response, it was increased at 100 and 200 ppm by 107 and 71%, respectively, and decreased at 400 ppm by 21%. Among nucleic acids, DNA was decreased at 50 and 200 ppm by 20 and 19%, respectively, and increased at 100 ppm by 54%. However, at 400 ppm, it remained unaffected with cypermethrin treatment. RNA content was decreased at 200 and 400 ppm and this decrease was 24 and 14%.

DISCUSSION

The present study revealed that administration of a single sublethal dose of various concentrations of cypermethrin into the eggs caused significant changes in biochemical constituents of the muscle of 16-days-old chick embryo.

Biochemical components. Cypermethrin treatment resulted in the significant increase in muscular glycogen of 16-day-old chick embryo. Findings of the present study are in agreement with the work reported by Gluth and Hanke (1985) who observed the elevation in muscle glycogen at 6 and 24 hours by atrazin, at 24 hours by methanol and also at 24 hours by 4-N-Phenol treatment in carp, *Cyprinus carpio.* Present results are also consistent with the results of Bakthavathsalam and Reddy (1983) who reported increased muscle glycogen content with Lindane (r-BHC) intoxication in the climbing perch, *Anabas testudineus.* Langslow and Hales (1971) and Hazelwood (1972) reported that the avian pancreas is richly endowed with glucagon that the plasma levels of glucagon in birds are higher than in man. In the light of these observations it can be speculated that cypermethrin might have interacted with glucagon and thus resulted in increased glycogen in the muscle. Another possibility for increased glycogen content in muscle comes from the findings of Reddy and Yellamma (1991a) who reported inhibition of phosphorylase b enzyme by cypermethrin treatment in the fish Tilapia mossambica. Since phosphorylase *a* and phosphorylase *b* enzymes are involved in glycogen break down, their inhibition can lead to decreased glycogen breakdown and thus increased glycogen synthesis. Other possibility for increased glycogen content could be the reduced metabolic activity caused by cypermethrin. Reddy and Yellamma (1991a) also reported an increase in lactate and decrease in pyruvate level by cypermethrin in fish Tilapia mossambica. Increase in lactate and decrease in pyruvate level also indicates slow carbohydrate metabolism. Generally, depletion in glycogen occurs under stress conditions when it is utilized for the detoxification purposes i.e. glucuronidation. However, in the present study, increase in muscle glycogen content seems to be due to the increased glycogenesis. This glycogen was not utilized by the muscle because of some other toxic effects of cypermethrin on muscle like, decreased muscular activity. Muscular activity meets its energy requirement from glucose and stored glycogen. Increase in glycogen content indicates non-utilization of glycogen and hence reduced muscular activity. Muscular activity depends upon the nervous system and the nervous system is affected with cypermethrin (Eells *et al*., 1992)

Table I. Toxicological effects of a single treatment of Cypermethrin of various concentrations (50, 100, 200 and 400ppm) administered in to the eggs at day '0' of incubation on some Enzymes Activities of the muscle of 16-day-old chick embryo

Parameters	Control n=6	50ppm n=6	100ppm n=6	200ppm n=6	400ppm n=6
Amylase SoU/g	20.5±2.4[a]	26.8±7.35	29.21±9.67	23.8±1.66	18.4±2.81
AkP KAU/g	0.73±0.24	0.45±0.05	0.64±0.10	0.63±0.06	0.42±0.07
AcP KAU/g	0.36±0.06	0.29±0.02	0.50±0.14	0.35±0.05	0.35±0.07
AST IU/g	5.13±0.56	6.6±0.81	7.90±1.6	6.1±0.35	6.53±1.16
ALT IU/g	0.18±0.04	1.7±0.3**	3.7±0.6***	3.72±0.3***	2.3±0.44***
LDH IU/g	36.31±7.15	32.9±4.72	37.46±7.43	29.02±1.53	24.4±3.64

Total protein, soluble proteins and free amino acid contents in muscles of the chick embryos were increased with cypermethrin treatment. Das and Mukherjee (2003) have noted a decrease in serum protein level in *Labeo rohita* with cypermethrin. Decrease in serum protein reflects increase in the tissue protein and thus is consistent with the results of present study. Similarly, increase in soluble protein and free amino acids contents of the muscle observed in the present investigation is also consistent with the work reported by Shakoori *et al*. (1988) who noted the increases in soluble protein and free amino acid in the liver of albino rats following cypermethrin administration and with that of Reddy and Yellamma (1991b) who observed increase in free amino acids in liver of the fish *Tilapia mossabica* with cypermethrin treatment. Elevation in both total and soluble protein and free amino acid contents of the muscle also indicate the reduction in protein and amino acid metabolism. The evidence in support of decreased amino acid metabolism also comes from the findings of the present study in which urea, the end product of amino acid metabolism, was also decreased.

Cypermethrin treatment has also caused drastic effects on total lipids and cholesterol contents of the embryonic muscle. Total lipid contents were decreased at 200 and 400 ppm, whereas, cholesterol content was decreased at all the dose levels tested. In contrast, Reddy *et al*. (1991) observed increases in total lipid, lipase, triglycerides and cholesterol content in brain, liver and gill tissues of *Tilapia mossambica* with cypermethrin treatment. However, increase in lipase activity can be attributed to the decrease in total lipid content. The decrease in both the total lipid and cholesterol content might have occurred as a result of their utilization to provide the energy since glycogen which is an instant source of energy next to glucose was not utilized (Table III).

Table II. Percent change in the Enzymes Activities of the muscle of 16-day-old chick embryo developed from eggs injected with a single dose of cypermethrin of various concentrations (50, 100, 200 and 400ppm) at day '0' of incubation.

Parameters	Control n=6	50ppm n=6	100ppm n=6	200ppm n=6	400ppm n=6
Amylase So U/g	-	-	-	-	-
AkP KAU/g	-	-	-	-	-
AcP KAU/g	-	-	-	-	-
AST IU/g	-	-	-	-	-
ALT IU/g	-	839	1955.56	1967	1172
LDH IU/g	-	-	-	-	-

DNA was decreased at 50 and 200 ppm and increased at 100 ppm. Decrease in DNA content indicates damage to the muscle, whereas, its increase may indicate the increased synthesis to repair the damaged muscle. RNA content was decreased at 200 and 400 ppm. Decrease in RNA content might have occurred either due to damage to the muscle or its decreased synthesis. Results of the present study regarding changes in nucleic acid contents are in agreement, with the findings of Sheela and Muniandi (1992) who reported decrease in RNA content in muscle of fish *Lepidocephalichthyes thermalis* with cypermethrin treatment and with the findings of Das and Mukherjee (2003) who reported cypermethrin-induced increase in DNA and decrease in RNA in muscle of Indian major carp, *Labeo rohita*. In contrast, uric acid content, the end product of nucleic acid metabolism showed a mixed response, it was increased at 100 and 200 ppm and decreased at 400 ppm. At 200 ppm, increase in uric acid content correlates well with the decrease in DNA and RNA contents observed at the same dose level indicating breakdown of DNA and RNA rather than their decreased synthesis as the result of cypermethrin toxicity. These changes in nucleic acid contents also indicate damage to the embryonic muscle caused by cypermethrin.

Enzyme activities. In the present study, only the activity of ALT was seriously affected with cypermethrin treatment, whereas, the activities of rest of the enzymes investigated were found unaltered. A marked increase in the ALT activity was noted at all the doses tested. Results of the present study are in agreement with the work of Reddy and Yellamma (1991b) who noticed cypermethrin-induced increases in ALT and AST activities in liver of the fish *Tilapia mossambica.* ALT, the alanine aminotransferase or glutamate pyruvate transaminases is the enzyme that catalyses the interconversion of alanine and pyruvic acid. Accumulation of pyruvic acid in the cells occurs when enzymes of the citric acid cycle and the enzymes which hydrolyses ATP i.e. ATPase to generate energy are inhibited. The activities of some of the citric

Table III. Toxicological effects of a single treatment of cypermethrin administered into the eggs at day '0' of incubation on some Biochemical Components of the muscle of 16-day- old chick embryo

Parameters	Control n=6	50ppm n=6	100ppm n=6	200ppm n=6	400ppm n=6
Glucose mg g^{-1}	1.86±0.40	1.92±0.52	2.02±0.66	1.43±0.08 2.00	2.0±0.18
Glycogen mg g^{-1}	3.32±0.65	5.08±0.25*	7.5±1.7*	6.18±0.93*	7.13±1.53*
Total Protein mg g^{-1}	71.6±4.5	81.7±6.0	128.6±14.4**	105.1±5.6**	95.6±9.18*
Soluble Protein mg g^{-1}	13.6±0.72	13.6±2.9	21.62±4.04	14.23±1.6	14.0±1.7
Free Amino acids mg g-1	3.27±0.74	1.63±0.2	9.13±2.0*	3.5±0.93	2.61±0.47
Total Lipids mg g^{-1}	104.51±11.53	66.5±5.6	99.62±17.86	71.6±6.71*	64.7±5.22**
Cholesterol mg g^{-1}	6.5±1.15	2.73±0.54*	3.2±0.62*	2.96±0.62*	3.26±0.27*
Urea mg g^{-1}	1.11±0.1	1.1±0.24	1.28±0.33	0.9±0.07	0.75±0.06*
Uric Acid mg g^{-1}	0.14±0.01	0.20±0.03	0.29±0.05*	0.24±0.02**	0.11±0.01**
DNA mg g^{-1}	1.75±0.08	1.4±0.01*	2.7±0.37*	1.42±0.07*	1.7±0.11
RNA mg g^{-1}	9.5±0.18	8.2±0.54	9.93±1.31	7.16±0.21***	8.12±0.36**

Table. IV. Percent change in the Biochemical Components of the muscle of 16-day-old chick embryo developed from eggs injected with a single dose of cypermethrin of various concentrations (50, 100, 200 and 400ppm) at '0' day of incubation

Parameters	Control n=6	50ppm n=6	100ppm n=6	200ppm n=6	400ppm n=6
Glucose mg g^{-1}	-	-	-	-	-
Glycogen mg g^{-1}	-	+53	+125	+70	+115
Total Protein mg g^{-1}	-	-	+80	+47	+27
Soluble Protein mg g^{-1}	-	-	+59	-	-
Free Amino acids mg g^{-1}	-	-	+179	-	-
Total Lipids mg g^{-1}	-	-	-	–31	–38
Cholesterol mg g^{-1}	-	–58	–51	–54	–50
Urea mg g^{-1}	-	-	-	-	–32
Uric Acid mg g^{-1}	-	-	+107	+71	–21
DNA mg g^{-1}	-	–20	+54	–19	-
RNA mg g^{-1}	-	-	-	–24	–14

acid cycle enzymes like succinate dehydrogenase, isocitrate dehydrogenase and the enzyme of the electron transport chain, the cytochrome c oxidase, is known to be inhibited by cypermethrin (Reddy & Yellamma, 1991a). Inhibition of succinic dehydrogenase at mitochondrial level with cypermethrin treatment has also been observed in various tissues of common carp, *Cyprinus carpio* (Kamalaveni *et al*., 2001). El-Toukhy and Girgis (1993) observed the inhibition of Na, K and Mg dependent ATPase activity in liver of albino rats with cypermethrin. Recently, Das and Mukherjee (2003) observed the inhibition of succinate dehydrogenase and ATPase activities in brain, kidney and liver of the Indian major carp, *Labeo rohita* following cypermethrin treatment. In addition to inhibition of the activities of these enzymes, damage to mitochondria and endoplasmic reticulum has also been observed in liver cells of male Wister rats with cypermethrin and this damage was prevented with antioxidant alpha-tocopherol (Aldana *et al*., 2001) indicating that cypermethrin causes damage through the generation of free radicals and reactive oxygen species (ROS). El-Demerdash *et al*. (2003) reported that cypermethrin induces free radicals in plasma, liver, brain and testes of male New Zealand white rabbits. Mitochondria are both the target and source of ROS and free radicals (Kroemer *et al*., 1997; Backway *et al*., 1997). These free radicals deplete glutathione, which renders mitochondria susceptible to dysfunction from oxidant stress (Hirano *et. al*., 1992) and induces mitochondrial structural degeneration (Martensson and Meister, 1989). The oxidative stress has also been observed in rat brain and liver following cypermethrin administration (Giray *et al*., 2001). Damage to mitochondria and inhibition of the enzymes of citric acid cycle and electron transport chain as well as ATP hydrolysing enzyme with cypermethrin can result in the increased pyruvate level. This increased pyruvate level may lead to the induction of the enzymes which convert pyruvic acid either back to lactic acid by lactate dehydrogenase or to alanine by ALT. In the present study the increase in ALT activity might have occurred as a result of its induction due to increased pyruvate level. Disturbance in metabolism with cypermethrin thus can result in the changes in the biochemistry of muscle as observed in the present study and hence leads to muscle dysfunction.

Table. I shows the toxic effects of cypermethrin on amylase, AkP, AcP, ALT, AST and LDH activities of the muscle of 16-day-old chick embryo. Cypermethrin of various concentrations (50, 100, 200 and 400ppm) dissolved

in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Muscles were taken out from 16-day-old chick embryos and analysed for the enzyme activities and some biochemical components.

a, Mean ± SEM; *, significantly different from controls at P < 0.05, **, at P < 0.01, ***, at P < 0.001, using student 't' test.

IU: International unit, the amount of enzyme, which under defined assay conditions, will catalyse the conversion of 1 micromole 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 minutes.

Table. II shows the percent change in the activities of amylase, AkP, AcP, AST and LDH in the muscle of 16th day old chick embryo developed from eggs administered with (0.05ml) of cypermethrin of various concentrations (50, 100, 200 and 400ppm). Cypermethrin was dissolved in acetone. Control eggs received acetone only. Muscles were taken out from 16-day-old chick embryos and analysed for the enzyme activities and some biochemical components. Only statistically significant changes were considered.

Table. III shows the toxic effects of cypermethrin on various biochemical constituents of the muscle of 16-day-old chick embryo. Cypermethrin of various concentrations (50, 100, 200 and 400ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Muscles were taken out from 16-day-old chick embryos and analysed for the enzyme activities and some biochemical components.

a, Mean ± SEM; *, significantly different from controls at P < 0.05, **, at P < 0.01, ***, at P < 0.001, using student 't' test.

Table.IV shows the percent change in various biochemical constituents of the muscle of 16-day-old chick embryo developed from eggs administered with (0.05ml) of cypermethrin of various concentrations (50, 100, 200 and 400ppm). Cypermethrin was dissolved in acetone. Control eggs received acetone only. Muscles were taken out from 16-day-old chick embryos and analysed for some biochemical components. Only statistically significant changes were considered.

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