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Effect of a Single Sublethal Dose of Permethrin on the Biochemical Components of Developing Muscle in Chick Embryo

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Abstract.- Present study was aimed at investigating the toxic effects of a single sublethal dose of various concentrations of permethrin insecticide (25, 50, 100 and 200 ppm) on the muscles of 16 day old chick embryo following administration into the eggs at day `0' of incubation. Muscles were analyzed for a few enzyme activities such as amylase, alkaline phosphatase (AkP), acid phosphatase (AcP), Aspartate aminotransaminase (AST), Alanine amino transaminase (ALT) and lactate dehydrogenase (LDH) and some of the biochemical components like, glucose, glycogen, total proteins, soluble proteins, free amino acids, total lipids, cholesterol, urea, uric acid, DNA and RNA. The activities of AkP and were reduced whereas the activity of LDH was elevated. The activities of amylase, AcP and remained unaltered. Glucose content decreased at 50 ppm and increased at 200 ppm whereas glycogen showed increase at all the doses. Total protein content decreased at 200 ppm, whereas soluble proteins remained unaffected. Free amino acid contents were increased at 25, 50 and 100 ppm but decreased at 200 ppm. Uric acid content was reduced at 100 ppm but increased at 200 ppm. Significant reduction in DNA content was observed at 50 and 100 ppm. RNA content increased at 200 ppm. Other biochemical components remained unaltered.

Key words: Permethrin, chick muscle, chick embryo.

INTRODUCTION

Poultry is a rich source of protein for human in the form of eggs and meat. Xenobiotics like insecticides, metals, fungicides and gases in the feed, as well as in the environment can affect their growth and production. Insecticides taken in by hens, through contaminated feed gain entry into eggs, and hence cause severe teratological abnormalities, biochemical changes, organ dysfunction and mortality in the young embryos. Many workers have undertaken the toxicological studies of pesticides and their metabolites in chick embryo and in adult chicks (Walker, 1971; Abuelgasim *et al*., 1981; Mufti and Nasim, 1987; Kudy *et al*., 1988; Seifort, 1989; Pikulska, 1988; Lanselink *et al*., 1993).

The toxins are metabolised via mixed function oxidase system. Hatolkar and Pawar (1992) studied the hepatic mixed function oxidase system in chick embryos exposed to phenobarbitol, 3-methylcholanthrene and 1, 1.1-trichloro 2, 2-bis (P-chlorophenyl ethane). Their results revealed increase in aminopyrine N-demethylase, acetanilide hydroxylase, aniline hydroxylase, cytochrome-C reductase, cytochrome b5 and cytochrome P-450 at all stages of development following administration of phenobarbitol and 3-methyl cholanthrene.

Kapoor *et al*. (1988) also studied the effect of permethrin on the mixed function oxidase system. He found that permethrin induces microsomal protein, cytochrome P-450 and NADPH cytochrome C reductase in chicks in dose dependent manner and also observed permethrin as a weak inducer of hepatic microsomal mixed function oxidases in chicks fed vitamin A deficient diet.

Many toxins are known to cause muscular abnormalities in chick embryos (Scheideler, 1993; Mufti and Nasim, 1987) as well as in adult chicks (De Bleecker *et al*., 1992). These muscular abnormalities include the abnormalities of the both sekeletal (Moretto *et al*., 1991) and cardiac muscles (Mufti and Nasim, 1987). Khan *et al*. (1989) and Scheideler (1993) reported the increased mortality and musculoskeletal abnormalities in chick embryos due to aflatoxin B1 toxicity. Weakening of muscle tone has also been reported in hens exposed to two herbicides metoxuron and monolinuron (Ermolin

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and Rabinovich, 1990). Moretto *et al.* (1991) observed the inhibition of peripheral nerve neurotransmitter esterase (NTE) by organophosphates, which resulted in deficit of retrograde axonal transport, axonal degeneration and paralysis. Muscle atrophy has been observed in chick embryo following exposure to neuromuscular blocking agents (Meiniel, 1981).

Permethrin is photostable and possess high insecticidal activity. Many workers have undertaken the toxicological studies of permethrin on chicks (Qadri *et al*., 1987; Kapoor *et al*., 1988; Ferguson and Audesirk, 1990). Observations made by Ferguson and Audserik (1990) showed that permethrin and DDT decrease the number of neurites/neuron, neurite length and number of neurites/cell through interference with intracellular calcium regulation.

Acute toxicity of permethrin to hemoglobin, red cell (RBC) count and chloride level has also been observed in chick blood (Qadri *et al*., 1987). Permethrin, besides being used as an insecticide is also used as prophylactic agent against scabies in humans (Chouela *et al*., 2002). Permethrin in commercially used in large quantity, therefore, the studies of the secondary effects of this insecticide in chicks are of great toxicological importance.

A little data is available on the effect of permethrin on the development of muscle in the chick. So the present study was designed to investigate the toxic effects of permethrin on the development of muscle, which is an important factor in the growth of chick.

MATERIALS AND METHODS

Fertilized eggs obtained from Government Poultry Farm at Muzaffarabad, Azad Kashmir, Pakistan were injected with different concentrations of permethrin insecticide. Dilutions were made in acetone. LD_{50} was obtained using probit analysis. After measuring the LD_{50}, which was found 676 ppm, a single sublethal dose (0.05 ml) of permethrin of various concentrations; 25, 50, 100 and 200 ppm was injected 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 with a relative humidity of 70% and proper ventilation. The eggs were rotated every two hours to avoid the sticking of the embryo to the shell membranes.

On day 16 of incubation, limb muscles from each embryo were taken out, weighed and 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 acids.

The saline homogenate was used for the estimation of various enzyme activities like AkP, AcP, LDH, AST, ALT and amylase and some of the biochemical components like, glucose, free total amino acids, urea, uric acid and soluble protein contents. 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 determined according to the method of Kind and King (1954). The activities of aspartate aminotransferase (AST; L, aspartate: 2 oxoglutarate aminotransferase, EC 2:6:1:1) and alanine amino transferase (ALT; L, alanine, 2 oxoglutarate amino transferase (EC 2:6:1:2) were estimated according to Reitman and Frankel (1957). The activity of amylase (1, 4 a-D glucanhydrolase, EC 3:2:1:1) was estimated according to the procedure described by Wootton (1964). The glucose content was determined by O-toluidine method of Hartel *et al*. (1969), soluble protein content was determined by the method of Lowry *et al*. (1951) and the amino acid content according to the Ninhydrin method of Moore and Stein (1957). Urea content was determined according to the diacetyl monoxime method as described by Natelson *et al*. (1951), and uric acid content according to the method described by Carraway (1963).

For the estimation of total protein contents, the protein extract was prepared by digesting freshly prepared saline homogenate in 0.5N NaOH for 24 hours. Total protein was estimated according to Lowry *et al*. (1951). Glycogen content in the supernatant left after centrifugation (removal of protein) was precipitated with ethanol and then dissolved in distilled water and estimated by the Anthrone method oc Consolazio and Lacono (1963).

For the extraction of total lipids and

cholesterol, the tissues were boiled in ethanol for a few hours then kept overnight. After centrifugation at 5,000 rpm for 10 minutes, the supernatant was obtained and used for the estimation of total lipids 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. RNA extract was made in cold PCA and estimated according to the Orcinol method. DNA and RNA contents were estimated as mentioned by Schneider (1957).

RESULTS

Table I shows the effect of single treatment of various concentrations (25, 50, 100 and 200) of permethrin administered into the eggs at day `0' of incubation on some enzyme activities and biochemical constituents of muscle of 16 day old chick embryo.

Of the enzyme activities, the AkP, ALT and LDH activities were significantly altered. AkP activity reduced (63 and 54%) at 100 and 200 ppm respectively. The activity of ALT altered only at 100 ppm where it declined by 6.3%. However, the LDH activity was elevated (183%) at 200 ppm. The activities of amylase, AcP and AST were not influenced by the insecticide.

Among other biochemical components glucose, glycogen, total protein, free amino acid, uric acid, DNA and RNA contents were found sensitive to the insecticidal treatment. Generally, however, the response in many of these parameters was not in stepwise and in consistent manner. Glucose content was elevated (124%) at 200 ppm. Glycogen content was significantly elevated at all the dose levels. Elevation was 185, 67, 56 and 411% at 25, 50, 100 and 200 ppm, respectively. Total protein was decreased whereas soluble protein remained unaltered. Decrease in total protein was observed only at the highest dose of 200 ppm that was 25%. Free amino aid content was elevated by 27, 122 and 22% at 25, 50 and 100 ppm, respectively, and lowered (37%) at 200 ppm. Uric acid content showed both the decrease and increase at the two different dose levels. It was decreased by 50% at 100 ppm and increased by 123% at 200 ppm.

Among nucleic acids, both the DNA and RNA contents were affected by permethrin treatment, DNA was decreased whereas RNA increased. DNA showed decrease of 23 and 24% at 50 and 100 ppm, respectively. RNA content showed increase (42%) only at the higher dose of 200 ppm. In addition to soluble protein, total lipids, cholesterol and urea contents were also found unaffected with permethrin treatment.

DISCUSSION

Activity of AkP showed a decrease only at the two higher doses of 100 and 200 ppm. AkP is a membrane bound enzyme, decrease in its activity may be taken as indication of the impaired energy process of the cell as well as parenchymal damage. Onikienko (1963) has taken its decrease as an index of parenchymal damage. In the present study, total protein content in the muscle was also decreased due to damage to muscle. ALT activity was decreased only at 100 ppm. Decrease in ALT can be considered as decreased amino acid metabolism as well as disturbed energy process. LDH activity was increased at 200 ppm. Since LDH catalyses the reversible conversion of lactate to pyruvate, it might have been induced in the cells under stress to switch on the conversion of lactate to pyruvae, an initial substrate of TCA cycle to meet their energy requirements.

Both glucose and glycogen contents were increased in this treatment. Glucose was increased in the present study. Muscular activity meets its energy requirement from glucose and stored glycogen. Increase in both glucose and glycogen indicates non-utilization of these components and hence reduced muscular activity. Gluth and Hanke (1985) 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*. Increased muscle glycogen content was also noted in Lindane (r-BHC) intoxication in the climbing perch, *Anabas testudineus* (Bakthavathsalam and Reddy, 1983).

Table I.- Effect of single administration of permethrin on the activities of some enzymes and other biochemical components of muscles of developing chicks. The insecticide was injected in the egg on day 0 of incubation and leg muscles of chick were analyzed on day 16 of incubation.

Biochemical components	Control (n=6)	25 ppm (n=6)	50 ppm (n=6)	100 ppm (n=6)	200 ppm (n=6)
Amylase (SoU/g)[b]	28.75±3.06[a]	22.21±1.81	28.35±2.66	25.15±2.2	25.34±2.03
AkP (KAU/g)	1.32±0.12	0.88±0.28	1.01±0.14	0.49±0.06***	0.61±0.29**
AcP (KAU/g)	0.57±0.07	0.74±0.08	0.82±0.13	0.74±0.21	0.5±0.24
AST (IU/g)	2.2±0.06	1.96±0.07	2.15±0.19	1.75±0.33	2.17±0.67
ALT (IU/g)	0.32±0.07	0.26±0.04	0.33±0.02	0.12±0.04*	0.52±0.2
LDH (IU/g)	8.57±2.67	9.26±1.28	11.95±1.99	10.69±2.22	24.75±5.0*
Glucose (mg/g)	0.76±0.06	0.63±0.03	0.52±0.06*	0.88±0.11	1.77±0.08***
Glycogen (mg/g)	0.27±0.04	0.77±0.2*	0.45±0.05*	0.42±0.05*	1.38±0.10***
Total protein (mg/g)	95.81±5.65	81.34±3.14	86.48±0.93	88.44±1.49	72.06±4.26*
Soluble protein (mg/g)	32.33±2.88	28.04±2.08	33.95±4.79	28.33±4.09	26.08±5.75
Free amino acid (mg/g)	1.17±0.05	1.48±0.11*	2.6±0.28**	1.43±0.07*	0.79±0.01***
Total lipids (mg/g)	28.3±3.62	41.84±0.2	46.3±7.02	37.8±5.32	36.02±0.88
Cholesterol (mg/g)	3.4±0.25	3.11±0.2	3.27±0.54	3.84±0.5	4.39±0.88
Urea (mg/g)	1.45±0.15	1.15±0.1	1.21±0.24	1.26±0.06	1.45±0.07
Uric acid (mg/g)	0.26±0.03	0.21±0.03	0.23±0.01	0.13±0.02**	0.58±0.13*
DNA (mg/g)	3.33±0.16	2.8±0.34	2.54±0.06**	2.52±0.19**	3.05±0.07
RNA (mg/g)	4.62±0.11	4.7±0.28	5.18±0.71	5.6±0.57	6.54±0.66*

[a]Mean±SE, Students `t' test; * P<0.05, ** P<0.01, *** P<0.001.
[b]IU, international unit, the amount of enzyme which under defined assay conditons will catalyse the conversion of one 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 one mig of phenol in 15 minutes.

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. The results of present study showing the high level of glucose and low level of glycogen in control animals are in agreement with the findings of these authors who observed increased glucagon synthesis in birds. So the changes in glucose and glycogen content observed in the present study could be due to the direct interaction of permethrin with glucagon. Total protein was decreased at the dose of 200 ppm, this decrease in total protein content of the muscle indicate muscular damage. Hoy *et al*. (2001) also observed the permethrin-induced disturbance in the locomotion of rats indicating the muscular damage with permethrin. Free amino acid contents were increased at 25, 50, 100 ppm and decreased at 200 ppm. Increased free amino acid contents at these doses could be due to non-utilization of these amino acids in the biosynthesis of protein that was also decreased non-significantly at these doses in the present study. However, decrease in free amino acid content at high dose of 200 ppm could be due to the decrease biosynthesis of the amino acids or breakdown of yolk protein to release free amino acids that are utilized in the various metabolic activities of the developing muscle. Urea was decrease at 50 ppm and this may be due to decreased urea metabolism as a result of permethrin toxicity. Uric acid was reduced at 100 and elevated at 200 ppm. Decrease in uric acid content at 100 ppm could be as a result of decreased uric acid biosynthesis. However, increase in uric acid contents might have occurred as a result of muscular damage as evidenced by the loss of total protein at this dose. The precursors of uric acid are generated from the breakdown of nucleic acids such as DNA. The increase in the uric acid content could be due to some other factors not the breakdown of DNA as in the present study the DNA content remained unaltered at this dose. Both the total lipids and cholesterol remained unaffected by the permethrin treatment in the present study. RNA content was significantly increased at the highest dose of 200 ppm indicating increased RNA synthesis, however,

in the present study, protein content showed decrease instead of increase.

The results of the present study indicate that permethrin causes muscular dysfunction through damaging muscles by changes in various biochemical constituents.

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