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Effect of Single Treatment of Permethrin on the Heart of Newly Hatched Chicks (*Gallus domesticus*)

KHURSHID ANWAR
Department of Zoology, University of Azad Jammu & Kashmir, Muzaffarabad (A.K), Pakistan

ABSTRACT

Toxic effects of single sub lethal doses (0.05 mL) of three different concentrations of permethrin insecticide injected in to the eggs at day '0' of incubation were investigated in the heart of newly hatched chick. Study included the estimation of a few enzyme activities like amylase, alkaline phosphatase (AkP), acid phosphatase (AcP), Glutamate Oxaloacetate Transaminase (AST), Glutamate Pyruvate Transaminase (ALT) and Lactate dehydrogenase (LDH) and some biochemical components like glucose, glycogen, total proteins, soluble proteins, free amino acids, total lipids, cholesterol, urea, uric acid, DNA and RNA contents. AkP activity was increased whereas, the activities of AST, amylase, AcP and ALT were decreased. In contrast, the activity of LDH did not show any change. AkP activity was increased at 100 and 200 ppm. Decrease, in AST activity was observed at all the doses, in amylase activity it was observed at 100 & 200 ppm, whereas, in AcP and ALT activities it was observed only at 200 ppm. Among biochemical components increase was observed in glycogen, cholesterol, total lipids and DNA contents at all the doses, and in free amino acid and uric acid contents at 200 ppm. Significant decrease was observed only in RNA at all the doses and in glucose content only at 200 ppm. Total protein, soluble protein and urea contents remained unaltered. All these biochemical changes indicate heart dysfunction.

Key Words: Permethrin; Heart; Chick; Biochemistry

INTRODUCTION

Poultry, a rich source of protein in the form of eggs and meat is an essential constituent of our food. Any harmful substance like insecticides, metals, fungicides and gases can affect their growth and production. Insecticides from hens, fed with contaminated feed are transported to young embryos through eggs, cause severe teratological abnormalities, biochemical changes and organ dysfunction and mortality in the young embryos. Many workers have undertaken toxicological studies of pesticides and their metabolites on chick embryos and adult chicks (Walker, 1971; Abuelgasim *et al*., 1981; Mufti & Nasim, 1987; Kudy *et al*., 1988; Pikulska, 1988; Seifort, 1989; Rao *et al*., 1992; Lanselink *et al*., 1993; Hill *et al*., 1994).

These toxins are metabolised via mixed function oxidase system. Hatolkar and Powar (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 65 and cytochrome P-450 at all stages of development during administration of phenobarbitol and 3 MC. Kapoor *et al*. (1988) found that permethrin induces microsomal protein, cytochrome P-450 and NADPH cytochrome C reductase in chicks in dose dependent manner and also is 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 & Nasim, 1987) as well as in adult chicks (De Bleecker *et al*., 1992). These muscular abnormalities include the abnormalities of the both skeletal (Moretto *et al*., 1991) and cardiac muscles (Mufti & 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 & Rabinovich, 1990). Moretto *et al*. (1991) have observed the inhibition of peripheral nerve neurotransmitter esterase (NTE) by organophosphates that 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).

Mufti and Nasim (1987) observed the histological changes in the heart of 7th day chick embryos injected with 2 mg/egg of insecticide dimicron. Histological changes in heart included the reduction in the myocardium part of ventricles. Bhattacharya *et al*. (1993) reported many post-mortem and histopathological changes in liver, kidney, spleen, heart and brain of chicks due to endosulfan toxicity. In heart, the changes included coagulative necrosis and fragmentation in cardiac muscle, coronary vessels filled with RBC and the presence of mononuclear cells in the spaces between the myofibrils. Majumder *et al*. (1994) studied the toxicity of fenvalerate insecticide in broiler chicks. He found that fenvalerate resides in blood, intestine, fat, brain, liver, kidney and heart. In heart it causes increases

in ALT, AST and AkP activities and decreases in AcP activity and glycogen content.

Permethrin (3-(2,2-dichloro-ethenyl)-2,2-dimethylcyclopropanecarboxylic acid-(3-phenoxyphenyl) methylester), being a most promising pyrethroid is photostable and possess high insecticidal activity. Being agricultural country insecticides like permethrin are widely used in Pakistan and there is increased risk of food being contaminated with the insecticide. Many workers have undertaken the toxicological studies of permethrin on chicks (Qadri *et al*., 1987; Kapoor *et al*., 1988; Ferguson & Audserik, 1990). In addition, permethrin is also used as prophylactic agent against scabies in humans (Chouela, *et al*., 2002).

The most common metabolites of permethrin found in rat plasma and urine are m-phenoxybenzoic acid and m-phenoxybenzoyl alcohol (Abu-Qare & Abou-Donia, 2001). As mentioned above, permethrin induces microsomal protein, cytochrome P-450 and NADPH cytochrome C reductase in chicks in dose dependent manner (Kapoor *et al*., 1988). Kostka *et al.* (1997) observed the induction of CYP 2B and slight increase in CYP1A in rats treated with 620 mg/kg of permethrin.

Permethrin is also known to affect the nervous system in mammals (Abdel-Rehman *et al.*, 2001). Observations made by Ferguson and Audserik (1990) showed that permethrin decreases the number of neurites/neuron, neurite length and number of neurites/cell through interference with intracellular calcium regulation. Abdel-Rehman *et al*. (2001) found that permethrin causes diffuse neuronal cell death and cytoskeletal in the cerebral cortex and hippocampus, and Purkinje neuron loss in the cerebellum in rats. Karen *et al.* (2001) also observed the permethrin-induced neurotoxicity in mammals through interfering with dopaminergic transmission. Acute toxicity of permethrin to hemoglobin, red cell (RBC) count and chloride level has also been observed in chick blood (Qadri *et al*., 1987). Sheets (2000) observed that young rats are more sensitive than old rats at lethal dose to pyrethroids and this greater susceptibility of the neonates to pyrethroids appear to be due to the limited metabolic capacity. Permethrin is commercially used in large quantity; therefore, the studies of the secondary effects of this insecticide in chicks are of great toxicological importance.

The purpose of this study was to evaluate the toxicity of permethrin in the heart of developing chick embryo. Heart is the pumping organ for circulation of blood in the body and any defect in it can lead to the abnormal circulation of blood, which ultimately affects the embryonic development and growth. Although the toxicological studies of a few insecticides on some enzyme activities and biochemical components in the heart of developing chick have been undertaken by many workers (as mentioned above) but no information is available about the toxicity of permethrin on the heart of newly hatched chick.. In the present study, the heart of the newly hatched chick was analysed for enzyme activities like amylase, AkP, AcP, AST, ALT and LDH and some of the biochemical components like glucose, glycogen, total protein, soluble protein, free amino acids, total lipids, cholesterol, urea, uric acid, RNA and DNA.

MATERIALS AND METHODS

Experimental design. Fertilised eggs were obtained from Government Poultry Farm at Muzaffarabad Azad Kashmir, Pakistan. Eggs were injected with different concentrations of permethrin insecticide. Dilutions were made in acetone. LD50 was obtained using probit analysis. After measuring the LD50, which was found 676 ppm, a single sublethal dose (0.05 mL) of the insecticide of various concentrations; 50 ppm, 100 ppm and 200 ppm was injected into the yolk of each egg at vegetal pole by disposable tuberculin syringe at day '0' of incubation. Equal amount of acetone was injected into the controls. The eggs were incubated at 38±0.5°C in incubators. The eggs were rotated every two hours to avoid the sticking of the embryos to the shell membranes.

Sampling. After hatching, the heart from the newly hatched chick was 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. Saline homogenate was made in ice-cold 0.89% saline using motor driven Teflon glass homogeniser. After centrifuging the saline homogenate at 4000 rpm for 10 minutes, extract was obtained that 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.

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) activities according to Kind and King (1954); lactate dehydrogenase (LDH, L, Lactate NAD oxidoreductase (EC 1:1:1:27) activity by a method based on Cabaud and Wroblewski (1958); 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) activities according to Reitman and Frankel (1957); and amylase (1, 4 a-D glucanhydrolase, EC 3:2:1:1) activity according to the procedure described by Wootton (1964).

Estimation of biochemical components. The saline extract was also used for the estimation of glucose content by O-toluidine method of Hartel *et al*. (1969), soluble protein content by the method of Lowry *et al*. (1951), amino acid content according to the Ninhydrin method of Moore and Stein (1957), urea content 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). Protein extract was prepared by digesting freshly prepared saline homogenate in 0.5N NaOH for 24 h. Total protein was estimated according to Lowry *et al*. (1951). Glycogen content in the supernatant left after centrifugation at 4000 rpm for 10 min, (removal of protein) was precipitated with ethanol and then dissolved in distilled water and estimated by the Anthrone method of Consolazio and Lacono (1963).

For extraction of the total lipid and cholesterol contents the tissues were boiled in the ethanol and then kept overnight. After centrifugation at 5,000 rpm for 10 min, the supernatant was used, for the estimation of total lipid by Vanillin reagent according to Zollner and Kirsch (1962) and cholesterol content according to Liebermann and Burchardt Reaction described by Henry and Henry (1974).

The nucleic acids were extracted according to the method described by Shakoori and Ahmad (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. The methods used for nucleic acid estimation were according to 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

Toxicological effects of a single dose of permethrin of various concentrations (50, 100 and 200 ppm) administered into the eggs at zero day of incubation on the heart of newly hatched chick are described here.

Heart weight. No significant change in the Body/Heart weight ratio was observed at any dose (Table I).

Biochemical analysis. Toxicological effect single dose of permethrin on heart of newly hatched chick has been ascertained in terms of activities of some enzymes like Amylase, AkP, AcP, AST, ALT and LDH and concentrations of glucose, glycogen, total protein, cholesterol, urea, uric acid, DNA and RNA contents. Three concentrations (50, 100 and 200 ppm) of the insecticide were injected in to the eggs at day '0' of incubation.

Enzyme activities. The effect of permethrin on various enzyme activities of the heart of newly hatched chick, are described in Table II and III. Amylase activity was inhibited by permethrin in dose dependent manner and the inhibition was 19% and 29% at 100 and 200 ppm, respectively. Both phosphates exhibited significant changes. AkP activity was increased by 123% at 100 ppm and by 23% at 200 ppm whereas, AcP activity showed change only at highest dose of 200 ppm, where it was decreased by 47%. Both the transaminases were significantly inhibited by permethrin treatment. AST decreased by 29, 12 and 35% at 50, 100 and 200 ppm, respectively, whereas, the ALT showed significant decrease of 46% only at 200 ppm. AST activity was apparently more sensitive to insecticidal treatment, as it was effected at all dose levels, while ALT activity was affected only with the high dose of permethrin. LDH activity was found insensitive to the insecticidal treatment in this study.

Biochemical components. Table IV and V indicate the effect of permethrin on some of the biochemical

Table I. Effect of permethrin on body/heart weight ratio of newly hatched chick

Parameters	Control (n=8)	50 ppm (n=4)	100 ppm (n=4)	200 ppm (n=4)
Body/Heart Wt Ratio	160.9±6.07	164.83±5.43	169.2±3.08	163.91±8.31

Table II. Toxicological effects of a single treatment of Permethrin administered into the eggs at '0' day of incubation on some enzyme activities of heart of newly hatched chicks

Parameters	Control (n=8)	50 ppm (n=4)	100 ppm (n=4)	200 ppm (n=4)
Amylase So U/g	67.97±3.38	63.77±3.98	54.86±4.19*	48.18±4.98*
AkP KAU/g	0.3±0.03	0.35±0.035	0.67±0.12*	0.37±0.02*
AcP KAU/g	1.45±0.15	1.51±0.07	1.7±0.14	0.77±0.04**
AST IU/g	41.18±1.76	29.13±3.46*	36.43±1.08*	26.65±2.77***
ALT IU/g	2.53±0.13	2.24±0.36	2.33±0.19	1.36±0.17
LDH IU/g	20.73±3.36	25.71±1.54	23.19±1.4	16.12±4.0

*significantly different from controls at P < 0.05, using student 't' test; **significantly different from controls at P < 0.01, using student 't' test; ***significantly different from controls at P < 0.001, using student 't' test; AkP Alkaline Phosphatase, AcP Acid Phosphatase, ALT Alanine aminotransferase, AST Aspartate aminotransferase, LDH Lactate dehydrogenase, IU/g International unit /gram, KAU/g, King amstrong unit/gram.

Table III. Toxicological effects of a single treatment of Permethrin administered into the eggs at '0' day of incubation on some enzyme activities of heart of newly hatched chick (Percent Increase/Decrease)

Parameters	Control (n=8)	50 ppm (n=4)	100 ppm (n=4)	200 ppm (n=4)
Amylase So U/g		-	-19	-29
AkP KAU/g		-	+123	+73
AcP KAU/g		-	-	-47
AST IU/g		-29	-12	-35
ALT IU/g		-	-	-46
LDH IU/g		-	-	-

Table IV. Toxicological effects of a single treatment of Permethrin administered into the eggs at '0' of incubation on some biochemical components of heart of newly hatched chick

Parameters	Control (n=8)	50 ppm (n=4)	100 ppm (n=4)	200 ppm (n=4)
Glucose mg/g	1.59±0.11	1.28±0.19	1.36±0.07	1.05±0.16*
Glycogen mg/g	1.06±0.03	1.58±0.19*	1.35±0.1*	1.45±0.1**
Total Protein mg/g	150.19±9.73	132.8±11.58	123.99±7.36	125.06±11.37
Soluble Protein mg/g	37.73±3.61	44.94±9.21	45.45±2.25	48.61±5.57
Free Amino acids mg/g	7.07±1.05	7.7±3.03	9.25±0.79	11.69±1.73*
Total Lipids mg/g	47.47±1.21	60.01±3.01**	70.92±0.81***	75.67±0.63***
Cholesterol mg/g	2.76±0.2	3.98±0.08***	4.94±0.63**	4.68±0.21**
Urea mg/g	1.13±0.05	1.14±0.07	0.97±0.06	1.0±0.09
Uric Acid mg/g	0.58±0.01	0.63±0.07	0.72±0.08	0.62±0.014*
DNA mg/g	1.94±0.09	2.34±0.14**	2.55±0.19*	2.78±0.2**
RNA mg/g	7.94±0.37	6.5±0.04**	5.52±0.41**	5.93±0.15**

*significantly different from controls at $P < 0.05$, using student 't' test; **significantly different from controls at $P < 0.01$, using student 't' test; ***significantly different from controls at $P < 0.001$, using student 't' test

Table V. Toxicological effects of a single treatment of Permethrin administered into the eggs at '0' of incubation on some biochemical components of heart of newly hatched chick (Percent Increase/ Decrease)

Parameters	Control (n=8)	50 ppm (n=4)	100 ppm (n=4)	200 ppm (n=4)
Glucose mg/g		-	-	-34
Glycogen mg/g		+49	+27	+37
Total Protein mg/g		-	-	-
Soluble Protein mg/g		-	-	-
Free Amino acids mg/g		-	-	+65
Total Lipids mg/g		+26	+49	+59
Cholesterol mg/g		+44	+79	+70
Urea mg/g		-	-	-
Uric Acid mg/g		-	-	+7
DNA mg/g		+21	+31	+43
RNA mg/g		-18	-30	-25

components of the heart of newly hatched chick. Among carbohydrates, tissue glucose was lowered by 34% at 200 ppm, whereas, glycogen was elevated at all doses, 49% at 50 ppm, 27% at 100 ppm and 37% at 200 ppm. Both total and soluble proteins were unaffected by insecticide. Significant F.A.A contents were elevated by 65% at 200 ppm. Total lipid and cholesterol contents were raised at all dose levels. Total lipid content showed the increase of 26, 49 and 59% whereas cholesterol content showed the increase of 44, 79 and 60% at 50, 100 and 200 ppm, respectively. Uric acid content showed elevation at higher dose of 200 ppm. Among nucleic acids, both DNA and RNA contents were seriously affected at all the doses. DNA content was increased whereas RNA content decreased. DNA content increased by 21, 31 & 43% at 50, 100 and 200 ppm, respectively. RNA content was decreased by 18, 30 and 25% at 50, 100 and 200 ppm, respectively. Urea content remained unchanged in this study.

DISCUSSION

As blood is pumped by heart to the various parts of the body so both the blood and heart may be affected first. Once the insecticide enters the body, it is transported to different parts through blood (Deichmann *et al*., 1968). Tang *et al*. (1987) studied the effect of deltamethrin on the cardiovascular system of rabbit. In the present study the activity of amylase decreased (19 and 29%%) significantly at the doses of 100 and 200 ppm and this decreased activity appears to be due to the damage to the cardiac tissue as a result of permethrin toxicity. AkP activity was significantly increased by permethrin treatment at all the doses. AkP is membrane bound enzyme, it is found on all cell membranes where active transport occurs and is hydrolase and transphosphorylase in function. This increase in AkP activity might have occurred as a result of regeneration after damage to the cardiac tissue with permethrin. AcP activity was decreased only at the highest dose of permethrin and this decrease may be due to the deletion of damaged cardiac tissue. Damage to cardiac tissue adversely affects the supply of blood to the embryo, which is in severe need of nutrients and oxygen supply for the rapidly growing tissues/organs. In contrast, Meena *et al*. (1978) observed the increased AcP activity in rats induced with the insecticide endrin. The activities of both the transaminases ALT and AST were significantly decreased at different dose levels and this decrease also reflects the damage to the cardiac tissue by permethrin. The LDH activity remained unchanged in this study.

Glucose was decreased and glycogen contents increased with permethrin treatment. Glucose was decreased only at the highest dose of 200 ppm, whereas, glycogen

increased at all the doses. Decreased glucose content at 200 ppm in the heart tissue may be due to the increased use of cardiac muscles under stress conditions. Glycogen content was increased at all the doses. This increase in glycogen content could be due to the increased glycogen synthetase activity which, might have resulted in decreased glucose content. Muscular activity meets its energy requirement from glucose and stored glycogen. Decrease in glucose and increase in glycogen indicates either increased glycogen synthesis or reduced break down of glycogen. Gluth and Hanke (1985) observed the elevation in muscle glycogen at 6 and 24 h by atrazin, at 24 h 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 & Reddy, 1983). Total and soluble protein contents remained unaffected with permethrin treatment. Increase in free amino acids contents either indicates the degradation of muscular protein or the reduced synthesis of muscle protein. These changes altogether indicate the necrosis in myocardial tissue.

Lipids are the essential constituents of the plasma membrane. Increase in total lipid indicates the reduced lipid metabolism. Cholesterol content was significantly increased at all the doses. High cholesterol content is also considered as an indicator of heart dysfunction. This increase in cholesterol content could be due to the interference of permethrin with cholesterol metabolism. Since uric acid is the major excretory product in chicks, its increase may indicate the disturbed metabolism.

Decreased in RNA content may be either due to tissue damage or due to decreased transcriptional process. When damage occurs to tissues, the tendency of the tissue to repair the damage is increased. In the present study, the increased DNA content may represent the increased mitotic figures to repair the injured tissue. In contrast, the observations made by Hadnagy *et al*. (1999) indicate that permethrin inhibits the cell cycle progression in lung cells. Similarly, Kostka *et al*. (2000) observed that permethrin inhibits G2 phase of cell cycle and suppresses the cell entering in to the stage of mitosis (M-phase) and does not increase the number of mitotic figures in hepatocytes. Both these authors studied the permethrin toxicity in lung and liver cells, none of them studied permethrin toxicity in heart.

Results of this experiment indicate that permethrin seriously damages the cardiac muscles of the heart and thus affects its function.

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