Effect of Single Sublethal Dose of Permethrin on the Development of Muscle in Newly Hatched Chick (Gallus domesticus)

Kurshid Anwar
Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Azad Kashmir, Pakistan

Abstract: Present study was aimed at investigating the toxic effects of a single sublethal dose of various concentrations of permethrin insecticide (50, 100 and 200 ppm) on the muscle of newly hatched chicks following administration in to the eggs at day ‘0’ of incubation. Muscles were analysed for a few enzyme activities such as amylase, alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and lactate dehydrogenase 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 contents. The activities of amylase (100 and 200 ppm), AKP (200 ppm) and ALT (50, 100 and 200 ppm) were decreased whereas the activity of LDH activity elevated (200 ppm). In contrast, the activities of AcP and AST remained unaltered. Among biochemical components glucose, soluble protein and urea were decreased, whereas glycogen, total proteins, total lipids, cholesterol and uric acid were increased. Total free amino acids, DNA and RNA contents remained unaltered. These biochemical changes indicate the muscle dysfunction in terms of its low motor activity.

Key words: Permethrin, chick muscle, biochemistry, Gallus domesticus

Introduction

Poultry a rich source of protein in the form of eggs and meat is an essential constituent of our food. Being an agricultural country insecticides like permethrin are widely used in Pakistan and there is increased risk of food being contaminated with the insecticide. This contaminated food may harm humans and the domesticated animals. In addition, permethrin is also used as prophylactic agent against scabies in humans (Chouela et al., 2002).

Permethrin being a most promising pyrethroid 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 Audesirk (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. Permethrin has been found to induce microsomal protein, cytochrome P-450 and NADPH cytochrome C reductase in chicks in dose dependent manner. This Insecticide also appeared as weak inducer of hepatic microsomal mixed function oxidases in chicks fed vitamin A deficient diet (Kapoor et al., 1988).

Treatment of rats with permethrin (620 mg Kg⁻¹) resulted in significant increase in the relative liver weight and the induction of CYP 2B and slight increase in CYP1A. (Kostka et al., 1997). Permethrin has been found to affect nervous system in mammals (Abdel-Rehman et al., 2001). Karen et al. (2001) also observed the neurotoxicity in mammals caused by permethrin. Young rats are more sensitive than old rats at lethal dose to pyrethroids. The greater susceptibility of the neonates appear to be due to limited metabolic capacity (Sheets, 2000).

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 is commercially used in large quantities therefore, the studies of the secondary effects of this insecticide in chicks are of great toxicological importance.

Many toxins are known to cause muscular abnormalities in chick embryos (Scheldeler, 1993) as well as in adult chicks (De Bleecker et al., 1992). Weakening of muscle tone has also been reported in hens exposed to two herbicides metoxuron and monolinuron (Ermolin and Rabinovich, 1990). Protein fractions from snake venom were proved as post-synaptic neurotoxins. They blocked the response of chick biventer cervicis muscle to both to nerve stimulation and acetyl cholinesterase activity but left response to direct nerve stimulation and K⁺ unaffected (Chen and Xiurona, 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. Treatment of parathion to chicks has resulted in respiratory paralysis, weakness in proximal limb and neck flexor muscle and territory of several motor cranial nerves (De Bleecker et al., 1992). Farage-Elawar and Blaker (1992) have noted the persistent locomotor alterations in chicks hatched from eggs injected with carbamate and carbaryl insecticides.

Lot of work has been done on the muscle toxicity of adult chicks by various toxins. A little data is available on the toxicity of permethrin on the muscle of adult 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
All the work was done in Biochemistry and toxicology Laboratory, Zoology Department, Azad Jammu and Kashmir University, Muzaffarabad, Azad Kashmir, Pakistan.

Experimental Design
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. 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, 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 amount of acetone was injected into the controls. 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 2 h to avoid the sticking of the embryo to the shell membranes.

Sampling
After 6-8 h of hatching, the muscle from each 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 min, extract was obtained that was used for the estimation of various enzyme activities like AKP, AcP, LDH, AST, ALT, 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) were determined according to the method of Kind and King (1954). The activity of lactate dehydrogenase (LDH, L, Lactate NAD oxidoreductase (EC 1:1:1:27) was determined by a method based on Cabaud and Wroblewski (1958). The activities of aspartate aminotransferase (AST; L, aspartate: 2 oxoglutarate aminotransferase, EC 2:6:1:1) and alanine aminotransferase (ALT; L, alanine, 2 oxoglutarate aminotransferase, (EC 2:6:1:2) were estimated according to Reitman and Frankel (1957) and the activity of amylase (1, 4 a-D glucan hydrolase, EC 3:2:1:1) according to the procedure described by Wootton and Freeman (1964).

Estimation of biochemical components
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), amino acid content determined 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 content the protein extract was prepared by digesting freshly prepared saline homogenate in 0.5 N 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 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 min the supernatant was obtained and used for the estimation of total lipid by Vanillin reagent (Zollner and Kirsch, 1974) and cholesterol content according to Liebermann and Burchard 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. RNA was extracted in 10% PCA at 4°C for 24 h, whereas, DNA was extracted done in 10% PCA at 65°C for 30 min. The hot PCA extract was used for estimation of DNA by diphenylamine method; while the cold PCA extract was used for the estimation of RNA by Oracinol method. Both these methods follow Schmidt and thannhauser described by Schneider (1957).

Reagents

All the reagents used were of analytical grade (BDH, MERCK and Sigma). Kits for the estimation of enzyme activities were from Roche and Glassware from Pyrex (U.K).

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).

Results

Effect of permethrin on enzyme activities

Tables 1 and 2 shows the changes in the activities of various enzymes (amylase, transaminases, phosphatases and dehydrogenase) in the muscle of newly hatched chicks developed from eggs injected with single sublethal doses of various concentrations (50, 100 and 200 ppm) of permethrin insecticide.

Amylase activity

Muscle amylase activity in vehicle-treated control group was 43.02±1.26 So U g⁻¹. Permethrin at the lowest dose (50 ppm) tested did not cause any significant change in amylase activity, however, at the doses of 100 and 200 ppm, it was decreased by 46 and 54%, respectively.

Phosphatases (AkP and AcP)

The activity of AkP was decreased whereas, the activity of AcP remained unaffected with

Table 1: Changes in some of the enzyme activities of muscles of newly hatched chick following administration at '0' day of incubation of a single dose of permethrin

<table>
<thead>
<tr>
<th></th>
<th>Control n=8</th>
<th>50 ppm n=4</th>
<th>100 ppm n=4</th>
<th>200 ppm n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase So U g⁻¹</td>
<td>43.02±1.26</td>
<td>35.17±8.11</td>
<td>22.66±2.07**</td>
<td>19.84±1.0**</td>
</tr>
<tr>
<td>AKP KAU g⁻¹</td>
<td>0.28±0.02</td>
<td>0.25±0.03</td>
<td>0.21±0.04</td>
<td>0.2±0.03</td>
</tr>
<tr>
<td>ACp KAU g⁻¹</td>
<td>1.36±0.09</td>
<td>0.81±0.04</td>
<td>1.07±0.27</td>
<td>0.97±0.26</td>
</tr>
<tr>
<td>AST IU g⁻¹</td>
<td>24.2±2.27</td>
<td>24.0±1.21</td>
<td>32.72±3.23</td>
<td>27.94±1.47</td>
</tr>
<tr>
<td>ALT IU g⁻¹</td>
<td>1.01±0.08</td>
<td>0.74±0.03**</td>
<td>0.5±0.04**</td>
<td>0.57±0.09**</td>
</tr>
<tr>
<td>LDH IU g⁻¹</td>
<td>7.59±0.91</td>
<td>10.8±2.75</td>
<td>10.13±2.9</td>
<td>12.39±2.00**</td>
</tr>
</tbody>
</table>

* significantly different from control at P < 0.05, ** significantly different from control at P < 0.01, *** significantly different from control at P < 0.001, AKP: Alkaline Phosphatase, AcP: Acid Phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, IU/g: International unit/gram, KAU/g: King armstrong unit/gram

Table 2: Percent changes in some of the enzyme activities of muscles of newly hatched chick following administration at '0' day of incubation of a single dose of permethrin

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>Control n=8</th>
<th>50 ppm n=4</th>
<th>100 ppm n=4</th>
<th>200 ppm n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase So U g⁻¹</td>
<td>-</td>
<td>-46</td>
<td>-54</td>
<td></td>
</tr>
<tr>
<td>AKP KAU g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-29</td>
<td></td>
</tr>
<tr>
<td>ACp KAU g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AST IU g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ALT IU/g</td>
<td>-27</td>
<td>-51</td>
<td>-44</td>
<td></td>
</tr>
<tr>
<td>LDH IU g⁻¹</td>
<td>-</td>
<td>-</td>
<td>+63</td>
<td></td>
</tr>
</tbody>
</table>

+= increase - = decrease

 permethrin treatment. The decrease in AKP activity was observed only at the highest dose (tested) of 200 ppm and that was 29% less as compared to that of vehicle-treated controls.

Transaminases (ALT and AST)

ALT activity was significantly decreased at all the doses tested, it was decreased by 27% at 50 ppm, 51% at 100 ppm and 44% at 200 ppm. In contrast, not a single dose of permethrin was able to cause any significant change in AST activities.

Lactate dehydrogenase (LDH)

LDH activity was increased and this increase was observed only at 200 ppm by 63%.

52
Effects of permethrin on biochemical components

Tables 3 and 4 show the changes in the concentration of macromolecules (glycogen, proteins, lipids, nucleic acids), metabolites (glucose, FAA, cholesterol) and excretory products (urea, uric acid) in the muscle of newly hatched chicks developed from eggs injected with single sub lethal doses of various concentrations (50, 100 and 200 ppm) of permethrin insecticide.

Table 3: Changes In some of the biochemical components of muscles of newly hatched Chick following administration at '0' day of incubation of a single dose of permethrin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control n=8</th>
<th>50 ppm=4</th>
<th>100 ppm=4</th>
<th>200 ppm=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg g⁻¹</td>
<td>1.95±0.15</td>
<td>1.44±0.05</td>
<td>1.59±0.31</td>
<td>1.13±0.12*</td>
</tr>
<tr>
<td>Glycogen mg g⁻¹</td>
<td>0.59±0.08</td>
<td>0.67±0.05</td>
<td>0.9±0.1*</td>
<td>1.06±0.03**</td>
</tr>
<tr>
<td>Total Protein mg g⁻¹</td>
<td>119.19±7.25</td>
<td>114.87±7.03</td>
<td>109.34±13.17</td>
<td>163.92±8.12**</td>
</tr>
<tr>
<td>Soluble Protein mg g⁻¹</td>
<td>48.7±3.18</td>
<td>38.8±3.85</td>
<td>30.1±5.05*</td>
<td>41.1±0.27*</td>
</tr>
<tr>
<td>Free Amino acids mg g⁻¹</td>
<td>14.12±1.22</td>
<td>14.05±1.46</td>
<td>14.85±1.11</td>
<td>10.22±3.22</td>
</tr>
<tr>
<td>Total Lipids mg g⁻¹</td>
<td>52.15±1.65</td>
<td>59.25±1.52*</td>
<td>66.58±4.21*</td>
<td>92.58±1.74**</td>
</tr>
<tr>
<td>Cholesterol mg g⁻¹</td>
<td>2.71±0.19</td>
<td>2.72±0.17</td>
<td>2.61±0.11</td>
<td>3.39±0.21*</td>
</tr>
<tr>
<td>Urea mg g⁻¹</td>
<td>0.47±0.06</td>
<td>0.1±0.01***</td>
<td>0.41±0.07</td>
<td>0.59±0.06</td>
</tr>
<tr>
<td>Uric Acid mg g⁻¹</td>
<td>0.2±0.01</td>
<td>0.42±0.01***</td>
<td>0.25±0.03</td>
<td>0.24±0.02*</td>
</tr>
<tr>
<td>DNA mg g⁻¹</td>
<td>1.21±0.04</td>
<td>1.16±0.1</td>
<td>1.07±0.07</td>
<td>1.21±0.15</td>
</tr>
<tr>
<td>RNA mg g⁻¹</td>
<td>5.08±0.21</td>
<td>4.8±0.23</td>
<td>5.4±0.23</td>
<td>5.17±0.17</td>
</tr>
</tbody>
</table>

* significantly different from control at P < 0.05, ** significantly different from control at P < 0.01, *** significantly different from control at P < 0.001

Table 4: Percent changes in some of the biochemical components of muscles of newly hatched chick following administration at '0' day of incubation of a single dose of permethrin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control n=8</th>
<th>50 ppm=4</th>
<th>100 ppm=4</th>
<th>200 ppm=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg g⁻¹</td>
<td>-</td>
<td>+53</td>
<td>+80</td>
<td>-</td>
</tr>
<tr>
<td>Glycogen mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Protein mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble Protein mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-9</td>
<td>-16</td>
</tr>
<tr>
<td>Free Amino acids mg g⁻¹</td>
<td>+14</td>
<td>+28</td>
<td>+78</td>
<td>-</td>
</tr>
<tr>
<td>Total Lipids mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+25</td>
</tr>
<tr>
<td>Urea mg g⁻¹</td>
<td>-66</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uric Acid mg g⁻¹</td>
<td>+110</td>
<td>-</td>
<td>+20</td>
<td>-</td>
</tr>
<tr>
<td>DNA mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RNA mg g⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+= increase, -= decrease

53
Glucose and glycogen contents

Permethrin treatment caused a significant decrease in glucose content and increase in glycogen content. Glucose content was only decreased at the highest dose of 200 ppm by 42%. In contrast, glycogen content was elevated at 100 and 200 ppm and this elevation was dose dependent. It was elevated at 100 ppm by 53% and at 200 ppm by 80%.

Proteins (Total and soluble proteins)

Permethrin treatment at the dose of 200 ppm caused a significant increase in total protein content of the muscle by 37%. However, at the doses of 50 and 100 ppm of permethrin no any change was observed in muscle protein. In contrast, soluble protein contents of the muscle were decreased and the decrease was only observed at 100 and 200 ppm by 39 and 16%, respectively.

Free amino acid contents

Free amino acid contents remained unaffected with permethrin treatment.

Total lipids and cholesterol contents

Both the total lipids and cholesterol contents of the muscle were increased with permethrin treatment. Total lipid contents were increased at all the doses of permethrin tested whereas, cholesterol content was increased at the highest dose tested of 200 ppm. Increase in total lipid content was dose dependent, it was 14, 28 and 78% at 50, 100 and 200 ppm, respectively. Cholesterol content showed increase of 25% at 200 ppm.

Nucleic acids (DNA and RNA)

Both DNA and RNA contents remained unaffected with permethrin treatment.

Excretory products (urea and uric acid)

Permethrin treatment at 50 ppm decreased the urea content whereas, at higher doses permethrin treatment did not cause any change in it. In contrast, permethrin treatment caused increase in the uric acid content. The increase was observed at two doses, at 50 ppm by 110% and at 200 ppm by 20%.

Discussion

Both the induction and inhibition of the enzymes were observed in the present study. The activities of amylase, AKP and AST were inhibited whereas, the activity of LDH was induced. The amylase activity was inhibited at 100 and 200 ppm, which reflect the decreased carbohydrate metabolism. Activity of AKP showed a decrease only at the highest dose of 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. However, Onklienko (1963) has taken its decrease as an index of
parenchymal damage. But in the present study, decrease in AKP activity cannot be considered as indication of muscular damage as the total protein of the muscle did not show any decrease. ALT activity was decreased at 50, 100 and 200 ppm in dose dependent fashion. Like AKP, decrease in ALT can also be considered as inhibition rather than decrease as a result of muscular damage. Inhibition of this enzyme may have resulted in disturbed energy process. LDH activity was increased at 200 ppm. LDH catalyses the reversible conversion of lactate to pyruvate. Increase in LDH activity after permethrin treatment might have switched on the aerobic TCA cycle to meet the energy requirements for the cells under stress.

Carbohydrate metabolism provides primary source for energy which is later followed by the utilization of lipids and proteins. In the present study, glucose content was decreased whereas glycogen content increased with permethrin treatment. Decreased glucose and increased glycogen contents indicate increased glycogenesis. Gluth and Hanke (1985) observed the elevation in muscle glycogen at 6 and 24 h by atrazine, at 24 h by methanol and also at 24 h 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). 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 were 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. Moreover, increase in muscle glucose can be linked with decreased muscular activity. Since muscular activity needs energy in the form of ATP generated through the oxidative phosphorylation of glucose, increase in muscular glucose content can be linked with decreased muscular activity that might have resulted from the inhibitory effects of permethrin on the enzymes involved in the oxidation of glucose. Decreased muscular activity is further supported by the decreased ALT activity, which is involved in energy process in addition to its role in transamination reactions. Evidences to support further the decreased muscular activity come from the findings of Vais et al., (2001) who found that pyrethroid insecticides cause molecular interactions with insect and mammalian sodium channels. Findings of other authors (Eells et al., 1993; Zhao et al., 1995; Hoy et al., 2000; Abdel-Rehman et al., 2001 and Karen et al., 2001) also suggest that permethrin acts as neurotoxic agent.

Increased total protein, decreased soluble protein and non-significant decrease of free amino acid contents were observed in this study. Increase in protein content may be due to increased rate of translation of protein. El-Sebaf et al. (1988) observed the increased protein synthesis in rabbit liver by the insecticides profenfos, fenvalerate and dimilin. Urea was decreased at 50 ppm and this may be due to decreased amino acid metabolism as a result of permethrin toxicity. Uric
acid was elevated at 50 and 200 ppm. Reason for elevation in uric acid content is not clear since the nucleic acids whose catabolism lead to uric acid production remained unaltered in this study. Both the total lipids and cholesterol were increased in the present study. This increase in total lipids and cholesterol contents indicates the less demand for energy by muscle as a result of its decreased locomotor function. Increased total lipid was also observed in DDT, endrin, dursban and lannate toxicity in rats whereas increased cholesterol content was noted in lannate treated rats (Borady et al., 1983).

These data suggest that permethrin elicit its toxic effects in the muscles of non-target vertebrates through affecting enzymes, like amylase, AKP, ALT and LDH and some other macromolecules responsible for normal muscular activity.

References


