

## Toxicological Effect of Single Treatment of Permethrin Injected into the Eggs on '0' Day of Incubation on the Liver of Newly Hatched Chick.

Khurshid Anwar

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

---

**Abstract:** Toxicity of permethrin was investigated in the liver of newly hatched chicks developed from the eggs injected with a single sublethal doses (0.05 ml) of three different concentrations of permethrin insecticide (50, 100 and 200ppm) on day '0' of incubation. Study included the estimation of a few enzyme activities and some biochemical constituents of liver. Among enzymes, the activities of amylase, alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase were significantly decreased whereas the activity of lactate dehydrogenase was increased. However, the activity of glutamate pyruvate transaminase remained unchanged. From among biochemical components total protein, total lipids and cholesterol were significantly elevated whereas urea and DNA contents were significantly reduced. Glucose, glycogen, soluble protein, free amino acids (FAA), uric acid and RNA contents remained unaltered. Permethrin-induced common histopathological changes observed were disturbance in the hepatic architecture, increased sinusoidal spaces, cytoplasmic vacuolations, hepatocytic nuclear condensation and fragmentation, hydropic degeneration and necrosis and the presence of anucleated hepatocytes. A mild blood cell infiltration in the hepatic parenchyma was observed.

**Key words:** Permethrin, chick liver, enzymes, biochemical components, histopathology, gallus domesticus

---

### Introduction

Insecticides are used against pests to increase the production of food grains and other agricultural-products like cotton, vegetable oils, vegetables and fruit etc. Pyrethroid insecticides, including permethrin are also used against pests. Besides their useful effects they produce deleterious effects on the non-target organisms including both vertebrates and invertebrates living in the exposed area (Anderson, 1983; Sibley and Kaushik, 1991; Ghosh, 1989; Majmunder *et al.*, 1994; Akhtar *et al.*, 1992) are seriously affected by the insecticides.

Domestic chicks, a rich source of animal protein in the form of eggs and meat are widely used all over the world. Any harmful substance like insecticides, metals, fungicides, gases and their residues can affect its growth and development. Insecticides from hens, fed on contaminated feed, can be transported to young embryos through eggs and thus can cause severe teratological abnormalities, biochemical changes, organ dysfunction and mortality in the

young embryos. Many workers have undertaken the toxicological studies with respect to pesticides and their metabolites in chick embryos and in adult birds (Walker, 1971; Abuelgasim *et al.*, 1981; Mufti and Nasim, 1987; Rao *et al.*, 1992; Lanselink *et al.*, 1993). A lot of information is available on the toxicity of insecticides, other than pyrethroids in chick embryo (Scheideler, 1993; Mufti and Nasim, 1987), whereas, a little information is available about the toxicity of pyrethroids including permethrin on the liver of developing chicks (Kapoor *et al.*, 1988).

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. Permethrin is 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). Many workers have undertaken the toxicological studies of permethrin on chicks (Qadri *et al.*, 1987; Kapoor *et al.*, 1988 and Ferguson and Audserik, 1990). Qadri *et al.* (1987) observed the acute toxicity of permethrin to hemoglobin RBC count and chloride level in chick blood.

In mammals, for example in rats, permethrin causes liver hypertrophy, change in microsomal enzyme activity and proliferation of smooth endoplasmic reticulum (Ishmael and Litchfield, 1988). Kostka *et al.* (1997) observed the induction of CYP 2B and slight increase in CYP 1A in rats treated with 620 mg Kg<sup>-1</sup> of permethrin. Abdel-Rehman *et al.* (2001) observed diffuse neuronal cell death and cytoskeletal in the cerebral cortex and hippocampus and Purkinje neuron loss in the cerebellum in rats as a result of permethrin toxicity. Karen *et al.* (2001) observed that permethrin induces neurotoxicity in mammals through interfering with dopaminergic transmission. 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 appears to be due to the limited metabolic capacity. Spencer and Berhane (1982) observed that permethrin induces fetal changes in rats.

Toxins are metabolised via mixed function oxidase system. Kapoor *et al.* (1988) 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. The most common metabolites of permethrin found in rat plasma and urine are m-phenoxybenzoic acid and m-phenoxybenzoyl alcohol (Abu-Qare and Abou-Donia, 2001).

Since liver is responsible for the detoxification of endogenously produced waste products or exogenously derived toxins and drugs and involved in the metabolism of proteins, carbohydrates and fats, its response to toxic insult may help to understand its role in the metabolism of toxic compounds including insecticides like permethrin. The present study was designed to evaluate the toxic effects of permethrin on the development of liver in newly hatched chicks at both biochemical and histological levels.

### Materials and Methods

Fertilized eggs (thirty) obtained from Government Poultry Farm, at Muzaffarabad Azad Kashmir Pakistan were injected with different doses of permethrin insecticide. Dilutions were prepared in acetone. LD<sub>50</sub> was obtained using probit analysis. A single sublethal dose (0.05 ml) of the insecticide of each concentration (25, 50, 100 and 200 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 hours to avoid the sticking of the embryo to the shell membranes.

On the day of hatching the liver from each chick was taken out, weighed and divided into three 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 third part was fixed in bouin's fluid for light microscopical studies. Saline homogenate was made in ice-cold 0.89% saline using motor driven Teflon glass homogeniser and used for the estimation of various enzyme activities and some other biochemical components.

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 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 (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 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 (Mettmert, 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

#### Liver weight

No significant change in the chick/liver weight ratio was observed. However, a non-significant decrease of 8% was observed at the dose of 200 ppm.

Table 1: Effect of Permethrin on body/liver weight ratio of newly hatched chick at the doses of 50, 100 and 200ppm injected into the eggs at '0' day of incubation

Parameters	Control n=8	50 ppm n=4	100 ppm n=4	200 ppm n=4
Body/Liver Wt Ratio	43.78±1.98	41.31±1.38	44.31±1.03	40.44±1.56

Table 1 shows the toxic effects of permethrin on Body/liver weight ratio of newly hatched chick. Permethrin of various concentrations (50, 100 and 200 ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Livers were taken out and weighed and their ratios with body weights were determined.

#### Biochemical analysis of Liver

Tables 2-5 show the effect of single treatment of various concentrations of Permethrin injected in to the eggs at '0' day of incubation on some enzyme activities and biochemical components of Liver of newly hatched chick.

#### Enzyme activities

Results are presented in tables 2 and 3. Among enzymes, the activities of amylase, AkP, AcP, AST were decreased whereas the activity of LDH was increased. However, the activity of ALT remained unchanged. Amylase activity decreased at all the doses. It was declined by 52%, 45% and 48% at 50, 100 and 200 ppm, respectively. Of the phosphatases, the activity of AkP decreased at all the doses (25% at 50 ppm and 30%, at 100 and 200 ppm), whereas the activity of AcP decreased only at 100 and 200 ppm by 30 and 48%, respectively. Of the transaminases, the AST activity decreased at 100 and 200 ppm by 7 and 17%, respectively, whereas, ALT activity remained unaltered. Activity of LDH increased by 18 and 25% at 100 and 200ppm, respectively.

Table 2: Toxicological effects of a single treatment of Permethrin of various concentrations (50, 100 and 200ppm) administered in to the eggs at '0' day of incubation on some Enzymes Activities of Liver of newly hatched chick

Parameters	Control n=8	50 ppm n=4	100 ppm n=4	200 ppm n=4
Amylase So U/g	61.1±4.16	29.48±2.8 <sup>***</sup>	33.67±4.38 <sup>**</sup>	31.76±1.9 <sup>***</sup>
AKP KAU/g	1.24±0.07	0.92±0.08 <sup>*</sup>	0.87±0.13 <sup>*</sup>	0.87±0.06 <sup>**</sup>
AcP KAU/g	3.18±0.19	3.52±0.18	2.24±0.19 <sup>**</sup>	1.65±0.4 <sup>**</sup>
AST IU/g	12.95±0.46	11.25±0.12	10.69±0.66 <sup>*</sup>	10.73±0.7 <sup>*</sup>
ALT IU/g	1.81±0.23	1.4±0.18	1.91±0.43	1.81±0.28
LDH IU/g	16.04±0.92	17.45±0.75	18.9±0.61 <sup>*</sup>	20.05±1.55 <sup>*</sup>

Table 2 shows the toxic effects of permethrin on amylase, AkP, AcP, ALT, AST and LDH activities of liver of newly hatched chick. Permethrin of various concentrations (50, 100 and 200 ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Livers were taken out from chicks and analysed for the enzyme activities and some biochemical components.

<sup>\*</sup>, significantly different from controls at P < 0.05, using student 't' test.

<sup>\*\*</sup>, significantly different from controls at P < 0.01, using student 't' test.

<sup>\*\*\*</sup>, significantly different from controls 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 one micro mole of substrate per minute

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

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

### Biochemical components

Tables 4 & 5 show the effect of permethrin treatment on biochemical components of liver. From among biochemical components total protein, total lipids and cholesterol were significantly elevated whereas urea and DNA contents were significantly reduced. Total protein and total lipid contents were increased at 200 ppm by 32 and 12%, respectively, whereas, cholesterol content was increased at all the doses, 7% at 50ppmm, 21% at 100 ppm and 44% at 200 ppm. Urea content was decreased at 50 ppm by 32% and DNA content at 100 and 200ppm by 23 and 38%, respectively. Glucose, glycogen, soluble protein, free amino acids (FAA), uric acid and RNA contents remained unaltered.

### Histopathological changes

Light microscopic examinations of hematoxylin and eosin stained liver sections from the control chicks revealed normal appearance (Fig. 1A and 1B). However, permethrin treatment had

Table 3: Percent change in the Enzymes Activities of Liver of newly hatched chicks developed from eggs injected with a single dose of permethrin of various concentrations (50, 100 and 200 ppm) at '0' day of incubation

Parameters	Control n=8	50 ppm n=4	100 ppm n=4	200 ppm n=4
Amylase So U/g		-52	-45	-48
AkP KAU/g		-25	-30	-30
AcP KAU/g		-	-30	-48
AST IU/g		-	-7	-17
ALT IU/g		-	-	-
LDH IU/g		-	+18	+25

Table 3 shows the percent change in the activities of amylase, AkP, AcP, AST and LDH in the liver of newly hatched chick developed from eggs administered with (0.05 ml) of permethrin of various concentrations (50, 100 and 200 ppm). Permethrin was dissolved in acetone. Control eggs received acetone only. Livers were taken out from the chicks and analysed for the enzyme activities and some biochemical components. Only statistically significant changes were considered.

Table 4: Toxicological effects of a single treatment of permethrin administered into the eggs at '0' day of incubation on some Biochemical Components of liver of newly hatched chicks

Parameters	Control n=8	50 ppm n=4	100 ppm n=4	200 ppm n=4
Glucose mg g <sup>-1</sup>	15.19±1.38	12.89±4.7	14.99±2.33	14.68±1.64
Glycogen mg g <sup>-1</sup>	2.23±0.8	3.97±2.41	2.41±0.95	2.97±1.91
Total Protein mg g <sup>-1</sup>	127.78±6.27	132.06±4.16	120.77±2.14	168.08±12.36*
Soluble Protein mg g <sup>-1</sup>	68.10±1.3	71.40±5.9	71.50±2.9	77.30±7.1
Free Amino acids mg g <sup>-1</sup>	2.72±0.24	2.30±0.24	2.36±0.34	2.16±0.99
Total Lipids mg g <sup>-1</sup>	117.95±2.54	127.56±6.35	122.96±4.6	132.64±1.99**
Cholesterol mg g <sup>-1</sup>	24.81±1.25	26.56±1.04*	29.90±1.14*	35.70±0.93***
Urea mg g <sup>-1</sup>	0.72±0.05	0.49±0.04*	0.65±0.03	0.71±0.05
Uric Acid mg g <sup>-1</sup>	1.64±0.13	1.54±0.05	1.60±0.18	2.10±0.19
DNA mg g <sup>-1</sup>	1.49±0.08	1.42±0.2	1.15±0.12	0.93±0.03***
RNA mg g <sup>-1</sup>	8.16±0.51	7.47±0.14	8.28±0.93	6.93±0.72

Table 4 shows the toxic effects of permethrin on glucose, glycogen, total proteins, soluble proteins, free amino acids, total lipids, cholesterol, urea, uric acid, DNA and RNA contents of liver of newly hatched chick. Permethrin of various concentrations (50, 100 and 200ppm) dissolved in acetone was administered into the eggs at day '0' of incubation. Control eggs received acetone only. Livers were taken out from the chicks and analysed for the enzyme activities and some biochemical components.

\*, 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 5: Percent change in the biochemical components of Liver of newly hatched chicks developed from eggs injected with a single dose of permethrin of various concentrations (50, 100 and 200 ppm) at '0' day of incubation

Parameters	Control n=8	50ppm n=4	100ppm n=4	200ppm n=4
Glucose mg g <sup>-1</sup>		-	-	-
Glycogen mg g <sup>-1</sup>		-	-	-
Total Protein mg g <sup>-1</sup>		-	-	+32
Soluble Protein mg g <sup>-1</sup>		-	-	-
Free Amino acids mg g <sup>-1</sup>		-	-	-
Total Lipids mg g <sup>-1</sup>		-	-	+12
Cholesterol mg g <sup>-1</sup>		+7	+21	+44
Urea mg g <sup>-1</sup>		-32	-	-
Uric Acid mg g <sup>-1</sup>		-	-	-
DNA mg g <sup>-1</sup>		-	-23	-38
RNA mg g <sup>-1</sup>				

Table 5 shows the percent change in total proteins, total lipids, cholesterol and DNA contents of liver of newly hatched chick developed from eggs administered with (0.05 ml) of permethrin of various concentrations (50, 100 and 200 ppm). Permethrin was dissolved in acetone. Control eggs received acetone only. Livers were taken out from the chicks and analysed for the enzyme activities and some biochemical components. Only statistically significant changes were considered.

induced some histopathological changes, which included increased sinusoidal spaces, cytoplasmic vacuolations, hepatocytic nuclear condensation, hydropic degeneration and necrosis of hepatocytes. Increased sinusoidal spaces were observed at all the doses. (Fig. 2A,B; 3A,3B and 4A,B). At 100 ppm, in addition to increased sinusoidal spaces, cytoplasmic vacuolations, hepatocytic nuclear condensation, hydropic degeneration, necrotic hepatocytes were also observed (Fig. 3A,B). At 200 ppm the changes included the vacuolation of hepatocytes, hepatocytic nuclear condensation and fragmentation, hydropic degeneration and necrosis of hepatocytes. Anucleated hepatocytes were also observed at this dose. One peculiar change at this dose was the severe disturbance in the hepatic architecture (Fig. 4A,B). A mild blood cell infiltration in the hepatic parenchyma was observed at both the doses of 100 and 200 ppm (Fig. 3A,3B and 4A,B).

### Discussion

Most of the toxins after they enter the body are metabolised in the liver by drug metabolising enzymes, which are mainly present in liver. These agents are transported to different parts of the body through blood (Deichmann *et al.*, 1968). Liver being a detoxifying

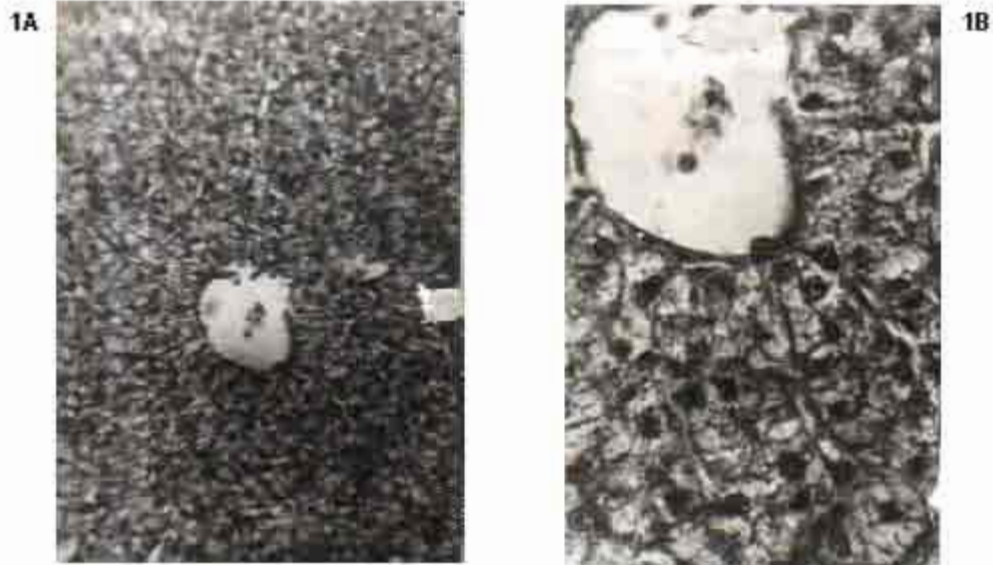


Fig. 1A, B: Show the histology of the liver of newly hatched chicks (Control) developed from the eggs injected with (0.2 ml) of vehicle acetone at '0' day of incubation. No any histopathological change is observed. All the hepatocytes are normal

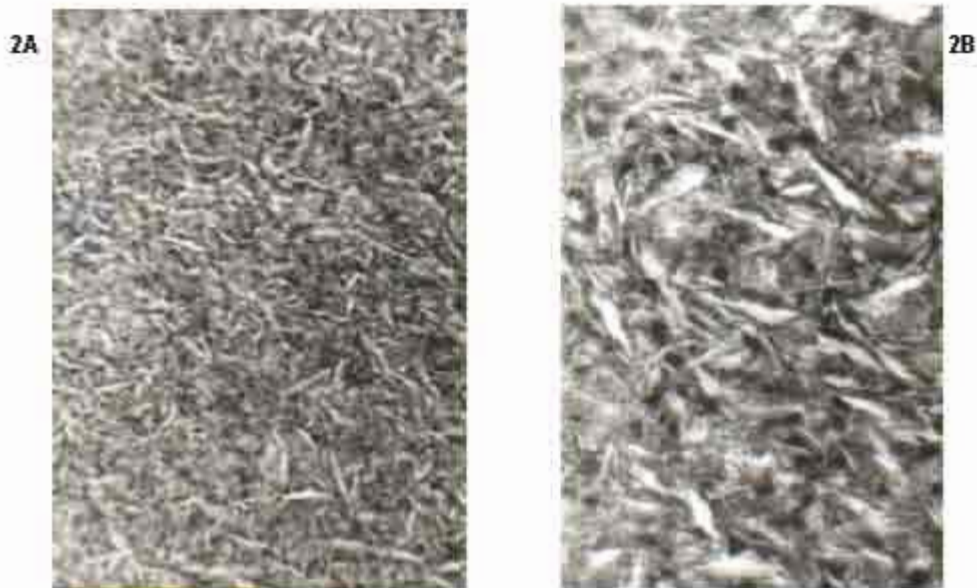


Fig. 2, B: Show the histological changes in the liver of newly hatched chicks developed from the eggs injected with 50 ppm (0.2 ml) of permethrin dissolved in acetone at '0' day of incubation. Note the increased sinusoidal spaces and destruction of hepatic architecture. Hepatocytic nuclear condensation is common. A few necrotic hepatocytes can also be seen



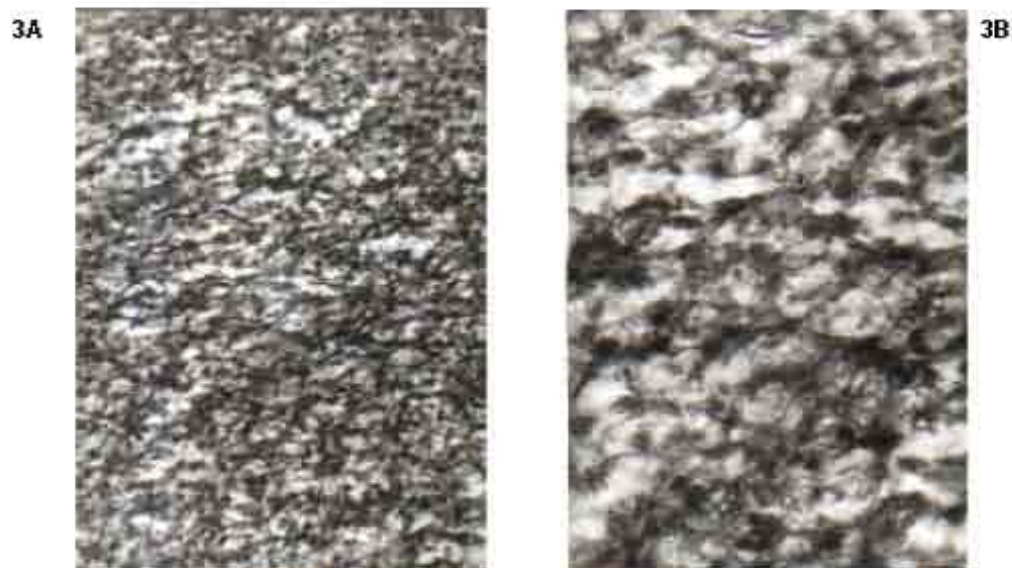


Fig. 3A, B: Show the histological changes in the liver of newly hatched chicks developed from the eggs injected with 100 ppm (0.2 ml) of permethrin dissolved in acetone at '0' day of incubation. Hepatocytic nuclear condensation and hydropic degeneration is common. Necrotic hepatocytes are also visible

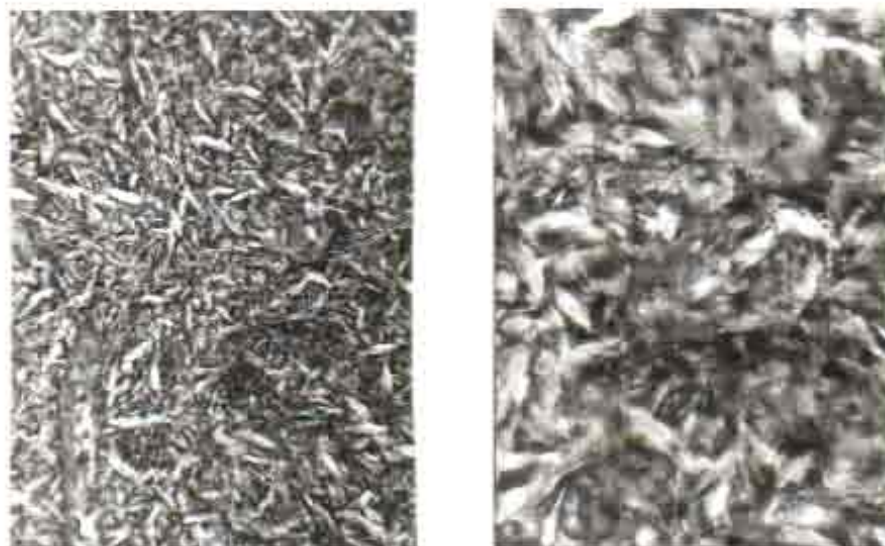


Fig. 4A, B: Show the histological changes in the liver of newly hatched chicks developed from the eggs injected with 200 ppm (0.2 ml) of permethrin dissolved in acetone at '0' day of incubation. Note the increased sinusoidal spaces and destruction of hepatic architecture. Hepatocytic nuclear condensation is common. Necrotic hepatocytes are also visible. Blood cells accumulation is also visible

organ is much more vulnerable to toxic insult and damage to it can produce secondary effects on the other systems of the body. In liver damage, for example, ammonia produced during the metabolism of proteins will not be incorporated in the urea by the liver and thus will cause cerebral edema and hence affect brain function. Similarly, decreased synthesis of clotting factors by damaged liver results in internal bleeding. Keeping in view the importance of liver in vertebrate body, the toxicity of permethrin was investigated in the liver of newly hatched chicks. Toxicity was evaluated in terms of biochemical and histological changes. Study of biochemical changes included the estimation of a few enzyme activities and some biochemical components.

### **Enzyme activities**

Of the enzyme activities, the activities of amylase, AKP, AcP and AST were significantly decreased whereas the activity of LDH was increased. However, the activity of ALT remained unchanged. Amylase activity was decreased at all the doses. Decreased amylase activity might be due to its inhibition by the permethrin treatment. Of the phosphatases the activity of AKP decreased at all the doses whereas the activity of AcP decreased at 100 and 200 ppm only. 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 may be taken as an index of parenchymal damage (Onikienko, 1963). In the present study, decrease in hepatic AKP activity could be due to hepatic parenchymal damage rather than its inhibition by the insecticide. Evidence for hepatic parenchymal damage is also provided by the light microscopic study of liver sections from the treated animals. Hepatic AcP activity was decreased only at the two higher doses of permethrin and this decrease may be due to the release of lysosomal enzyme from the liver tissue in to the blood stream as a result of parenchymal damage. Decreased AcP activity was also observed in the rat liver following treatment with fenvalerate (Majmunder *et al.*, 1994). The activity of AST was decreased at 100 and 200 ppm and this decrease also reflects the damage to the hepatic tissue by permethrin. In the present study the activity of ALT remained unchanged. It is in contrast with the findings of Anadon *et al.* (1988) who reported the increased activity of ALT in rat liver as a result of permethrin toxicity. Activity of LDH increased only at 100 and 200 ppm. Recent findings that apoptosis, a programmed cell death, in addition to necrotic cell death, play an important role in cell/tissue damage have made revolutionary changes in understanding the mechanism of tissue damage, which might have occurred by endo or exotoxins. Anwar *et al.* (1998) observed hepatocyte apoptosis in mice with paracetamol treatment. Apoptotic damage can be detected by TUNEL staining which pin point even a single apoptotic cell and DNA fragmentation as observed by gel electrophoresis. In the present study, since non of these procedures were applied to study apoptotic changes, only the observation of hepatic nuclear condensation which is also a hallmark of apoptosis indicate that permethrin might have caused apoptosis in addition to necrosis in the hepatocytes. El-Hassan *et al.* (2001) observed that mitochondria are also involved in the paracetamol-induced hepatocytic apoptosis. Though this study was performed on paracetamol toxicity but it still indicates the involvement of mitochondria in hepatocyte damage.

Damage to mitochondria may result in the accumulation of lactic acid, which in turn can induce the increased synthesis of the LDH enzyme to convert lactic acid to pyruvic acid, an initial substrate of the Krebs cycle. So the increase in LDH activity can be attributed to the hypoxic damage of the hepatocytes. It can be presumed that decreased AST might have occurred as a result of damage to some of the mitochondria in the hepatocytes.

#### **Biochemical changes**

Both glucose and glycogen remained unaffected by the permethrin treatment. Total hepatic protein content was increased only at 200 ppm. This increase in total hepatic protein content might have occurred to repair the injured tissue. The evidence for the permethrin-induced damage to hepatic parenchyma is further supported by the reduced hepatic DNA content observed in the present study. Hepatocytic nuclear condensation and the presence of anucleated hepatocytes indicate damage to DNA as well as its loss from the hepatocytes. Urea content was decreased with permethrin treatment. Since urea is synthesised in the liver its decrease in the liver may indicate damage to the liver. Both the hepatic total lipids and cholesterol contents were increased with permethrin in the present study. Increase in total lipid and cholesterol contents may be due to fatty change in the liver. Light microscopic study of the liver also reveals the fatty degenerative changes in the liver.

#### **Histopathological changes**

Permethrin treatment has resulted in increased sinusoidal spaces, fatty change in hepatocytes, hypertrophied hepatocytes and some necrotic changes in the hepatocytes (Fig. 2-4). Increase in sinusoidal spaces occurs as a result of toxic insult and its purpose is to increase the blood flow in the liver lobule to meet the energy requirements as well as to increase the supply of oxygen to hepatocytes under stress. Fatty changes occur as result of disturbance in fatty acid metabolism. Increased hepatic total lipid contents correlates well with the fatty change in the hepatocytes. Hydropic degeneration and necrotic changes were also observed in the hepatocytes. Hydropic degeneration and necrosis occurs as a result of disturbance in  $Ca^{+2}$  homeostasis. Mitochondria have a key role in the regulation of intracellular calcium ( $Ca^{2+}$ ) (Carafoli, 1987) and the disturbance of mitochondrial function is linked with increased  $Ca^{2+}$  and hence multiple downstream effects in necrosis (Trump and Berezsky, 1992). So in the present study hepatocyte necrosis might have resulted from disturbance in  $Ca^{+2}$  homeostasis. In addition, there is an evidence in the support of another kind of cell death, the programmed cell death or apoptosis, occurring in the hepatocytes as a result of permethrin treatment. Nuclear condensation and DNA fragmentation is the hallmarks of apoptosis (Ray *et al.*, 1996). Hepatocytic nuclear condensation has been observed in the present study with permethrin toxicity. Histological parameters also indicate hypertrophied hepatocytes.

Abu-Qare and Abou-Donia, (2001) observed the two metabolites of permethrin M-phenoxybenzoic acid and m-phenoxybenzoyl alcohol in rat plasma and urine. Kapoor *et al.* (1988) observed that permethrin induces the microsomal proteins cytochrome P-450 and NADPH reductase responsible for the bioactivation of permethrin. Kostka *et al.* (1997) observed that

permethrin induces the activity of cytochrome P-450 1A and 2B in rat liver. The metabolites of permethrin may be detoxified through conjugating with glutathione. This conjugation is catalysed by glutathione-S-transferases (GSTs), a family of enzymes found at high levels in the liver that play an important role in the detoxification of electrophilic alkylating agents (Mannervik and Danielson, 1988). Vontas *et al.* (2001) observed Glutathione-s-transferases as antioxidant defence against pyrethroid-induced lipid peroxidation, protein oxidation and depleted reduced glutathione in insects indicating that permethrin causes damage through the generation of reactive oxygen species. In the present study the damage to hepatic parenchyma might have resulted from the oxidative damage of tissue macromolecules by reactive oxygen species generated during the metabolism of permethrin. Results of this experiment indicates that permethrin seriously damages the liver in chicks.

### References

- Abdel-Rehman, A., A.K. Shetty and M.B. Abou-Donia, 2001. Subchronic dermal application of N, N-diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal in the cerebral cortex and hippocampus and Purkinje neuron loss in the cerebellum. *Exp. Neurol.*, 172: 153-171.
- Abuelgasim, A., R. Ringer and V. Sanger, 1981. Toxicosis of Mirex for chick embryos and chickens hatched from eggs. *Avian. Dis.*, 26: 34-39.
- Abu-Qare, A.W. and M.B. Abou-Donia, 2001. Simultaneous determination of chlorpyrifos, permethrin and their metabolites in rat plasma and urine by high-performance liquid chromatography. *J. Anal. Toxicol.*, 25: 275-279.
- Akhtar, M.H., C. Danis, H.L. Trenholm and K.E. Hartin, 1992. Deltamethrin residues in milk and tissues of lactating dairy cows. *J. Environ. Sci. Health*, 1327: 235-253.
- Anadon, A., M. J. Diez, M. Sierra, J. A. Sanchez and M. T. Teran, 1988. Microsomal enzyme induction by permethrin in rats. *Vet. Hum. Toxicol.*, 30: 309-312.
- Anderson, R.L., 1983. Toxicity of fenvalerate and permethrin to several non-target invertebrates. *Govt. Reports, Announcements and Index (GRA and I)*, pp: 15.
- Anwar, K., G.E.N. Kass and R.H. Hinton, 1998. Gadolinium pretreatment decreases paracetamol-induced apoptosis and toxicity in mice liver. *Human. Exp. Toxicol.*, 17: 469-534.
- Cabaud, P.G. and B. Wroblewski, 1958. Colorimetric measurement of lactate dehydrogenase activity of body fluid. *Am. J. Clin. Path.*, 30: 234.
- Carafoli, E., 1987. Intracellular calcium homeostasis. *Annu. Rev. Biochem.*, 56: 395-433.
- Carraway, W.T., 1963. *Standard methods of clinical chemistry* (Ed. Seligson, D). Academic Press, New York and London, 4: 239.
- Chouela, E., A. Abeldano, G. Pellerano and M.I. Hernandez, 2002. Diagnoses and treatment of scabies: a practical guide. *Am. J. Clin. Dermatol.*, 3: 9-18.
- Consolazio, C. F. and J. M. Lacono, 1963. Carbohydrates. In *methods of nutritional biochemistry with applications and interpretations* (Ed, A. A. Albanese). Academic Press, New York and London, 1: 317-367.

- Deichmann, W.B., I. Dressler, M. Keplinger and W.E. Macdonald, 1968. Retention of the dieldrin in blood, liver and fat of rats fed dieldrin for 6 month. *Indian. Med. Surg.*, 37: 837.
- El-Hassan, H., P. Macanas-Pirard, K. Anwar, P.C. Rhodes, S.C. Price, R.H. Hinton and G.E.N. Kass., 2001. Liver apoptosis by paracetamol (acetaminophen) is mediated by mitochondria. *Toxicology*, 168: 112-114.
- Ferguson, C.A. and G. Audesirk, 1990. Effects of DDT and Permethrin on neurite growth in cultured neurons of chick embryo brain and *Lymnaea stagnalis*. *Toxicology in vitro.*, 4: 23-30.
- Ghosh, T.K., 1989. Influence of cypermethrin on the oxidative metabolism of *Labeo rhita*. *Proc. Indian. Natl. Sci. Acad.*, 55: 115-119.
- Hartel, A., R. Helger and H. Lang, 1969. A method for determination of glucose. *Z. Klin. Chem. Klin. Biochem.*, 7: 183.
- Henry, R.J. and M. Henry, 1974. *Clinical chemistry*. Harper and Row Publishers, New York.
- Ishmael, J. and M.H. Litchfield. 1988. Chronic toxicity and carcinogenic evaluation of Permethrin in rats and mice. *Fundam. Appl. Tixocol.*, 11: 308-322.
- Kapoor, R.K., S.S. Chauhan, N. Sing and U.K. Misra, 1988. Induction of hepatic mixed function oxidases by permethrin and cypermethrin in chicks fed Vitamin A deficient diet. *Pestic. Biochem and Physiol.*, 32: 205-211.
- Karen, D.J., W. Li, P.R. Harp, J.S. Gillette and J.R. Bloomquis, 2001. Striatal dopaminergic pathway as a target for the insecticides permethrin and chlorpyrifos. *Neurotoxicology.*, 22: 811-817.
- Kind, P.R.N. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. *J. Clin. Path.*, 7: 322-326.
- Kostka, G., D. Palut and B. Wiadowska, 1997. [The effect of permethrin and DDT on the activity of cytochrome P-450 1A and 2B molecular forms in rat liver. *Rocz Panstw Zakl Hig.*, 48: 229-237.
- Lanselink, D.R., E.M. John and G.L.K. Olesari, 1993. Teratogenesis associated with oxydemettow methyl in the stage 12 chick embryo. *Teratology.*, 48: 207-211.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Majmunder, D., A.K. Chakraborty, T.K. Mandal, A. Bhattacharya and K. Basak, 1994. Sub acute toxicity of fenvalerate in broiler chicks. *Indian. J. Exp. Biol.*, 32: 752-756.
- Mannervik, B. and U.H. Danielson, 1988. Glutathione transferases-structure and catalytic activity. *CRC. Crit. Rev. Biochem.*, 23: 283-337.
- Moore, S. and W.H. Stein, 1957. A modified ninhydrin reagent for photometric determination of amino acids and related compounds. *J. Biol. Chem.*, 211: 907-913.
- Mufti, S.A. and R. Nasim, 1987. Avian embryo toxicity of Dimicron. A commonly used insecticide. *Biologia.*, 33: 109-121.
- Natelson, S., M.L. Scott and C. Beffa, 1951. A rapid method for determination of urea in biological fluids by means of the reaction between diacetyl and urea. *Am. J. Chem. Path.*, 21: 275.
- Onikienko, E.A., 1963. Enzymatic changes from early stages of intoxication with small doses of chloroorganic insecticides. *Gigienari. Fiziol. Truda. Taksikol. Klinikackiev Gos. IZ. Med. Git. Ukr. USSR.*, p:77.

- Qadri, S.S.H., K. Jabeen, M. Mahboob and M. Mustafa, 1987. Haemotoxicity to chicken (*Gallus gallus-domesticus*) by technical and formulation grades of some phosphoric and synthetic pyrethroid esters. *J. Appl. Toxicol.*, 7: 367-371.
- Rao, J.V., A.N. Swamy, S. Yamin, S.H. Rao and M.F. Rahman, 1992. *Food Chem. Toxicol.*, 30: 945-951.
- Ray, S.D., Mumaw, V.R, R.R. Raje and M.W. Fariss, 1996. Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesteryl hemisuccinate pre-treatment. *J. Pharmacol. Exp. Ther.*, 279: 1470-1483.
- Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. *Am. J. Clin. Path.*, 28: 56.
- Scheideler, S.E., 1993. Effects of various types of Aluminosilicates and Aflatoxin B1 on Aflatoxin toxicity, chick performance and mineral status. *Poultry. Sci.*, 72: 282-288.
- Schneider, W. C., 1957. Determination of nucleic acids in tissues by pentose analysis. *In methods in enzymology* (Eds S. P. Colowick and N. O. Kaplan). Academic press, New York, 3: 680-684.
- Shakoori, A. R. and M. S. Ahmed, 1973. Studies on the liver of chicken, *Gallus domesticus*. 1. Liver growth and Nucleic acid content. *Pakistan J. Zool.*, 5: 111-117.
- Sheets, L.P., 2000. A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroids insecticides. *Neurotoxicology*, 21: 57-63.
- Sibley, P.K. and N.K. Kaushik, 1991. Toxicity of microencapsulated permethrin to selected non-target invertebrates. *Arch. Environ. Contam. Toxicol.*, 20: 168-176.
- Spencer, F. and Z. Berhane, 1982. Uterine and fetal characteristics in rats following a post-implantational exposure to Permethrin. *Bull. Environ. Contam. Toxicol.*, 29: 84-88.
- Trump, B.F. and I.K. Berezsky, 1992. The role of cytosolic Ca<sup>2+</sup> in cell injury, necrosis and apoptosis. *Curr. Opin. Cell. Biol.*, 4: 227-232.
- Vontas, J.G., G.J. Small and J. Hemingway, 2001. Glutathione-s-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem. J.*, 357: 65-72.
- Walker, N.I., 1971. The effect of Malathion and Malaxon on esterases and gross development of the chick embryo. *Toxicol. Appl. Pharmacol.*, 19: 590-601.
- Wootton, I.D.P. and H. Freeman, 1964. *Microanalysis in medical biochemistry*, Churchill Livingstone, London.
- Zollner, N. and K. Kirsch, 1962. Microdetermination of lipids by the sulphophospho vanillin reaction. *Z. Ges. Exp. Med.*, 135: 545-561.