International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

Histopathological Changes In The Gills And Liver, And Biochemical Changes In Blood Of Common Carp (Cyprinus Carpio L. 1758), Exposed To Insecticide (Deltamethrin)

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Abstract
The study was conducted during the tenth month October 2023. Deltamethrin is a synthetic pyrethroid insecticide, used widely in farms. fields and home for prevention and treatment of harmful insects. The histopathological changes of deltamethrine on the (liver and gills) tissues in Cyprinus carpio were determined by light microscope. These changes characterized by severe epithelial sloughing and necrosis of lamellar epithelia, rupture of secondary lamellae and epithelial lifting, lamellar epithelial hypertrophy and hyperplasia with telangiectasis of some capillaries presence of mucinous material. Hepatic lesions in the liver tissues observed in the hepato-pancreatic tissue with multiple necrotic foci and loss of it is parenchyma, hemorrhage and blood vessels dilation, vacuolation with moderate MNCs, ductal dilation with necrotic debris and moderate accumulation of pigmented macrophages. In addition, serum glutamic pyruvic trans-aminase (GPT), glutamicoxaloacetic tran-aminase (GOT) and total protein were measured and compared with control group showed an increase ($p<0.05$) in these parameters in exposed fish to deltamethrin, but total protein was decreased in same groups. In conclusion, Deltamethrin as toxic substance that consider as stress factor in the aquatic environment.
Key words: *Histopathological changes, Gills, Liver, Biochemical changes, Cyprinus carpio, Deltamethrin.*

INTRODUCTION

Fishes are considered as a basis of protein, fat, phosphate, iron, calcium, amino acids and vitamins which are soluble in water and soluble in fat. Also, containing all the ten essential amino acids, fishes are consider as a drug against many diseases for example Psoriasis that is treatment by fish oil (omega-3) as well as treatment of heart diseases (Stanković et al., 2011 and Ali, 2020). Deltamethrin is a synthetic pyrethroid insecticide used widely in farming, home pest treatment and control it, food protection (Shikha et al., 2018). In recent years the application of deltamethrin as insecticide and anti-parasitic in the field has very increased, as well as in medicine and veterinary medicine to treat parasitic crustacean infestations. Also, it is used to maintain the public and animal health, that as a target organisms its (Valesca et al., 2020). The main feature of pyrethroids is their photostability and high activity in low concentrations (Velisek et al., 2006). Deltamethrin, pyrethroids are a group of compounds of natural origin isolated from flowers of plant Tanacetum cinerariaefolium (Chrustek, et al., 2018). Deltamethrin, a pyrethroids act on voltage-gated sodium channel, which cause an influx of sodium ions into nerve cells and permanent depolarization. They also influence activities of enzymes, especially in nerve and liver cells. Contact of deltamethrin with the skin, digestive tract, and respiratory system results in their penetration into the body (Chrustek, et al., 2018; Holynska-Iga and Szewczyk-Golec, 2020). A large number of pesticides are commonly used to control various agricultural pest. However, their toxicological impact also extends to no target species like fishes (Naqvi and Vaishnavi, 1993). Fishes are good indicator of aquatic contamination because its biochemical stress responses are quite similar to those found in mammals (Mishra and Shukla, 2003). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field study. So, should be examining specific target organs, including gills and liver that are responsible for vital function, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in fishes (Hadi and Alwan, 2012). Histopathological changes considered a rapid method to detect effects of irritants, especially chronic ones in various tissues and organs (Narges, 2013). Deltamethrin caused oxidative stress in fishes species like C. carpio, tilapia, and trout. it is vastly absorbed and excreted in urine and feces

during 24 hours (Enis and faith, 2011; Soderlund, 2012). Gills in fishes considered the main passage for entrance of pollutant substance to the internal body organs like kidney, liver, brain an muscle through the blood stream (Seham et al., 2014). Hence, this study was undertaken to check the changes of deltamethrin concentrations on histological aspects of gills and liver of freshwater fish, Cyprinus carpio. Liver is most important metabolicorgan, like protein, carbohydrate, and lipid metabolism, metabolic activity within liver controlled by enzymes such as GPT and GOT, that play an important role in detoxification mechanism of toxic and poisonous substances of fishes (Soreg and Seidman, 2001; Nevien, et al., 2015; Valesca et al., 2020). Highest value of transaminase enzymes are seen in case of hepatocyte necrosis occurring in case substances poisoning and viral hepatitis. The goal of this study was aimed to determine the effect of deltamethrin on histological composition and biochemical parameters like glutamatoxalacetat transaminase GOT, glutamatpyurvat transaminase GPT and total protein in liver tissue of common carp C. carpio.

MATERIALS AND METHODS

Cyprinus carpio were obtained from the fish farm in Baghdad province, Iraq, during the period of the October 2023. These specimens were transferred alive to the research laboratory in Fish and Animal Resource Department/ Agriculture Research Center/ Scientific Research Commission/ Ministry of Higher Education and Science Research. These fishes were identified and their scientific names were determined by (Coad, 2010 and Eschmeyer, 2018). A total of 90 fish were used. They weighed (90-100) gm. with different lengths (18-20) cm. The fishes were acclimated to the laboratory conditions for at last 14 days. Fish samples were exposed to 3% of salt concentration for five minutes until signs of stress for sterilization. The experiment fish were put them in glass aquariums (60 × 30 × 30) cm. each containing 6 fish in 40 L. with dechlorinated water. Water quality characteristics were determined, which were as follow: temperature 23 °C, PH 7.5, dissolved oxygen 7.5 mg/L. The fishes were fed daily with commercially balanced fish food.

Experimental Design

Fishes were divided into two groups, as follows: Control group with dechlorinated water and deltamethrin group. A set of 20 fish specimens were randomly exposed to deltamethrin concentrations (0.020, 0.030, 0.050, 0.060. 0.080) ppm. after 72 h. the value of LC50 of Deltamethrin for C. carpio was determined (0.050 ppm). Depending on the LC50 values, the fishes were then exposed for 72 hrs. to sub-lethal concentration of deltamethrin (0.010 ppm.), after acute exposure 72 h. the water was exchanged.

Histopathological Examination

At the end of exposure period, fishes were dissected and small pieces of exposed and control organs (gills and liver) were immediately isolated and fixed in formalin solution 10% was used for fixation for 24-48 hours. The fixed samples were washed by water for 30 seconds. The samples were placed in ethyl alcohol (70, 80, 90 and 100%) for two hours for each concentration except for 100% which was left over night. The samples were cleared by xylene A and xylene B for 30 minute to each one. Paraffin wax with a melting point of 54-56 °C was used by putting them in the oven 60 °C for three hours. The samples were embedded in the blocks with few amount of glycerin. The liquid wax was poured in these blocks and left in a cold climate until become solid. Afterwards, the blocks were put in the ice box. The samples were sectioned with Rotary micrortome with a thickness of 4-6 µm. The tissue slices were put in water bath, then the slide was put in the oven 70 °C to remove the additional wax. The slide was left in front of an air-conditioner for 24-48 hours. The staining was carried out by using the haematoxylin and eosin stain technique (Carson, 2007 and Seham et al., 2014). Stained mounted sections were examined under light microscope. Photographs were taken at x40 magnification using the camera at 50 mm. focal length.

Serum Samples

Blood samples were obtained from the caudal vein of fishes by using a 23-guage needle and 3 ml syringe (Blaxhall and Daisley, 1973; Douglas and Wardrop, 2010). Blood samples were used non-heparinized tubes for serum biochemical analysis, centrifuged at 3000 rpm for 10 min. and the obtained serum were aspirated into sterile vials and kept in deep freeze (-20°C) till biochemical analysis including: GPT, GOT by (Reitman and Frankel, 1957) and total protein according to (Watanabe et al., 1987). Data were

statistically analyzed by paired samples (T-Test). Comparison between means was done using stander error mean (SEM), by the (SPSS). Was used.

RESULTS AND DISCUSSION

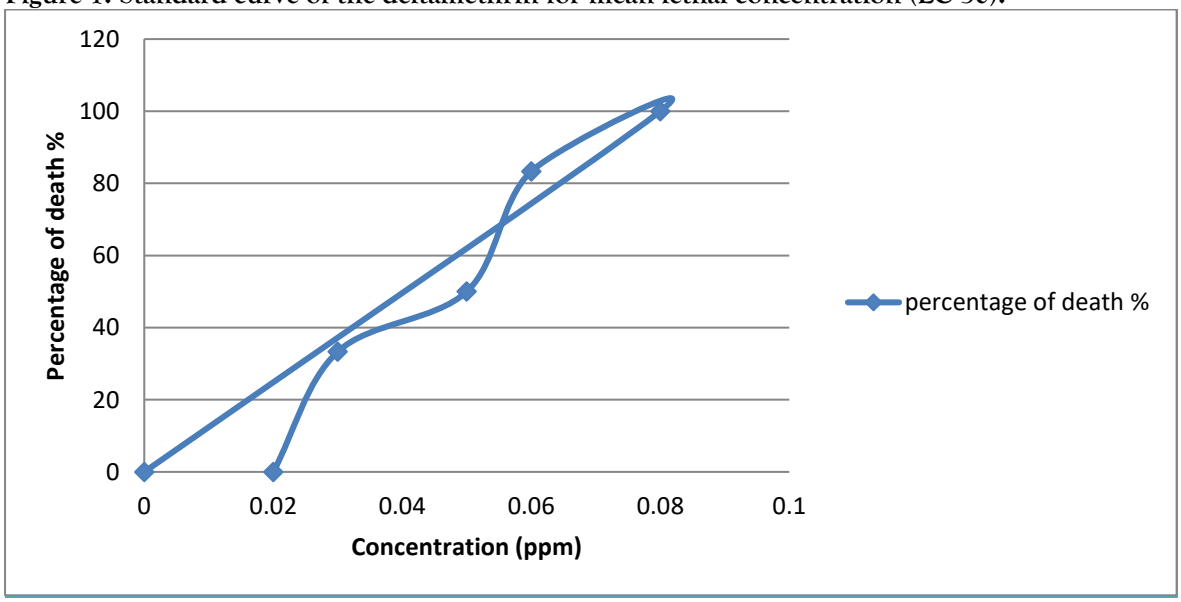
This study was determined the toxic effect of the insecticide deltamethrin on some histological composition and biochemical parameters in the fish culture C. carpio, its cause high toxicity and bioaccumulation in non-target organisms. They are 1000 times more toxic to fishes than to mammals and birds (Holynska-Iga and Szewczyk-Golec, 2020). Throughout the 72 hrs. of the experience, behavioral changes such as rapid and circular swimming, spasms, stability on the water surface and increased opercula activity were observed in experimental group, except the control group no behavioral changes were observed. The results are summarized in tables and figures as following: (Table 1): Explains that results deltamethrin toxicity on fish carp when exposed to different concentrations of pesticide, after passing (72) hrs. to determine median lethal concentration LC50 (0.050 ppm). according to linear regression method between effective material and duration of exposure to the pesticide (Figure 1). The following concentration were used: (0.020, 0.030, 0.050, 0.060, 0.080) ppm. in addition to control group with pesticide free water (tab water).

Table1: Shows the results of the deltamethrin concentrationon common carp.

Concen. of deltametrin ppm.	Total no. of fishes	No. of dead fishes	Percentage of death %
0.020	6	0	0
0.030	6	2	33.33
0.050	6	3	50
0.060	6	5	83.33
0.080	6	6	100
Control	6	0	0

Figure 1 explains different concentrations of deltamethrin according to linear regression method between effective material and duration of exposure to the pesticide.

Figure 1: Standard curve of the deltamethrin for mean lethal concentration (LC 50).



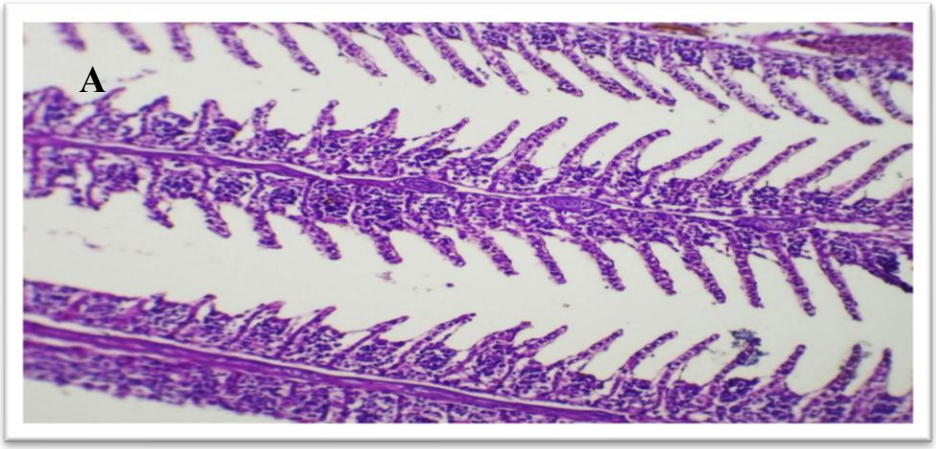
Microscopic Examination

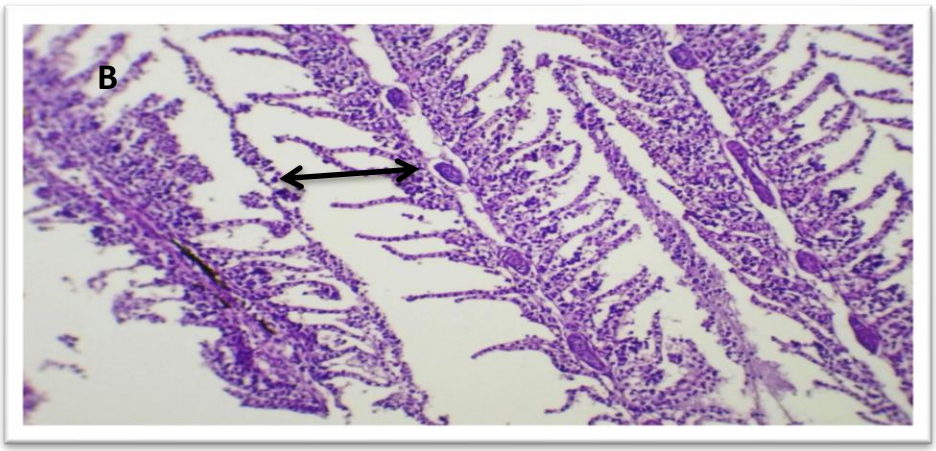
Gill findings

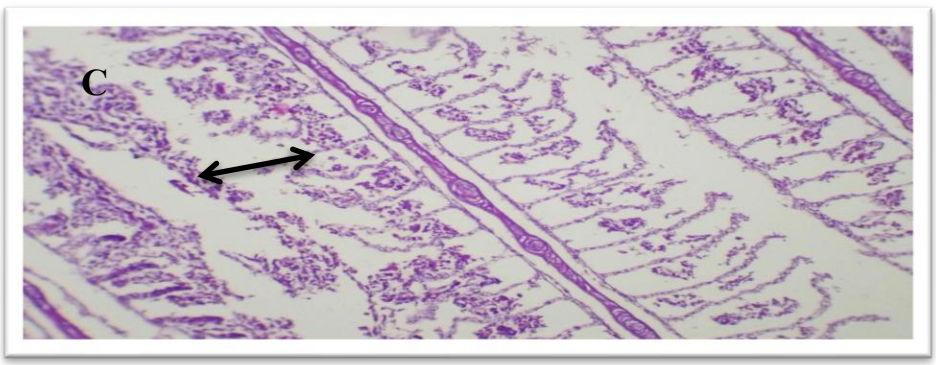
No histopathological changes were observed in all organs examined in all control groups. The microscopically of examined gill tissue revealed normal structure primary and secondary lamellae (fig. 2-A).

Gill section of experiment fish showed severe epithelial sloughing mainly of secondary lamellae (fig. 2-B) with rupture of secondary lamellae and epithelial lifting (fig. 2-C), while other findings revealed lamellar epithelial hypertrophy and hyperplasia with telangiectasis of some capillaries (fig. 2-D), in addition to presence of mucinous material as basophilic mass in the inter lamellas space (fig. 2-E).

Deltamethrine is one of stress factors in fish health, therefore, its increase the secretion of glucocorticoid that inhibits the immune system (Roberts, 1978 and Bennett and Wolk, 1987). The main histopathological changes due to deltamethrin which act on respiratory surfaces like gill and therefore affects oxidative phosphorilation that leading to entry the water and cause severe damage in organ tissues, cells are unable to utilize oxygen leading to survival or accumulation oxyhemoglobin in blood and severe disorder of osmoregulation (Mayer and Ellersieck, 1986; Holynska-Iwan and Szewczyk-Golec, 2020).







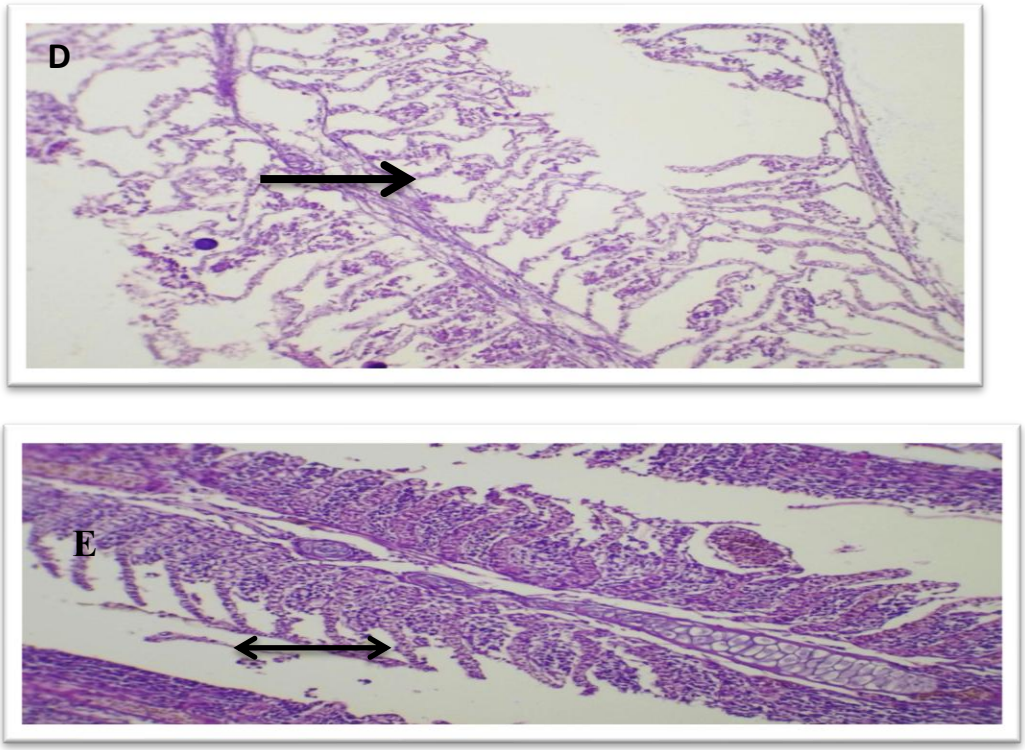
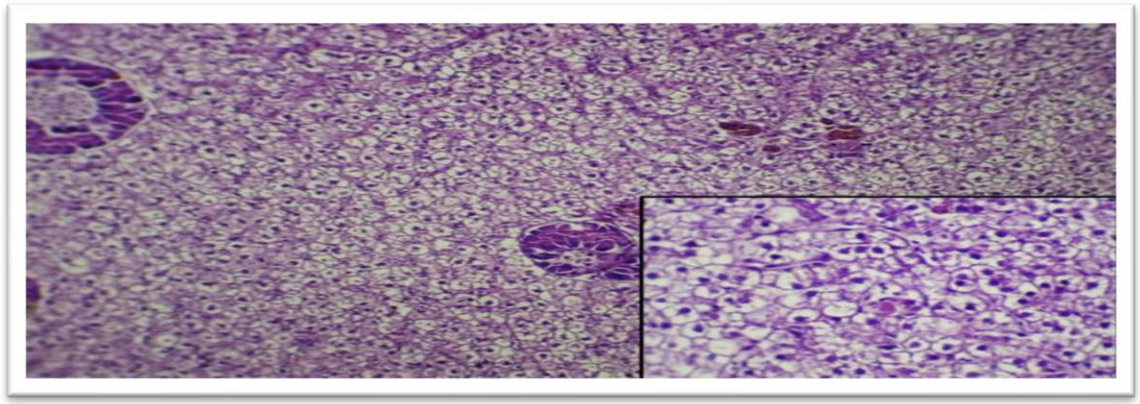


Figure 2: gill section of control fish group showing normal structural details (A), gill section of treated fish group showing sever epithelial sloughing and necrosis of secondary lamellar epithelia with cellular infiltration in the inter lamellar space (arrow) H&E X10 (B), rupture of secondary lamellae & epithelial lifting (arrow) H&E X10 (C), showing lamellar epithelial hyperplasia with telangiectasis of some capillaries (arrow) H&E X10 (D), massive destruction of primary lamellae with mucinous material as basophilic mass in the inter lamellas space (arrow) H&E X10 (E).

Liver findings

Also normal structural findings were recorded in liver control group (hepatic cell running toward the central vein with pancreatic tissue (fig. 3-A).

While in experiment fish showed extensive structural lesion observed in the hepato–pancreatic tissue with multiple necrotic foci and loss of it is parenchyma (fig. 3-B), with wide areas of hemorrhage and vessel dilation (fig. 3-C), other finding showed marked vacuolation with moderate MNCs (fig.3-D) also moderate ductal dilation with necrotic debris and moderate accumulation of pigmented macrophages (MM) (fig. 3-E). All histopathological changes of Deltamethrin in liver cause it influence activities of enzymes especially in liver cells, also cause injury of rough endoplasmic reticulum that responsible on the protein industry which reduce lipoprotein like choline and methionine leading to a decrease phospholipid and then increase diglycerides and triglycerides in the cytoplasm of hepatocytes (Bradury, 1987; Sharma et al., 2014; Saoudi et al., 2017; Holynska-Iwan and Szewczyk-Golec, 2020).



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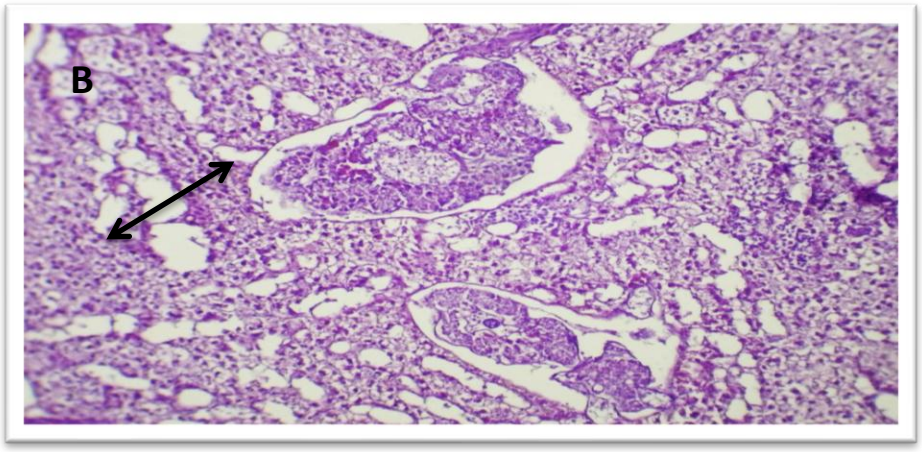

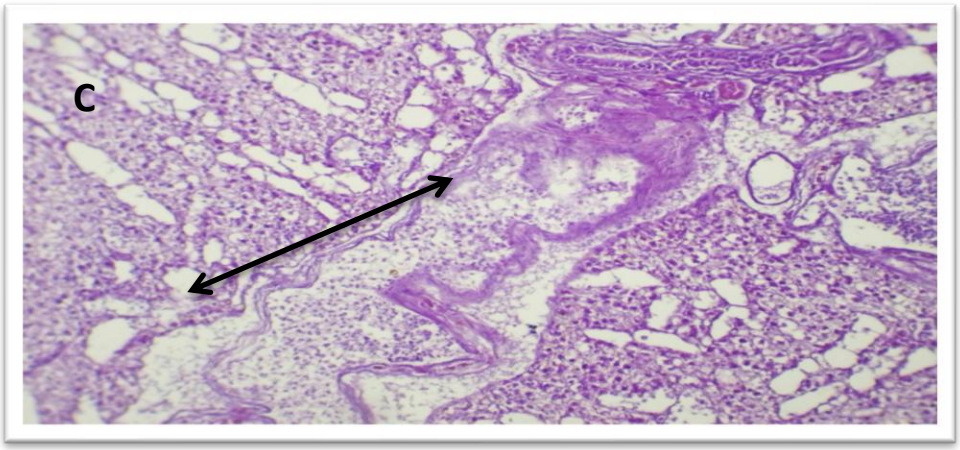

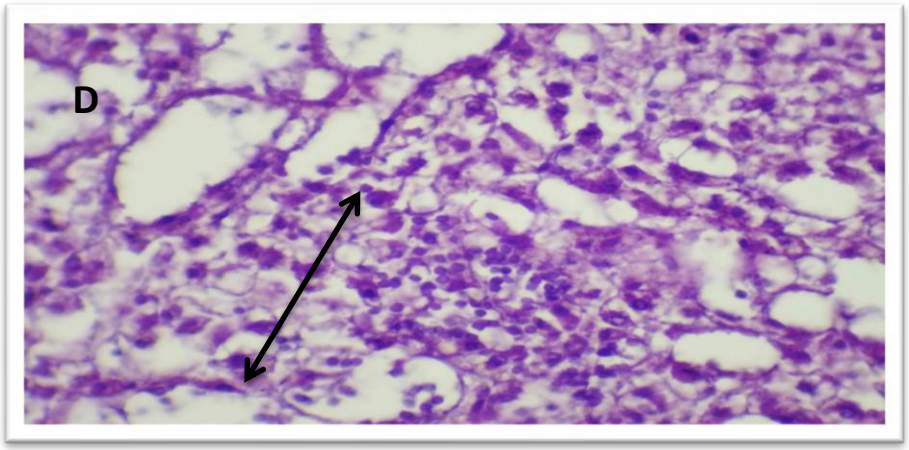

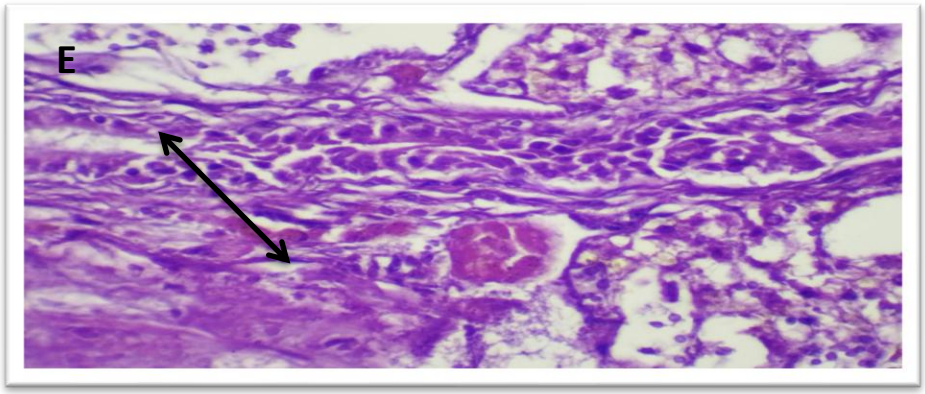

Figure 3: liver section of control fish group showed normal structural details (arrow) (H&E stain X10 & X40) (A), liver section of treated fish group showed multiple necrotic foci and loss of it is parenchyma in the hepato–pancreatic tissue (arrow) (B), wide areas of hemorrhage and vessel dilation (H&E stain X10) (C), marked vacuolation of hepatic cells with moderate MNCs (arrow) (H&E X40) (D), showed moderate ductal dilation with necrotic debris and accumulation of pigmented macrophages (arrow) (H&E stain X10) (**E**).

Results of the present study revealed that deltamethrin had very toxic to fishes (C. carpio) due to its lipophilic character, and its caused oxidative stress in carp, tilapia, and trout, its high absorption rate through the gill filaments and excreted in urine and feces during 24 hours (Enis and Fatin, 2011; Moraes et al., 2013). Activity of deltamethrin was found in brain, muscle, liver and gills in fish culture, its toxicity on nervous system investigated included on telenocephalon, optic tectum and cerebellum, its inhibitory effect on acetyle-colin esterase activity, its has a high toxicity to fishes under laboratory environment, but in field conditions under normal conditions of use fishes may not harmed. Deltamethrin had an impact on aquatic herbivorous insects, fishes and other aquatic organism (Svobodova et al., 2003). The ability of all chemical material to cause harm or damage or injury to an organisms by mechanical means (Camargo and Martinez, 2007; Cshikha, 2018). Behavioral clinical signs that you showed on fishes to test that characterized by increase movement and neurological signs that appeared on experimental fish which are attributed to neurotoxic of deltamethrin which act on voltage-gated sodium channels, which cause an influx of sodium ions into the nerve cells and permanent depolarization (Holynska-Iwan and Szewczyk-Golec, 2020). The explanation of the toxic effect of pyrethroids on the organs examined, it´s back to irritation of pyrethriod chemicals on epithelial cells of organs examined. Also, cause hypoxia to the cells of organs examined (Narges et al., 2013). According to the results on the organ tissues, deltamethrin caused histopathological changes in the organ examined. These changes due to continuous irritation and toxic effect of deltamethrin. The toxic nature of pyrethriods has been caused severe damage in all organ´s tissue and the accumulation of these substance over time in the bodies of animals can cause serious illness and may be carcinogenic to other (Wester et al., 2004). All these histopathological changes have been related to a stress response which might result from exposure to toxic compounds (Marigomez et al., 2006).

Biochemical Analysis

Table 2: demonstrated that deltamethrin (0.010 ppm) an increased the level of serum GPT and GOT to the acute phase 72 hrs. compared to control group, showing increased level of serum GPT and GOT (54.40, 392.93 ppm.) respectively during the exposure period compared to control group, while significantly decreased the level of serum total protein (2.90 gm/dl) compared to control group. The current results showed significant increase in plasma GPT and GOT activities (Table 2). These changes are due to toxicity of deltamethrin on liver functions due to pathological effects of all toxins on liver. Also, all toxic substances cause cell membrane damage, necrosis of hepatocytes, increasing cell membrane permeability lead to the enzyme leakage and disorder of metabolic processes of liver enzymes (Alaa El-din et al., 2022). The decrement of plasma total protein (Table 2) agree with (Amin and Hashem, 2012; Essam et al., 2020). This decrease in plasma protein back to changes in protein and free amino acids metabolism and their synthesis in hepatocytes. Also, the observed decreases in plasma total proteins could be ascribed to the damaging effect of deltamethrin on hepatocytes. Liver plays an important role in detoxification of poisonous and toxic substances through urea cycle and biotransformation of the hydrophobic substances such as toxic substances occur in the liver (Hadi and Alwan, 2012). Diagnosis of transaminase enzymes play an important role in early diagnosis of liver cell necrosis like toxic diseases. So that highest values of these enzymes seen in case of fungal poisoning, liver cirrhosis and viral hepatitis. In these cases release of GOT and GPT from cell mitochondria that indicator of cell damage (Valesca et al., 2020; Alaa El-Din et al., 2022).

Table 2: Effects of deltamethrin (0.010 ppm) on serum liver enzymes activities and total protein in common carp.

Groups	GPT U/L	GOT U/L	Total protein (gm/dl)
Control	48. 40 ± 0.21	220.80 ±0.24	5.26 ±1.30
Deltamethrin	54.40 ±0.36*	392.93 ± 0.24*	2.90 ±0.07*

Values are means ± SEM (n=6) * Means with different superscripts are significantly different (P≤ 0.05), while means with same superscripts indicate non-significant changes.

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