Original Article

Biochemical and Histopathological Responses of Oreochromis Niloticus and Cyprinus Carpio to Sub-lethal Exposure of Ictacrune Pesticide

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ABSTRACT

Aim of the work: Determining the negative impact of sub-lethal concentrations on biochemical and histological of Oreochromis niloticus and Cyprinus carpio.

Introduction: Ictacrune pesticide (Profenofos), is an organophosphate pesticide over the last two decades were used in agricultural for controlling pests. The toxicity of profenofos is the inhibition of the acetylcholine esterase activity resulting in neuro toxicity to aquatic vertebrates and humans.

Results and Discussion: Oreochromis niloticus and Cyprinus carpio were subjected to different concentrations (0.036 – 0.252 mg/l) and (0.0072-0.108 mg/l), respectively, of Ictacrune pesticide for 96 hours andthe lethal concentration (LC50) values of Ictacrune pesticide for the two studied fishes were 0.144 and 0.02662 mg/l Ictacrune, respectively, for 96 hours of exposure. The values of lipid, glucose, T. protein and albumin were significantly ($P<0.05$) decreased for Cyprinus carpio from (650.6, 85.5 mg/dl, 3.2 and1.5 g/dl, respectively), for control group to 255.3, 42.1 mg/dl, 2.15 and 0.95 g/dl and 158.15, 31.6 mg/dl, 2.15 and 0.85 g/dl after 45 days exposure for 0.25&0.50 LC50, while, O. niloticus (870.0, 88.50 mg/dl, 3.60 a3nd 1.90 g/dl, respectively for control group to 285.0, 55.9 mg/dl, 2.45 and 0.90 g/dl and 195.0, 44.55 mg/dl, 2.15 and 0.80 g/dl after 45 days exposure for 0.25&0.50 LC50 of Ictacrune pesticide. The values ALT, AST, urea and uric acid were significantly ($P<0.05$) increased for O. niloticus from 15.30, 33.24 IU/ml, 25.0 and 4.0 mg/dl, respectively, of control group to 51.70, 94.40 IU/ml, 162.90 and 21.90 mg/dl and 67.50, 112.35 IU/ml, 194.30 and 24.60 mg/dl, respectively, after 45 days exposure for 0.25&0.50 LC50 of Ictacrune pesticide. While as these values were significantly ($P<0.05$) increased for Cyprinus carpio, from (17.30, 39.24 IU/ml, 45.50 and 6.50 mg/dl), respectively, in control group to (87.50, 107.40 IU/ml, 123.10 and 25.50 mg/dl) and (92.75, 132.35 IU/ml, 142.80 and 30.90 mg/dl), respectively, after 45 days exposure for 0.25&0.5 LC50 of Ictacrune pesticide.

The histological examination of the liver, muscles and gills obtained from the two studied fishes exposed to Ictacrune pesticide for 45 days in present study revealed many alterations including, degeneration, necrosis, piknosis, congestion, edema, fibrosis, hyperplasia, curling, separation and hemorrhage. The degree of these alterations depended on dose and the period of Ictacrune pesticide exposure.

Conclusion: there is a direct relationship between pesticide exposure and histopathological alterations that observed in studied organs. The degree of these alterations depended on dose and the period of Ictacrune pesticide exposure.

Received: 30 December 2019, **Accepted:** 26 January 2020

Key Words: Biochemistry, fish, histopathology, pesticide.

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ISSN: 1110-0559, Vol. 43, No.3

INTRODUCTION

Fish is an economic source of protein in developing countries and is extensively cultured in inland water bodies. It is important to study the effects of the lethal exposure of pesticides on biochemical parameters and histological structures as a result of their accumulation in various fish organs and its effects on humans as a final user[1,2]. Pesticides are dangerous pollutants which cause harmful effect on the aquatic environment[3].

Ictacrune pesticide (Profenofos), is an organophosphate pesticide over the last two decades were used in agricultural for controlling pests. The toxicity of profenofos is the inhibition of the acetylcholine esterase activity resulting in neuro toxicity to aquatic vertebrates and humans. Microbial degradation of profenofos is part interesting because of the high mammalian toxicity of these compounds and their widespread and highly use. The most important step in detoxifying organophosphate compounds is hydrolysis which makes the compounds more vulnerable to further degradation. The enzyme responsible for this reaction catalyzing is referred as phosphotriesterase or esterase[4].

Ictacrune pesticide (Profenofos) is classified as moderately dangerous (toxicity class II) pesticide by WHO and it has a moderate order of acute toxicity following oral

 DOI: 10.21608/ejh.2020.21602.1224

and dermal administration. The acute toxicity of profenofos come from inhibiting the activity of acetyl cholinesterase[5-8]. Using of different groups of pesticide make toxicological and environmental problems, like impacts on many aquatic species, as fish[9]. Profenofos (O-4-bromo-2-chlorophenyl-O-ethyl S-propyl phosphoro-thioate), is a broad spectrum organophosphate pesticide widely used for agricultural and household purposes in India[10-12]. It is effective against wide range of mites sucking insects and chewing on many crops especially on cotton plants[13]. Its half-life in soil is approximately one week[14]. One reason for the heavily use of PFF is a deceptive view of its short half-life in soil, but, it has been recognized as very toxic and persistent pesticide even at low concentrations[15]. It is proposed that the alteration in protein, fat, ash, and carbohydrate may be used as a biomarker to assess the level of pesticide stress[2].

The study was aimed to assess the impact of sub-lethal concentrations of Ictacrune pesticide on biochemical and histological characteristics of Oreochromis niloticus and Cyprinus carpio.

MATERIALS AND METHODS

A total number of 180 apparently healthy live Common carp, Cyprinus carpio, average body weight (30±5 g), and 170 Oreochromis niloticus fish, average body weight (25±2 g), were obtained from El-Serw Station for Fish Research, Inland Water and Aquaculture branch, National Institute of Oceanography & Fisheries for determination of 96 hrs LC_{50} of Ictacrune and the impact of sub-lethal concentrations of Ictacrune on the biochemical and histological characteristics of Oreochromis niloticus and Cyprinus carpio. Fish were acclimated to the laboratory conditions for period of two weeks

Aquaria

Glass aquaria (80 × 30 × 25 cm) were used in this study. Each aquarium was supplied with dechlorinated tap water, continuous aeration. Aquaria were cleaned periodically. Fish were kept at dissolved oxygen 8.0± 0.5 mg/l, pH 8 ± 0.5, water temperature 28 ± 2 °C, along the period of the study.

Fish Basal Diet

A basal diet in form of dry pellets was obtained from National institute of Oceanography and fishers. This diet was offered to the fish by 2-3% of the total biomass of fish, twice daily.

Pesticides

Ictacrune: It is abroad-spectrum organophosphate insecticide, widely used to control Lepidoptera in cotton and soybean with strong effects against mining and sucking insects as well as mites. Each liter of it contains 72 g of active substance (profenofos).

Experiment Design

Determination of 96 hrs-LC_{50} of Ictacrune

A total number of 90 Cyprinus carpio, with an average body weight (30± 5 g) were divided into 9 groups (10 fish/group). First group was left as a control, while, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th and 9th were exposed to 0.0072, 0.0144, 0.0216, 0.0288, 0.036, 0.0432, 0.072 and 0.108 mg/l of Ictacrune, respectively. As well as, a total number of 80 Oreochromis niloticus fish with an average body weight (25± 2g) were divided into 8 groups (10 fish/group). First group was left as a control, while, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th were exposed to 0.036, 0.072, 0.108, 0.144, 0.180, 0.216 and 0.252 mg/l of Ictacrune, respectively. These concentrations were selected as trails for determination of 96 hrs-LC_{50} of Ictacrune. Fish were observed at 12 hrs interval up to 96 hrs. The dead fish was removed immediately up on discovery. Mortalities and survival time were recorded, and then LC_{50} was calculated according to equation[16].

Effect of sub-chronic toxicity of Ictacrune in Cyprinus carpio and Oreochromis niloticus fishes.

A total number of 90 Cyprinus carpio, with average body weight 30±5 g and 90 fish of Oreochromis niloticus with average body weight 25±2 g were divided into 3 groups. Each group has 3 replicates (10 fishes/replicate). Ictacrune was prepared to produce the required concentrations (0.036 mg/l (0.25 LC_{50}) and 0.072 mg/l (0.50 LC_{50}), where, LC_{50} of Ictacrune = 0.144 mg/l for Oreochromis niloticus fish and 0.006 mg/l (0.25 LC_{50}) and 0.013 mg/l (0.50 LC_{50}), where, LC_{50} of Ictacrune = 0.0.026 mg/l for Cyprinus carpio.

Blood samples were collected in eppendorf tubes from the caudal vein after15, 30 and 45 days of exposure of Oreochromis niloticus and Cyprinus carpio fishes to Ictacrune pesticide and one group control. The collected samples centrifuged at 3000 rpm for 15 minutes then the supernatant serum obtained by using micropipette and stored at 4˚C till determination of glucose, total protein, albumin, total lipid, urea, uric acid concentrations and AST and ALT activities

Determination of serum glucose level was measured according to GOD-PAP enzymatic colorimetric method[17], (Spectrum Kit).The serum total proteins and albumin were measured colorimetrically according to the method described by[18,19], respectively. Kidney function tests (determination of urea and uric acid). While, The concentration of serum urea and uric acid were measured enzymatically according to[20]. Liver function tests (Determination of serum ALT and AST): The serum activity of AST and ALT were determined colorimetrically using readily made kits according to the method described by[21]. The serum total lipids were according to[22].

Histopathological Studies

Samples from liver, muscles and gills were collected at 15, 30 and 45 days from exposure to Ictacrune for histopathological examinations and samples control.

Immediately after dissection of the studied fish, parts of liver, muscles and gills were carefully removed and fixed in 10 % formalin at 40C, for 48 hours then the samples were dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick using Euromex Holland Microtome, then stained according to Harris Hematoxylin and Eosin method. Finally, the sections were examined microscopically and photographed by a microscopic camera according to[23,24].

Statistical Analysis

SPSS software - Ver.16 statistical package was used for statistical analyses. The statistical analysis used to detect the significant differences between the groups for control and Ictacrune pesticide treated one by using one way-ANOVA. All statistical analyses based on at $P<0.05$ significance level.

RESULTS

Oreochromis niloticus and Cyprinus carpio were subjected to different concentrations (0.036-0.252 mg/l) and (0.0072-0.108 mg/l), respectively, of Ictacrune pesticide for 96 hours to detect the lethal concentration (LC50) values of Ictacrune pesticide. These values were 0.144 and 0.02662 mg/l Ictacrune forthe two studied fishes (Oreochromis niloticus and Cyprinus carpio), respectively, (Tables 1,2).

Biochemical analysis

(Figures 1-8) showed the effects of Ictacrune on biochemical analysis of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal concentrations(0.036-0.252 mg/l) and (0.0072-0.108 mg/l) of 0.25 and 0.50 LC50 respectively, during different exposure periods and control group. The results showed that, Cyprinus carpio, recorded the highest values of AST, ALT, urea and uric acid (39.24 IU/ml, 17.30 IU/ml, 45.50 mg/dl and 6.50 mg/dl), respectively, for the control group, while, Oreochromis niloticus showed the highest values of lipid, glucose, protein and albumin (870, 88.50, 3.60 and 1.90 mg/dl), respectively, for the control group. On the other hand, our results indicated that, there were significantly ($P<0.05$) increased in AST, ALT, urea and uric acid values of both Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal concentrations of Ictacrune compared to the control group by increasing the exposure periods.

The values of AST, ALT, urea and uric acid were significantly ($P<0.05$) increased for Oreochromis niloticus exposed to 0.25 LC50 and 0.50 LC50of Ictacrune, from 33.24 IU/ml, 15.30 IU/ml, 25.0 mg/dl and 4.0 mg/dl, respectively, in control group to 94.40 IU/ml, 51.70 IU/ml, 162.90 mg/dl and 21.90 mg/dl; and 112.35 IU/ml, 67.50 IU/ml, 194.30 mg/dl and 24.60 mg/dl, respectively, after 45 days of Ictacrune exposure. While as this values were significantly ($P<0.05$) increased for Cyprinus carpio, exposed to 0.25 LC50 and 0.50 LC50 from 39.0 IU/ml, 17.30 IU/ml, 45.50 mg/dl and 6.50 mg/dl, respectively, in control group to 107.40 IU/ml, 87.50 IU/ml, 123.10 mg/dl and 25.50 mg/dl; and 132.35 IU/ml, 92.75 IU/ml, 142.80 mg/dl and 30.90 mg/dl, respectively, after 45 days of Ictacrune exposure. The results showed that, AST, ALT, urea and uric acid values increased with increasing in the exposure period and concentration of Ictacrune pesticide in Oreochromis niloticus and Cyprinus carpio.

The values of lipids, glucose, protein and albumin were significantly ($P<0.05$) decreased in Oreochromis niloticus exposed to 0.25 LC50 and 0.50 LC50 from 870, 88.50, 3.60 and 1.9 mg/dl, respectively, in control group to 285 mg/dl, 55.90 mg/dl, 2.45 g/dl and 0.90 g/dl; and 195 mg/dl, 44.55 mg/dl, 2.15 g/dl and 0.80 g/dl, respectively, after 45 days of Ictacrunee exposure. While, as this values were significantly ($P<0.05$) decreased for Cyprinus carpio, exposed to 0.25 LC50 and 0.50 LC50 from 650.6 mg/dl, 85.50 mg/dl, 3.20 g/dl and 1.50 g/dl, respectively, in control group to 255.30 mg/dl, 42.10 mg/dl, 2.15 g/dl, and 0.95 g/dl; and 158.15 mg/dl, 31.60 mg/dl, 2.15 g/dl and 0.85 g/dl, respectively, after 45 days of exposure. The results showed that, lipid, glucose, T. protein and albumin concentrations decreased with increasing in the exposure period of Ictacrune pesticide in Oreochromis niloticus and Cyprinus carpio.

Histopathological changes

Many histopathological alterations observed in liver, muscles and gills organs due to pesticide exposure.

Liver

As shown in (Figures 9a,b), in the control group of O. niloticus and C. carpio, respectively, the liver showed, hepatic cells are near normal (H). The normal liver almost uniform in appearance. It is soft in consist and uniformly dark red in color. It is enclosed within a fibro connective tissue capsule. The alterations in the liver (Figure 10a-h) included: degeneration (D), necrosis (N), and piknosis of the nucleus of hepatocytes. Congestion (C) in blood sinusoid was also observed. Dilation (Di) edema (E) and fibrosis (Fb) in blood vessels were noticed in the liver.

Muscles

As shown in (Figures 11a,b), in the control group of O. niloticus and C. carpio, respectively, muscles showed normal Myomeres (M). The alterations in the muscles (Figure 12a-h) included: degeneration (D), necrosis (N) and edema (E) in muscle bundles.

Gills

As shown in (Figures 13a,b), in the control group of O. niloticus and C. carpio, respectively, the gills

showed Primary lamellae (Pl) and Secondary Lamellae (Sl) of Oreochromis niloticus and Cyprinus carpio fish, respectively. The alterations in gills were showed in (Figure 14a - h) which included: Hemorrhage (Hr) and necrosis (N) in primary lamellae. Necrosis (N), curling (Cr), separation (S), hemorrhage (Hr), bumped base (B) and hyperplasia (H) in epithelial cells of secondary lamellae.

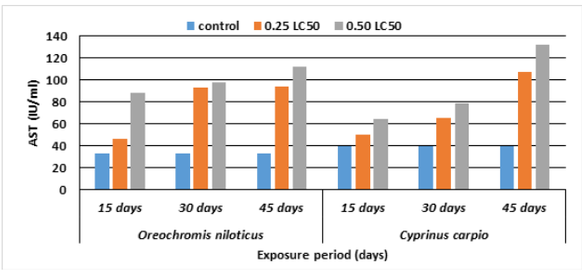

Fig. 1: Effect of Ictacrune (profenofos) on AST activities of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

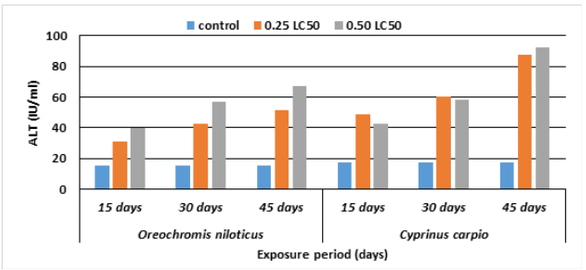

Fig. 2: Effect of Ictacrune (profenofos) on ALT activities of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

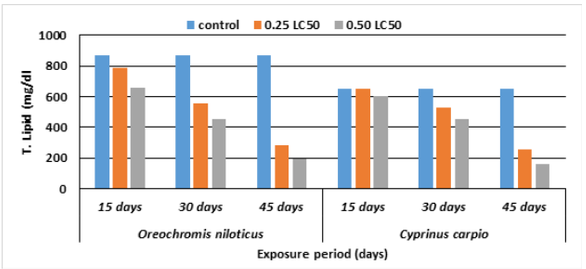

Fig. 3: Effect of Ictacrune (profenofos) on T. lipid concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

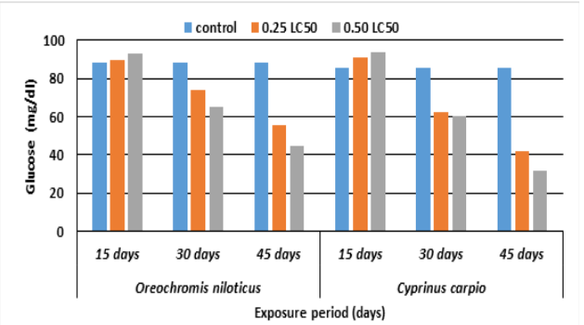

Fig. 4: Effect of Ictacrune (profenofos) on glucose concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

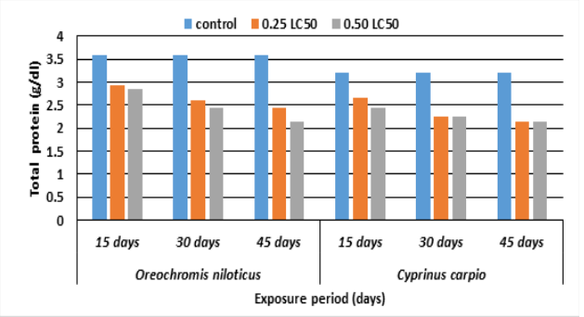

Fig. 5: Effect of Ictacrune (profenofos) on total protein concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

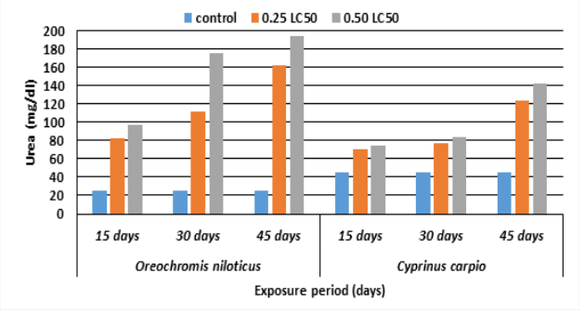

Fig. 6: Effect of Ictacrune (profenofos) on urea concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

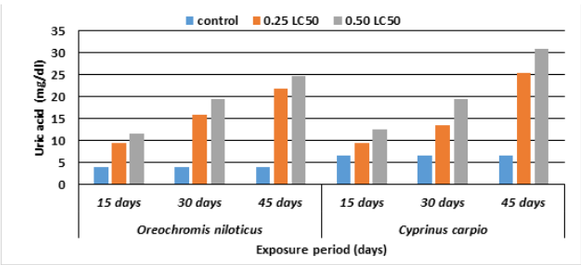

Fig. 7: Effect of Ictacrune (profenofos) on uric acid concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

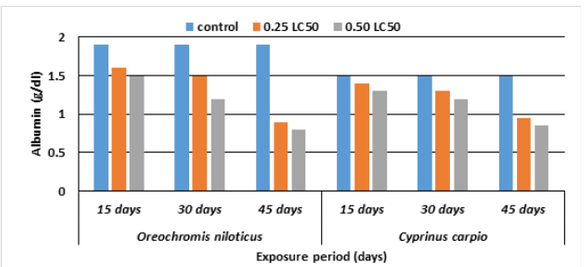

Fig. 8: Effect of Ictacrune (profenofos) on albumin concentrations of Oreochromis niloticus and Cyprinus carpio, exposed to different sub-lethal (0.25 LC50 and 0.50 LC50) concentrations during different exposure periods.

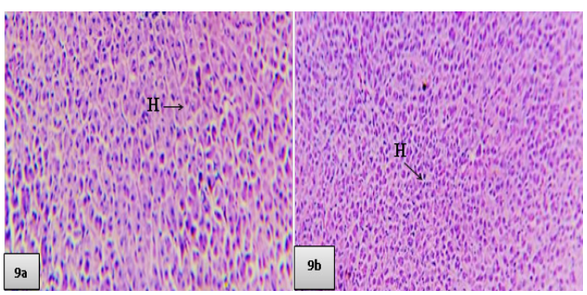

Fig. (9 a,b): Liver of control O. niloticus and C. carpio, respectively showing hepatic cells are near normal (H).

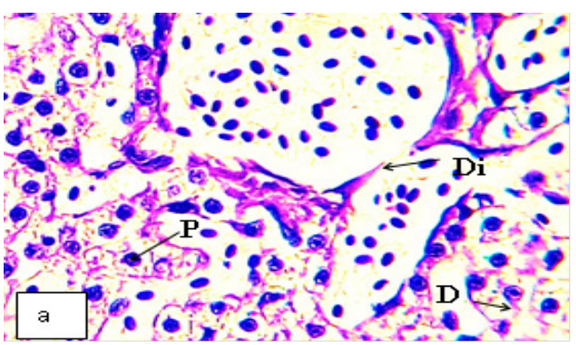

Fig. (10a): Liver section of Cyprinus carpio exposed to0.25 LC50 of Ictacrune for 15 days showed: Dilation (Di) in blood vessels, degeneration (D) and nuclear piknosis (P) in hepatic cells.

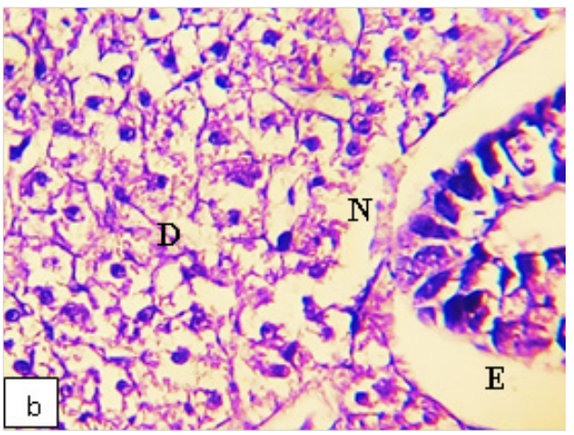

Fig. (10b): Liver section of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 15 days showed: Edema (E) around blood vessels, degeneration (D) & necrosis (N) in hepatic cells.

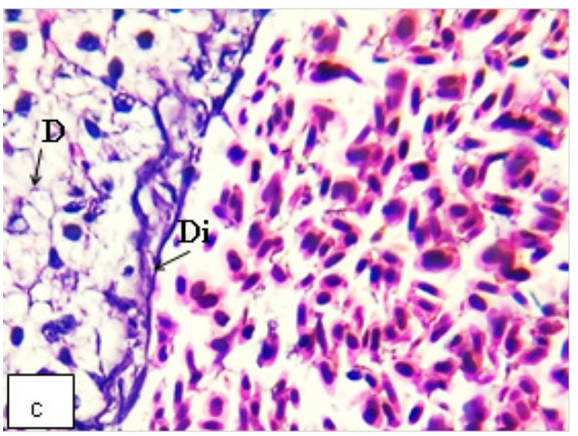

Fig. (10c): Liver section of Cyprinus carpio exposed to 0.25 LC50 of Ictacrune for 45 days showed: Dilation (Di) in blood vessels, degeneration (D) in hepatic cells.

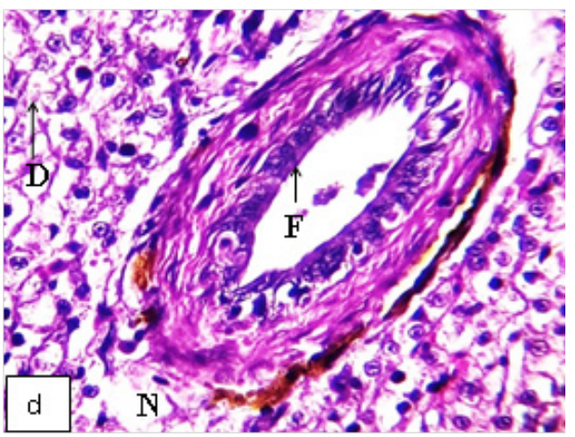

Fig. (10d): Liver section of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 45 days showed: Fibrosis (F) blood vessels wall, degeneration (D) and Necrosis (N) in hepatic cells.

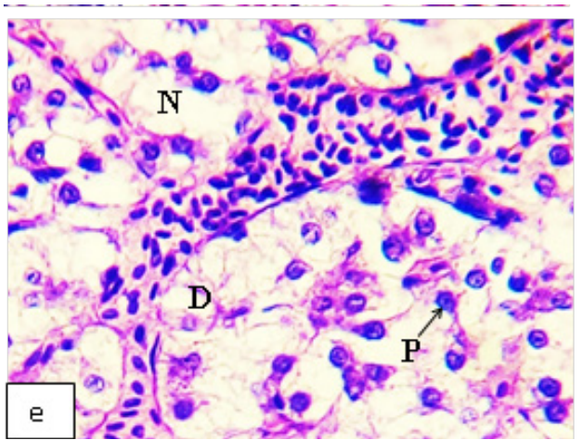

Fig. (10e): Liver section of Oreochromis niloticuse exposed to 0.250 LC50 of Ictacrune for 15 days showed: Degeneration (D), necrosis (N), and nuclear piknosis (P) in hepatic cells.

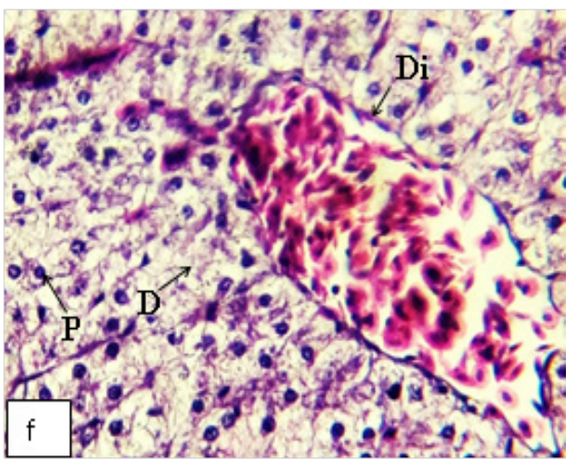

Fig. (10f): Liver section of Oreochromis niloticus exposed to 0.50 LC50 of Ictacrune for 15 days showed: Dilation (Di) in blood vessels, degeneration (D) & nuclear piknosis (P) in hepatic cells.

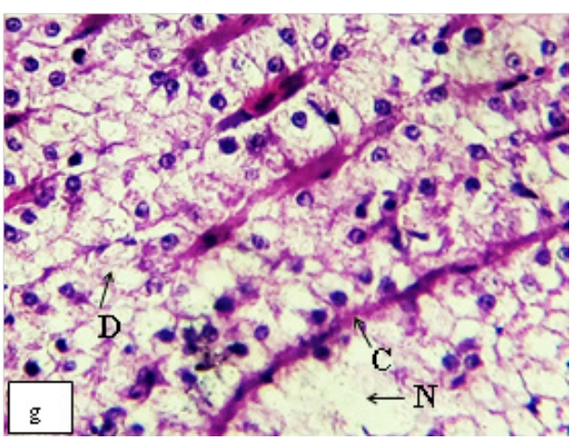

Fig. (10g): Liver section of Oreochromis niloticus exposed to 0.250 LC50 of Ictacrune for 45 days showed: Degeneration (D), necrosis (N)) in hepatic cells, congesion (C) in blood sinusoid.

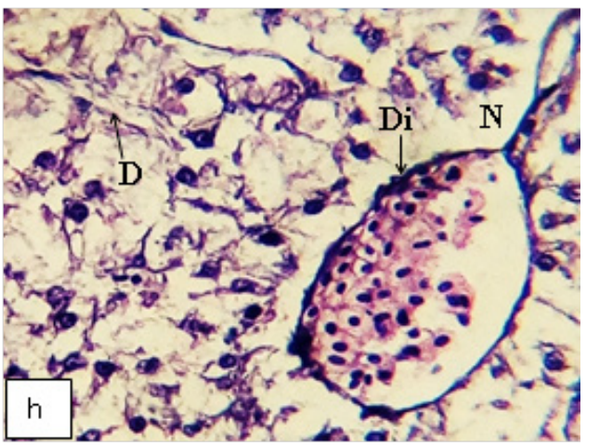

Fig. (10h): Liver section of Oreochromis niloticus exposed to 0.50 LC50 of Ictacrune for 45 days showed: Dilation (Di) in blood vessels, degeneration (D) & necrosis (N) in hepatic cells.

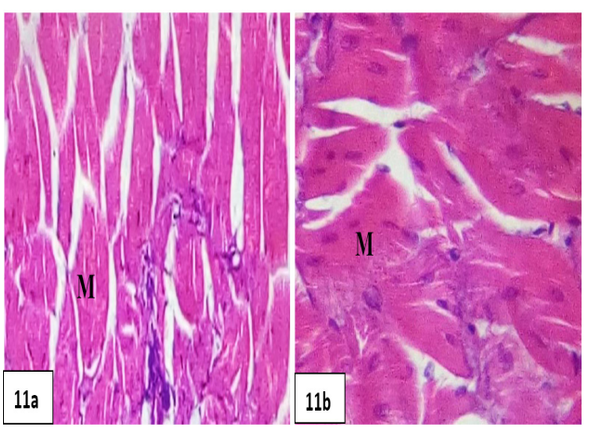

Fig. (11 a,b): Muscles of control O. niloticus and C. carpio, respectively showing myomeres (M).

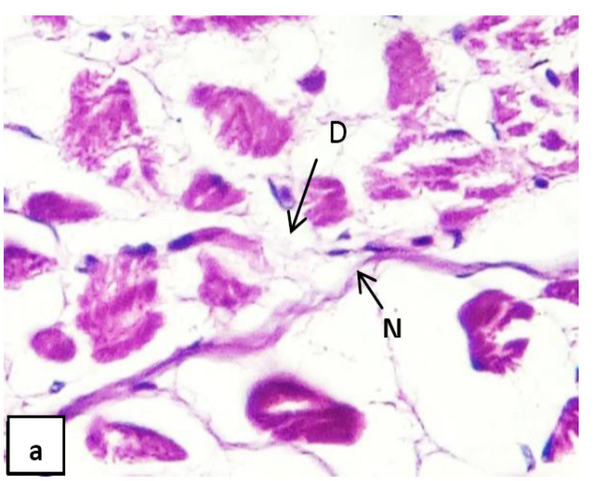

Fig. (12a): V.S. in muscles of Cyprinus carpio exposed to 0.250 LC50 of Ictacrune for 15 days showed: Degeneration (D) and necrosis (N) in muscles bundles.

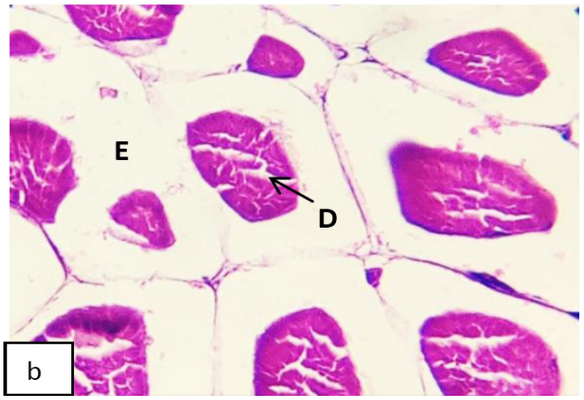

Fig. (12b): V.S. in muscles of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 15 days showed: Edema (E) and degeneration (D) in muscles bundles.

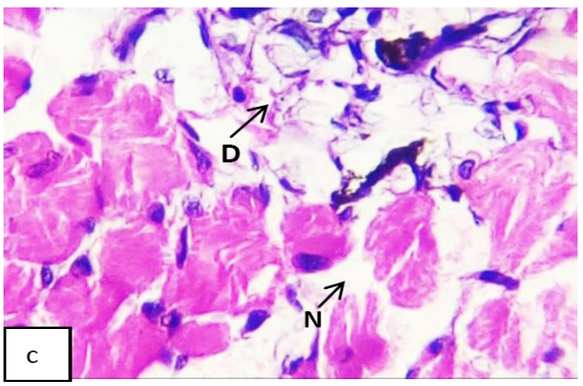

Fig. (12c): V.S. in muscles of Cyprinus carpio exposed to 0.250 LC50 of Ictacrune for 45 days showed: Degeneration (D) and necrosis (N) in muscles bundles.

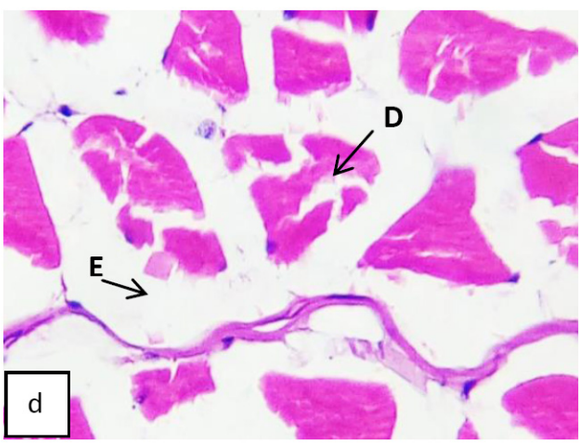

Fig. (12d): V.S. in muscles of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 45 days showed: Degeneration (D) and edema (E) in muscles bundles.

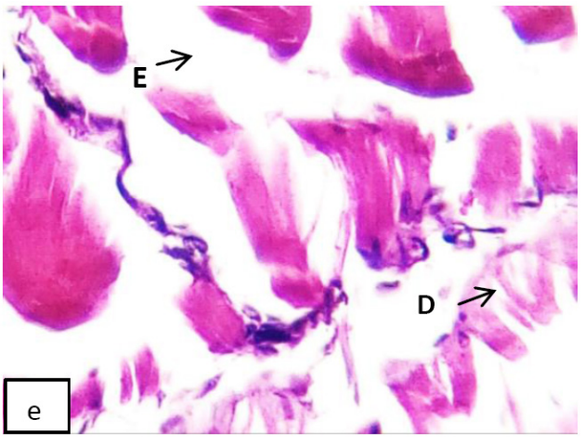

Fig. (12e): V.S. in muscles of Oreochromis niloticus exposed to 0.25 LC50 of Ictacrune for 15 days showed: Degeneration (D) and edema (E) in muscles bundles.

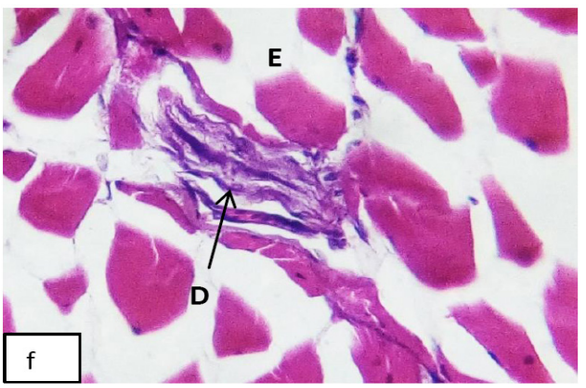

Fig. (12f): V.S. in muscles of Oreochromis niloticus exposed to 0.50 LC50 of Ictacrune for 15 days showed: Degeneration (D) and edema (E) in muscles bundles.

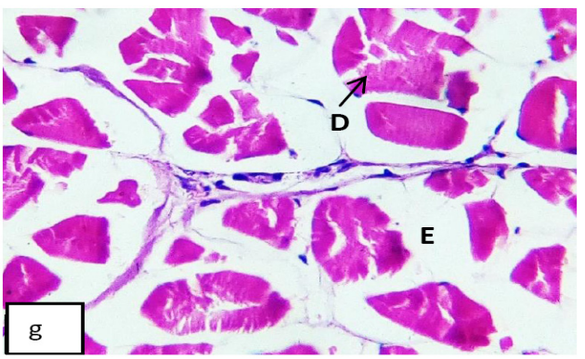

Fig. (12g): V.S. in muscles of Oreochromis niloticus exposed to 0.25 LC50 of Ictacrune for 45 days showed: Degeneration (D) and edema (E) in muscles bundles.

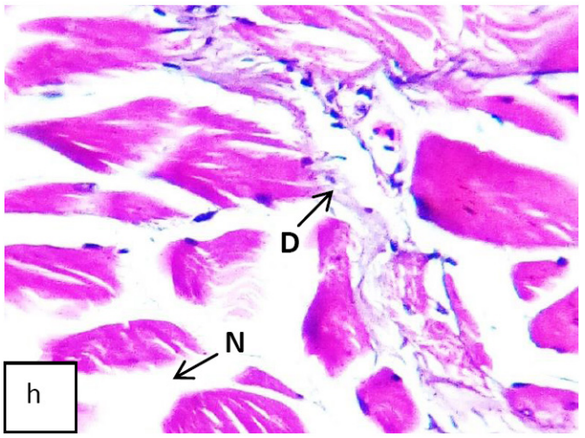

Fig. (12h): V.S. in muscles of Oreochromis niloticus exposed to 0.50 LC50 of Ictacrune for 45 days showed: Degeneration (D) and necrosis (N) in muscles bundles.

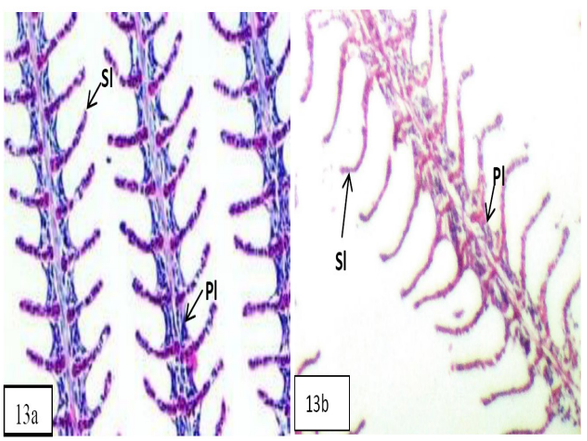

Fig. (13 a,b): Gills of control O. niloticus and C.carpio, respectively showing primary lamellae (Pl) and secondary lamellae (Sl).

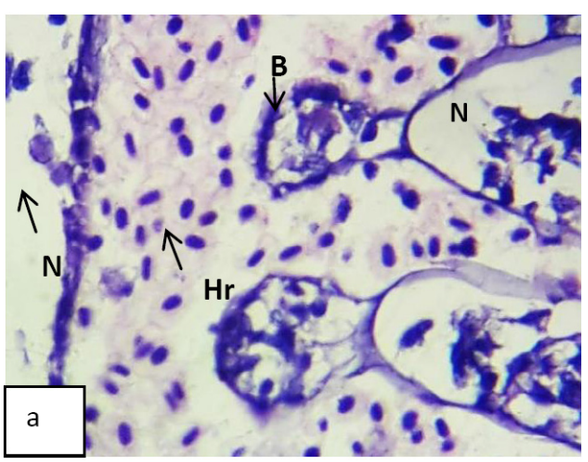

Fig. (14a): L.S. in gills of Cyprinus carpio exposed to 0.25 LC50 of Ictacrune for 15 days showed: Necrosis (N) & hemorrahage (Hr) in primary lamellae, Necrosis (N) & bumped base (B) in secondary lamellae.

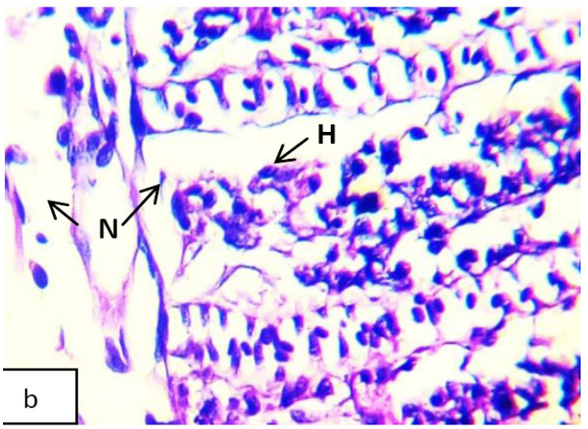

Fig. (14b): L.S. in gills of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 15 days showed: Necrosis (N) in primary and secondary lamellae, hyperplasia (H) in epithelial cells of secondary lamellae.

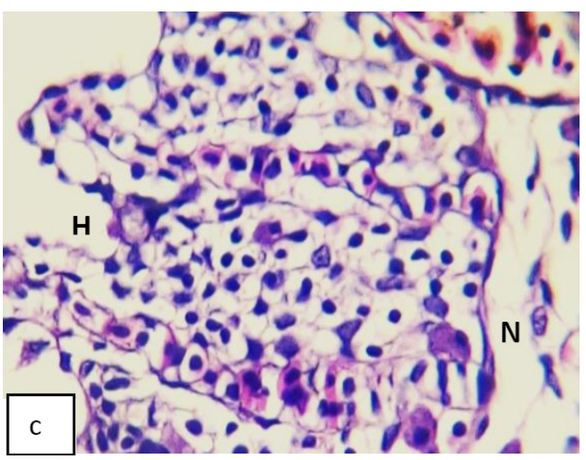

Fig.(14c): L.S.in gills of Cyprinus carpio exposed to 0.250 LC50 of Ictacrune for 45 days showed: Necrosis (N) in primary lamellae, hyperplasia (H) in epithelial cells of secondary lamellae.

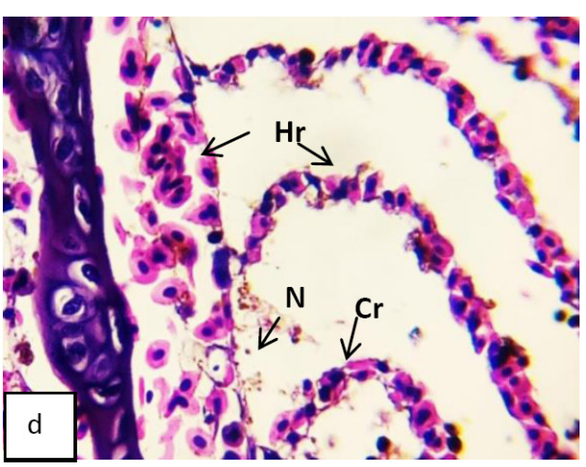

Fig. (14d): L.S. in gills of Cyprinus carpio exposed to 0.50 LC50 of Ictacrune for 45 days showed: Hemorrahage (Hr) in primary and secondary lamellae with curling (Cr) and necrosis (N).

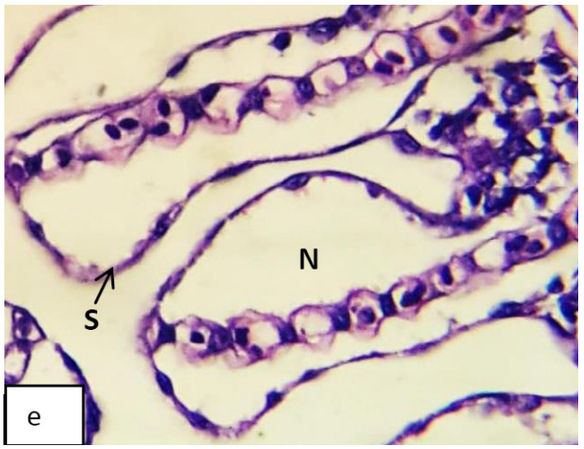

Fig. (14e): L.S.in gills of Oreochromis niloticus exposed to 0.25 LC50 of Ictacrune for 15 days showed: Necrosis (N) and separation (S) in epithelial cells of secondary lamellae.

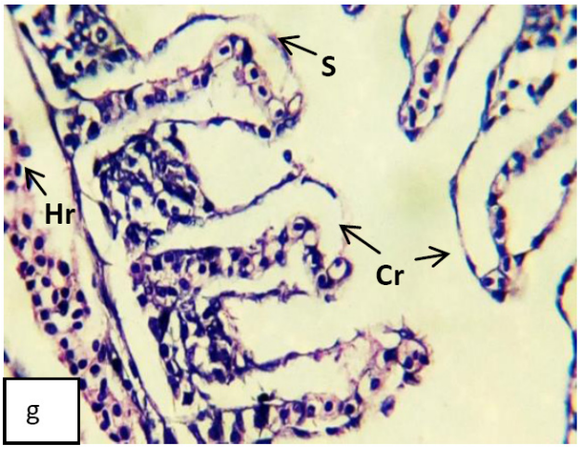

Fig. (14g): L.S.in gills of Oreochromis niloticus exposed to 0.25 LC50 of Ictacrune for 45 days showed: Separation (S) and curling (Cr) in secondary lamellae, necrosis (N) and hemorrahage (Hr) in primary lamellae.

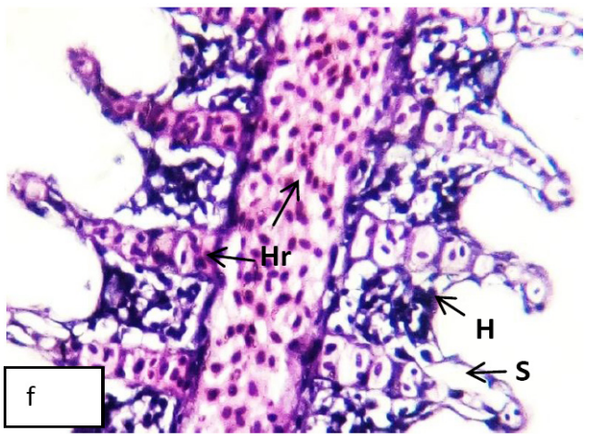

Fig. (14f): L.S.in gills of Oreochromis niloticu sexposed to 0.50 LC50 of Ictacrune for 15 days showed: Hemorrahage (Hr) in primary and secondary lamellae, hyperplasia (H) and separation (S) in epithelial cells of secondary lamellae.

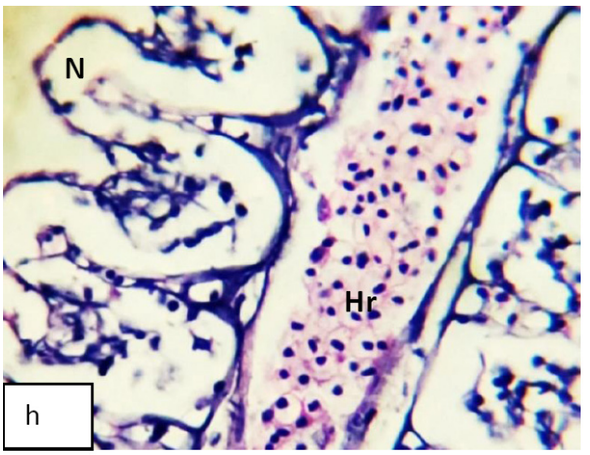

Fig. (14h): L.S.in gills of Oreochromis niloticus exposed to 0.50 LC50 of Ictacrune for 45 days showed: Hemorrahage (Hr) in primary lamellae and necrosis (N) in secondary lamellae.

Table 1: Preliminary trials for zero and hundred % mortalities in O.niloticus exposed to different concentrations of Ictacruneand actual estimation of 96 hrs-LC50.

Concentrations mg/l	Mortality number during 96 hrs				Total mortality number	Total mortality %	dead fish at 96 hrs	a	b	axb
	1st day	2nd day	3rd day	4th day						
0.036	0	0	0	0	0	0	0	0	0	0
0.072	0	0	1	1	2	20	2	0.036	1	0.036
0.108	0	0	1	1	2	20	2	0.036	2	0.072
0.144	2	1	0	1	4	40	4	0.036	3	0.108
0.180	3	3	1	1	8	80	8	0.036	6	0.216
0.216	5	2	1	1	9	90	9	0.036	8.5	0.306
0.252	5	2	2	1	10	100	10	0.036	9.5	0.342
					Σ axb					1.08

a= constant factor between two successive doses.
Σ axb= sum of axb.
b= the mean of dead fish in each group.
N= Number of fish in each groups.
96 hrs-LC50= Highest dose - Σ axb/n
= 0.252 – 1.08/10 = 0.144 mg/l of Ictacrune.

Table 2: Preliminary trials for zero and hundred % mortalities in Cyprinus carpio exposed to different concentrations of Ictacrune and actual estimation of 96 hrs-LC50.

Concentrations mg/l	Mortality number during 96 hrs				Total mortality number	Total mortality %	dead fish at 96 hrs	a	b	axb
	1st day	2nd day	3rd day	4th day						
0.0072	0	0	0	0	0	0	0	0	0	0
0.0144	0	1	1	1	3	30	4	0.0072	2	0.0144
0.0216	1	1	2	1	5	50	6	0.0072	5	0.036
0.0288	2	2	2	1	7	70	7	0.0072	6.5	0.0468
0.0360	3	2	1	1	7	70	8	0.0072	7.5	0.054
0.0432	4	1	1	1	8	80	9	0.0072	8.5	0.0612
0.0720	5	2	1	1	19	90	9	0.0288	9	0.2592
0.1080	5	3	1	1	10	100	10	0.036	9.5	0.342
					Σ axb					1.08

a= constant factor between two successive doses.
Σ axb= sum of axb.
b= the mean of dead fish in each group.
N= Number of fish in each groups.
96 hrs-LC50= Highest dose - Σ axb/n
= 0.108 – 0.8136/10 = 0.02664 mg/l of Ictacrune

DISCUSSION

Assessment of the biochemical parameters in fish and other organisms is broadly used to monitor the water contaminants and their effect on health conditions. Moreover, biochemical parameters have been used as biomarkers for toxicant exposure and resultant effects in fish[25].

The present study showed that, total protein and albumin dropped as the concentration of the toxicant increased. This finding is comparable to the report on Oreochromis niloticus exposed to Pendimethalin herbicide that showed a decrease in total protein and albumins[26]. The decrease was ascribed to the inhibitory effect of pesticide on the protein synthesis. Also, the reduction in total protein concentrations was attributed to the damaging effects of pesticide on liver cells, as was confirmed by the increase in the activities of serum AST and ALT in the study. Also[27], reported a lower in total protein and albumin of Oreochromis mossambicus with elevation in concentration of Pyrazosulfuron–ethyl toxicant. While, studied record increase in total protein and albumins with increase in pesticides concentration and the long exposure period[28]. The reduction of protein (hypoproteinaemia) in this study suggested an increase in proteolytic activities and possible utilization of their products for metabolic purpose to overcome stress. While,the reduction in serum albumin (hypoalbuminaemia) can be attributed to the liver damage. In the present study, serum glucose values showed declining trend at both concentrations of Ictacrune in the exposed fish. The decrease in glucose level might be due to the increase of glucose oxidation to meet the higher energy demands during exposure. Similar findings have been suggested by[29].

The elevation of urea level may be attributed to gill dysfunction[30]. The kidney damage may result in reduced renal blood flow with reduction in glomerular filtration rate, resulting in azotemia characterized by increase in blood urea[31]. Also, the increase the uric acid indicated several disturbances in kidney[32]. On the other hand, in our study, blood analysis revealed a significant increase in uric acid of fish, this elevation might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism[33]. The elevation of serum AST and ALT may be due to liver dysfunction. In addition, the increase of serum AST and ALT may be attributed to the hepatocellular damage or cellular degradation, perhaps in liver, heart or muscle[34]. Also, the increase of blood enzymatic activity may be due to increased enzymes synthesis[35].

Similar increased activities of AST and ALT were reported by other authors. Increase in the AST and ALT concentrations was observed in Oreochromis niloticus exposed to stomp Pendimethalin herbicide, the increase in these parameters indicated stressed based tissue impairment[26]. According to[36], the change in activities of transaminases indicates amplified transamination processes and an increase in transamination occurs with amino acid input into the TCA cycle to cope with the energy crisis during pesticide stress[37]. The sub-lethal concentrations of Vestaline (Pendimethalin) herbicide are harmful directly or indirectly to Clarias gariepinus juveniles because changes were observed in total protein, albumin, alanine amino transferase and aspartate amino transferase and were also dose dependent. The results of serum biochemistry parameter values indicated that the exposed fish were faced serious metabolic crisis.

Histopathological changes

Histopathological studies are very important in showing alterations in target organs such as liver, muscles and gills of the two studied fishes as results of exposure to Ictacrune (profenofos) pesticides.

Liver of fish is responsible for the digestion,filtration and stored of glucose.Also, the liver produces many

enzymes that stored in the gall bladder .These enzymes assistance in the breakdown of food. Generally, the liver is considered as the principal organ of detoxification in vertebrates and especially in fish. Also, fish liver is a good index of aquatic environmental pollution;it is considered the most important liver function tocleanly of any poisons or pollutants in the blood coming of the intestine.

The muscular system constitutes the biggest portion of the toleost body. The basic function in overall body is movement, coordinated locomotion of skeletal elements, pumping of blood and peristaltic constriction of visceral organ and their associated structures.

Gills are responsible for respiration, excretion, osmoregulation, ion regulation and acid-base balance. Since the gill represents the respiratory and osmoregulatory organ of fish, the cellular damage induced by the continuous entry of the toxicants might retard the respiratory function of organ by reducing its surface area.

The present study revealed that the exposure of Cyprinus carpio and Oreochromis niloticus fishes to 0.25 LC50 & 0.50 LC50 of Ictacrune pesticide for 45 days showed many alterations in histological characteristics of liver, muscle and gills organs. These alterations differ in their degree according to dose and the period of exposure. Our finding goes in parallel with that obtained by[34,38,39,40,41,42]who revealed these alterations in same fishes and same organs after exposure to heavy metals and pesticide.

In conclusion, there is a direct relationship between pesticide exposure and histopathological alterations that observed in studied organs.

CONFLICTS OF INTEREST

There are no conflicts of interest

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الملخص العربى

الاستجابات البيوكيميائية والنسيجية المرضية لأسماك البلطي النيلي والمبروك العادى للتعرض شبه المميت لمبيد آكتاكرون

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المعهد القومى لعلوم البحار والمصايد

يهدف البحث الى تأثير المبيد الحشرى اكتاكرون على أسماك البلطى النيلى وأسماك المبروك العادى حيث تم التعرض الأسماك لتركيزات مختلفة من المادة الفعالة للمبيد كالتالى (٠,٣٦ – ٠,٢٥٢ مجم/لتر) للبلطى النيلى وبتركيزات (٠,٠٠٧٢ – ٠,١٠٨ مجم/لتر) لأسماك المبروك العادى لمدة ٩٦ ساعة لتحديد قيمة التركيز المميت (LC_{50}) للأسماك تحت الدراسة وكانت ٠,١٤٤ مجم/لتر لأسماك البلطى النيلى، ٠,٠٢٦٦٢ مجم/لتر لأسماك المبروك العادى.
تم عمل مجموعتين كل مجموعة تحتوى على ثلاثة أحواض وبكل حوض عشرة أسماك متجانسة الاوزان تقريبا وتم تعرض مجموعات الأسماك الى ٠,٢٥LC_{50} & ٠,٥٠LC_{50} (ربع ونصف) من الجرعة المميتة لمدة ٤٥ يوما ومجموعة أخرى تحتوى على ثلاثة أحواض بكل حوض عشرة أسماك وهى مجموعة الكنترول بدون تعرض لمبيد الاكتاكرون.
لوحظ إنخفاض قيم الدهون، الجلوكوز، البروتين الكلى والألبيومين بشكل ملحوظ $P<0,05$ بالنسبة للمبروك العادى حيث كانت فى مجموعة الكنترول (٦٥٠,٦, ٨٥٥,٠ مجم/ديسيلتر، ٣,٢ and ١,٥ جرام/ديسيلتر) إنخفضت الى (٢٥٥,٣ مجم/ديسيلتر، ٤٢,١ مجم/ديسيلتر، ٢,١٥ جرام/ديسيلتر ، ٠,٩٥ جرام/ديسيلتر) فى تركيز ٠,٥٠LC_{50} بينما إنخفضت الى (١٥٨,١٥ مجم/ديسيلتر، ٣١,٦ مجم/ديسيلتر، ٢,١٥ جرام/ديسيلتر، ٠,٨٥ جرام/ديسيلتر) فى تركيز ٠,٢٥LC_{50}. بينما فى أسماك البلطى النيلى إنخفضت القيم من (٨٧٠ مجم/ديسيلتر، ٨٨,٥ مجم/ديسيلتر، ٣,٦ جرام/ديسيلتر، ١,٩ جرام/ديسيلتر) فى الكنترول الى (٢٨٥ مجم/ديسيلتر، ٥٥,٩ مجم/ديسيلتر، ٢,٤٥ جرام/ديسيلتر، ٠,٩ جرام/ديسيلتر) فى تركيز ٠,٢٥LC_{50} وإنخفض الى (١٩٥ مجم/ديسيلتر، ٤٤,٥٥ مجم/ديسيلتر، ٢,١٥ جرام/ديسيلتر، ٠,٨ جرام/ديسيلتر) فى تركيز ٠,٥٠LC_{50} على الترتيب.
تم تسجيل زيادة قيم كلا من ALT, AST واليوريا واليورك اسيد بشكل كبير($P<0,05$) حيث كانت بمجموعة الكنترول (١٥ ml/IU ، ٣٣,٢٤ ml/IU، ٢٥ مجم/ديسيلتر، ٤ مجم/ديسيلتر) وزادت القيم الى (٥١,٧ ml/IU، ٩٤,٤ ml/IU، ١٦٢,٩ مجم/ديسيلتر، ٢١,٩ مجم/ديسيلتر) فى تركيز ٠,٢٥LC_{50} والى (٦٧,٥ ml/IU، ml/IU ١١٢,٣٥، ١٩٤,٣ مجم/ديسيلتر ، ٢٤,٦ مجم/ديسيلتر) فى تركيز ٠,٥٠LC_{50} بعد التعرض لمدة ٤٥ يوما لمبيد اكتاكرون لأسماك البلطى النيلى. وزادت هذة القيم فى أسماك المبروك العادى من (١٧,٣ ml/IU، ٣٩,٢٤ ml/IU، ٤٥,٥ مجم/ديسيلتر، ٦,٥ مجم/ديسيلتر) فى مجموعة الكنترول الى (٨٧,٥ ml/IU، ١٠٧,٤ ml/IU، ١٢٣,١ مجم/ديسيلتر، ٢٥,٥ مجم/ديسيلتر) بالنسبة لتركيز ٠,٢٥LC_{50} والى (٩٢,٧٥ ml/IU، ١٣٢,٣٥ ml/IU، ١٤٢,٨ مجم/ديسيلتر، ٣٠,٩٩ مجم/ديسيلتر فى تركيز ٠,٥٠LC_{50} بعد التعرض لمدة ٤٥ يوما من المبيد الحشرى اكتاكرون.
بالفحص الهستولوجى للكبد، العضلات والخياشيم والتى تم الحصول عليها من أسماك البلطى النيلى وأسماك المبروك العادى كل ١٥ يوما من التجربة لوحظ وجود تغيرات هستولوجية وحالات مرضية حادة تزداد بزيادة تركيز المبيد وفترة تعرض الأسماك للمبيد.