Zagazig J. Agric. Res., Vol. 45 No. (1) 2018 165-175

Plant Protection and Pathology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

TOXICOLOGICAL AND HISTOPATHOLOGICAL EFFECTS OF DIAZINON AND SODIUM BENZOATE ON THE NILE TILAPIA FISH, O*reochromis niloticus* L.

Refaat M. Abd El-Sameaa*, A.A. Aioub, A.E.A. Elsobky and M.A. Hendawy

Plant Prot. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 30/10/2017 ; Accepted: 24/12/2017

ABSTRACT: The acute and subchronic toxicity of diazinon and sodium benzoate to the Nile tilapia fish, O*reochromis niloticus* L. 60 g weight were studied. The obtained results showed that the LC_{50} after 24, 48, 72 and 96 hr., post treatment to the insecticide diazinon were 8.377, 6.852, 6.200 and 5.679 mg/l, respectively. Exposed fish to sublethal concentration of diazinon (0.56 mg/l) and sodium benzoate at 150 mg/l for 21days caused some biochemical and histopathological changes in some blood components and organs. Diazinon increased alanine amino transferase (ALT), aspartate amino transferase (AST), glucose, total protein, albumin, creatinine and cholesterol activities, while acetylcholinesterase (AchE) and uric acid were decreased. Sodium benzoate caused an increase of ALT, AST, glucose, total protein, albumin, creatinine, uric acid and cholesterol activities. The histopathological studies showed degeneration of hepatocytes, congestion of blood vessels and revealed degenerative changes on some organs and cystic dilatation of some renal tubules together with congestion of renal blood vessels and mild to moderate lymphocytic infiltration were detected compared with control fish (untreated)

Key words: *Oreochromis niloticus*, Nile tilapia fish, diazinon, sodium benzoate, toxicological and histopathological effects.

INTRODUCTION

Over the last 30 years, the agrochemical industry has turned from organchlorines to other neurotoxic organophosphate and carbamate pesticides. Use of these toxic nerve poisons, however, continues to grow. In California, about 17 million pounds of organophosphate and carbamate pesticides are applied annually in urban and agricultural setting (**EPA, 2000**). The aquatic environments continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities.

Pesticides are one of the major classes of toxic substances used for management of pests in agricultural lands and control of insect vectors of human diseases **(Begum, 2004).** The runoff from treated areas enters the rivers and aquaculture ponds are likely to be contaminated by pesticides. Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes the most sensitive and relatively early events of pollutants and to delineate mechanisms of pollutant action and possibly ways to mitigate adverse effects. Sodium benzoate remains widely applicative as preservatives in a number of products consumed by humans **(Winkler *et al.*, 2006; Abd El-Rahman, 2009)**. Fish, generally appreciated as one of the health highest sources of protein, have amino acid composition that are higher in cysteine than most other sources of protein. In the 1900, tilapia species were introduced into most of the world from their original ranges in Africa and the Middle East. They are now grown in commercial farming operations in almost 100 countries. Tilapia is likely to be the most

* **Corresponding author: Tel. :** +201099283214
E-mail address: refaatelshikh@yahoo.com

important aquaculture species of the 21st century. **FAO (1997)** estimated that world aquaculture production of tilapia had reached 659000 ton in 1995. *O. niloticus* was chosen as a model organism due to its high sensitivity in detecting potential adverse effects of chemicals (**Uner *et al.*, 2006)**. Moreover, the change in plasma glutamate-oxaloacetate transaminase (GOT) and plasma glutamate pyruvate transaminase (GPT) activities can also indicate the impacts of water pollution on fish (**Haider and Rauf, 2014)**. Therefore, the present work aimed to study the acute and subchronic toxicity of diazinin and sodium benzoate to the Nile tilapia fish. In addition to the effects of tested chemicals on some biochemical and histopathological parameters of the Nile tilapia fish after 21 days of exposure.

MATERIALS AND METHODS

Tested Animal

The Nile tilapia fish, *O. niloticus* of average body weight (60 ± 2 g) and total body length (13.5 ±0.5 cm) were a kind gift of the Arabian Fish Breeding Company in Abbasa, Sharkia Governorate, Egypt. Fish were transferred alive to the Laboratory of Pesticide Toxicology, Faculty of Agriculture, Zagazig University within 2 hr., in plastic bags supplied with sufficient oxygen. Test glass aquaria (60-liter capacity) were used for holding fish (10 fish per aquarium) throughout the experimental period. Fish were left in the test aquaria one week for acclimatization before starting the experimental study. All the aquaria were kept under the same conditions of temperature (27 ± 2°C), pH (7.2 ± 0.1) and photoperiod (12 hr. light/12 hr. dark) and dissolved oxygen pump. Fish were fed with the standard diet *adlibitum* every day a week once a day.

Tested Chemicals

Diazinon

An emulsified concentrate of organophosphate insecticide

-IUPC name: (0,0-diethyl-O-2isopropyl-6-methylpyrimidin-4- ylphosphorothioate) - commercial name Diazinon EC® (*a.i.* Diazinon, 600g/l), -a kind gift of the Egyptian Ministry of Agriculture.

Sodium benzoate

Sodium benzoate was obtained from Merck Darmstadt Germany. All chemicals used were analytical reagent grade or higher quality and purchased from Sigma, Aldrich Chemicals.

Acute Toxicity

Toxicity tests were performed according to the USEPA procedure **(EPA, 1975)**. Fish individuals were starved for 48 hr., before treatment and during the experiment (96 hr.). Mortality was less than 10% during the acclimatization period. Preliminary screen test was carried out to determine the appropriate concentration for the test compound. Each test consisted of control and four concentrations. Three replicates for each concentration with ten fish in each replicate were used. 60-liter glass aquaria were used for fish (60 g). At the beginning of tests and every 24 hr., the symptoms during holding period (4 days) were recorded. Preliminary study was conducted to establish the 96-h LC_{50} of the organophosphate insecticide diazinon against the 60 ± 2 g fish which found to be 5.6 mg/l. The results of LC_{50} were computed using the EPA probity analysis programs. Concerning the sodium benzoate toxicity, it was found that the mortality percentages did not exceed 10% after 96 hr., of treatments at 150 mg/l which was recommended in meat industrial factories.

Biochemical studies

To detect the role of increasing the exposure period of fish individuals to a sublethal concentration of toxicants on disturbance of the detected biochemical aspects, fish individuals were continuously exposed to a sublethal concentration of the insecticide diazinon and concentration of 150 mg/l sodium benzoate. In this respect, the tested fish were divided into three groups, 30 fish of each in three replicates, and then each replicate was placed in glass aquaria (60 liter). The first group was kept in pesticide free water as control, whereas the second group was exposed to pesticide solution with the concentration of 0.56 mg/l. The concentration used represents the value of 0.1 of 96-hr., LC_{50}. The third group was treated with sodium benzoate with the concentration of 150 mg/l. At the end of experiment, fish were transferred to clean aquaria containing clean

untreated dechlorinated tap water for 14 days (*i.e.,* recovery period). Fish samples were taken from each group after 1, 2 and 3 weeks as well as 14 days after the recovery period. Three individuals were taken from each group then blood samples were taken from caudal vein of treated and untreated fish using clean syringe, collected in centrifuged tubes and left at room temperature until it has clotted. After that, the blood samples were centrifuged at 3000 rpm for 15 minutes and the serum was separated for estimation of some blood serum contents as follows: transaminases (AST and ALT), total protein, uric acid, glucose, creatinine, albumine, Ach.E, triglyceride and cholesterol according to the methods of **Belfield and Goldberg (1971), Trinder (1969), Fossati *et al.* (1980), Trinder (1969), Henry (1974), Henry (1974), Ellman *et al.* (1961), Jacobs and Vandemark (1960) and Roeschlau *et al*. (1974)**, respectively.

Histopathological studies

This study was conducted to investigate the effects of the sublethal concentration of diazinon (0.56 mg/l) and 150 mg/l sodium benzoate on the Nile tilapia fish tissues. Some organs (gill, liver, ovary, brain, kidney, testis, intestine and muscle) were removed from samples of treated and untreated fish after 21 days of treatment, as well as the end of the recovery period, and kept in plastic cassettes and preserved in 10% formalin. Fish tissues were processed in an automated tissue processor. The processing consisted of an initial 2 steps fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of ethyl alcohol (70%, 90% and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for a hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out in paraffin blocks. Then these blocks were sectioned at 4µ thickness. The paraffin sections were stained with hematoxylin and eosin stain for histopathological examination, the conventional staining technic. Stained sections were examined for necrosis, inflammation, vascular changes along with presence of granulomas, degenerative and or fatty change in different tissues of different groups **(Suvarna *et al.*, 2013).**

During the experimental period to study the biochemical and histopathological effects of the tested materials every 4 days the solution of each aquarium was replaced by the freshly prepared sublethal concentration of diazinon (0.56 mg/l) as well as the tested concentration of soduim benzoate till 21 days.

Statistical Analysis

All data were expressed as mean ± standard error for 30 fish in each group. All the obtained data were statistically evaluated with **CoStat (2004)**. Hypothesis testing methods included one-way analysis of variance (ANOVA). P value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Acute Toxicity

Diazinon

Results in Table 1 indicate that the LC_{50} of diazinon to the Nile tilapia fish were calculated after different periods *e.g.* 24, 48, 72 and 96 hr., post- treatment. The LC_{50} after 24 hr., was 8.377 mg/l. whereas the corresponding LC_{90} after the same period was 28.28 mg/l. The LC_{50} values after 48, 72 and 96 hr., post-treatment were 6.852, 6.200 and 5.679 mg/l to the previous periods, respectively. LC_{90} values which determined for the same periods were 18.38, 13.01 and 10.65 mg/l. The slope values ranged between 2.425 to 4.693 which refer to homogenecity of tested fish.

Biochemical Study

Diazinon

In general, diazinon was anticholinesterase potential. AChE activities measurement in the Nile tilapia fish compared with control at different

Table 1. Acute toxicity of diazinon to the Nile tilapia fish, *Oreochromis niloticus* L. after different periods of exposure

Time of exposure (hr.)	LC_{50} (mg/l)	LC_{90} (mg/l)	Slope
24	8.377	28.28	2.425
48	6.852	18.38	2.989
72	6.200	13.01	3.981
96	5.679	10.65	4.693

exposure periods were presented in Table 2. Exposure of diazinon to dose depicted a general dose-dependent inhibition of AchE in blood serum. The comparison of mean values of AchE in blood serum showed significant differences for the inhibition of AchE. Results showed the decrease from 1644.6 U/l to 115.3 U/l after 21 days.

Results also indicated that diazinon treatments increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities after 7, 14 and 21 days of treatment, the increase was high after 21 days recorded 207.33 and 115 U/l to ALT and AST, respectively, as for as recovery period (14 days). The ALT and AST activities were returned to the normal level compared with untreated group.

Results also showed increases of total protein, albumin, creatinine and cholesterol activity after 7, 14 and 21 days of treatment, the increase was high after 21 days, while at the end of recovery period (14 days) the values of these parameters were returned to be normal level compared with untreated group. Uric acid was decreased in treated fish compared with untreated group and recorded 1.133 mg/dl after 21 days compared with 1.900 mg/dl in control group.

It is well known that; many organophosphate compounds show selective toxicity among fish species**.** The great range of acute toxicity levels among insecticides for many species or for one compound among species may be the result of the differences in inhibitory potency for the target and non-target enzymes and metabolism. Analyses of plasma constituents have proved to be useful in the detection and diagnosis of metabolic disturbance and disease **(Al-Ghanim, 2014)**. Many factors affect the biochemical composition of fish such as fishing area, type of food, water quality and pollution **(El-Tantawy *et al.*, 2006; Cong *et al.*, 2009; Ahmad, 2011).**

The present study showed significant changes in plasma total protein, albumin, globulin, total lipids, cholesterol, AST, ALT, uric acid, creatinine and glucose activities in the untreated control and treated fish. However, these results reflect the healthy status of the cultured fish at this treatment. Protein plays an important role in the metabolism and regulation of water balance **(Heath, 1995)**. It is the basic building nutrient of any growing animal and also used as an indicator of their state of health (**Lea-Master *et al.*, 1990)**. Regarding the plasma total protein of the Nile tilapia, (*O. niloticus*) collected from the different studied sites is clear that there is significant difference in the plasma total protein in the untreated control and treated fish.

Uric acid is formed from the metabolism of nucleic acid. Liver cells metabolize purine nitrogenous bases to uric acid. Serum uric acid is produced by the oxidation of hypoxanthine and xanthine by xanthine oxidase and dehydrogenase enzyme, it is less toxic than urea, and less soluble. High uric acid is associated with higher risk of type 2 diabetes independent of obesitsy, dyslipidemia and hypertension. Increased dietary acids and purine intake increased uric acid formation. High levels of uric acid in blood can cause solid crystals to form within joints. This causes painful condition called gout. It can also form crystals or kidney stones that can damage the kidney.

Sodium benzoate

Results in Table 3 indicate that sodium benzoate treatments increased the enzymes alanine aminotransferase (ALT) and aspartate

Table 2. Effect of diazinon on some biochemical parameters of the exposed Nile tilapia fish, *Oreochromis niloticus* L. to the sublethal concentration (0.56 mg/l) for 21 days

Exposure period (day)	Glucose (mg/dl)	Uric acid (mg/dl)	Albumine (mg/dl)	ALT (U/l)	AST (U/l)	Cholestrol (mg/dl)	Triglycride (mg/dl)	Creatinine (mg/dl)	Total protien (mg/dl)	Ach.E (u/l)
Control	90.00±0.02E	1.900±0.025A	1.70±0.01C	190.0±0.005E	100.00±0.001D	250.0±0.01C	157.0±0.1D	0.500±0.057B	3.600±0.002C	438±0.025A
7	100.33±2.08C	1.500±0.10B	2.033±0.15AB	200.67±1.52 C	108.0±1.00E	255.3± 1.52B	162.0±1.00B	0.500±0.10B	3.766±0.35BC	226.0±1.00B
14	103.00±1.0B	1.300±0.10C	2.100± 0.200A	205.00±1.00B	113.67±1.52A	258.0± 1.00A	163.0±1.00B	0.633±0.057AB	4.033±0.11AB	165.3±1.5C
21	106.67±1.52A	1.133±0.057D	2.133±0.208A	207.33±1.52A	115.0±1.00A	259.6± 1.52A	165.3±0.57A	0.700±0.10A	4.233±0.057A	115.3±1.52D
Recovery (14 days)	95.0±1.00D	1.633±0.057B	1.800±0.10BC	193.00±1.00D	102.3±0.57C	251.6±1.52C	158.6± 0.57C	0.533±0.057B	3.733±0.057BC	110.3±1.52 E

All values represent mean ± SE; those in the same column differ significantly (Duncan's multiple-range test, $p<0.05$).

Table 3. Effect of sodium benzoate at concentration 150 mg/l on some biochemical parameters of the exposed Nile tilapia fish, *Oreochromis niloticus* L. for 21 days

Treatment	Glucose (mg/dl)	Uric acid (mg/dl)	Albumine (mg/dl)	ALT (U/l)	AST (U/l)	Cholestrol (mg/dl)	Triglycride (mg/dl)	Creatinine (mg/dl)	Total protien (Mg/dl)
Untreated fish	90.00±0.02B	1.900±0.025B	1.70±0.01B	190.0±0.005B	100.00±0.001B	250.0±0.01B	157.0±0.1B	0.500±0.057B	3.600±0.002B
Treated fish	104.00±1.00A	2.63±0.057A	2.500± 0.100A	209.0±1.00A	111.67±1.52A	260.3±1.52A	165.0±1.00A	0.800±0.10A	4.066± 0.057A

All values represent mean ± SE; those in the same column differ significantly (Duncan's multiple-range test, $p<0.05$).

aminotransferase (AST) activities after 21 days post-treatment recorded 209 u/l and 111.67 u/l for ALT and AST, respectively

The present results indicated that an increase of glucose, total protein, albumin, uric acid, creatinine and cholesterol values after 21days of treatment was noticed. The previous parameters recorded 104.00 mg/dl for glucose compared with 90.00 mg/l in the control, 4.066 mg/l for protein compared with 3.600 mg/dl in the control, 2.500 mg/l for albumin compared with 1.70 mg/dl to control, 2.63 mg/l for uric acid compared with 1.900 mg/dl in the control, 0.800 mg/dl for creatinine compared with 0.500 mg/dl in the control and 260.3 mg/dl for cholesterol compared with 250.0 mg/dl in the control after 21 days.

Ibekwe *et al*. (2007), Oluwole *et al.* (2012) and Hamdy *et al*. (2015) reported that, the plasma total protein was significantly increased at high doses sodium benzoate, and this result may be due to the fact that androgens regulate protein synthesis by binding to cytosolic or nuclear receptors for steroids than modulates transcription. Regarding plasma albumin and globulin of the Nile tilapia, *O. niloticus*. Lipids, as an important source of energy, play an important role in toelest fish **(Sinha and D'souza, 2010; Abdel Aziz and Zabut, 2012).** In contrast to mammals fish prefer to utilize lipids rather than carbohydrates as a main source of energy. Lipids are important metabolites for locomotory and reproductory activities of fish. Albumin maintains the amount of blood in the veins and arteries. When albumin levels become very low, fluid can leak out from the blood vessels into nearby tissues, causing swelling in the feet and ankles. Very low level of albumin may be a sign of liver damage (**Wikipedia, 2011).**

Histopathological Changes

Control group

Examined sections of this group revealed normal liver with preserved morpho-histological structures and normal hepato-pancreases (1,2). Other organs including gills (3,4), intestine (5), kidney (6), brain (7), ovary (8) and muscle (9), all were apparently normal with preserved morpho-histological structures.

Plates 1-4 indicated the histapathological changes in all tissue of the Nile tilapia fish, *O. niloticus* in case of both diazinon and sodium benzoate treated fish as compared with untreated (control group).

These results agree with those obtained by **Joseph and Raj (2011**).

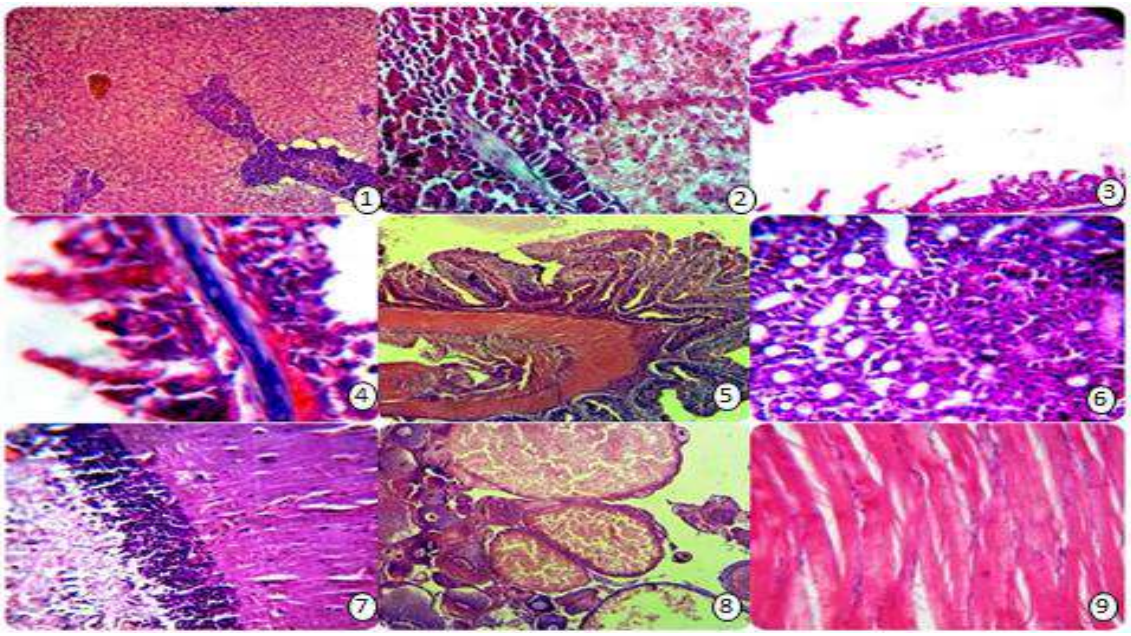

Plate 1. Normal histomorphological structures in all tissues of the Nile tilapia fish, *Oreochromis niloticus* L., H and E X 200, 400 (control group)

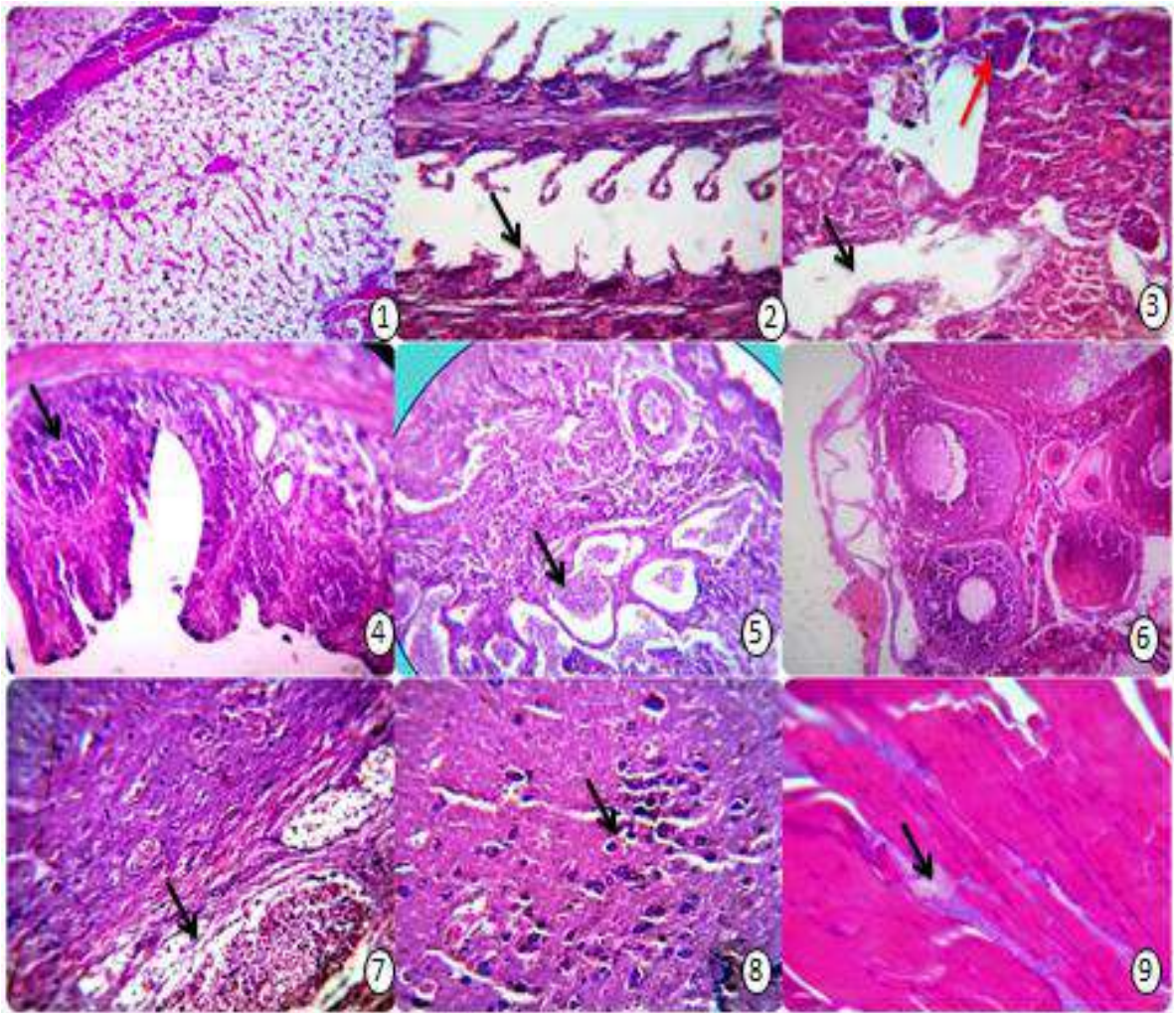

Plate 2. Diazinon- treated group: hematoxylin and eosin stain of fish group showing: (1) Liver revealed extensive hydropic degeneration of hepatocytes, congestion of blood vessels, H and E, X 200, (2) Gills revealed desquamation and sloughing of the lining epithelium of the primary and secondary gill filament (arrow), H and E, X 200, (3) Kidney revealed glomerular shrinkage (red arrow), perivascular edema, hemorrhage, necrosis and degenerative changes (arrow). H and E, X 200, (4) Intestine revealed lymphocytic enteritis "lymphocytic aggregation in the mucosa and submucosa" and dilatation of blood vessels (arrow), H and E, X 200, (5) Testis most of the seminiferous tubules revealed mildly active and contained spermatozoa, others were inactive and showed very few number of spermatozoa with a moderate number of spermatocytes (arrow), H and E, X 200, (6) Ovary (7,8) Brain tissue revealed congested cerebral blood vessels (arrow), focal coagulaive necrosis infiltrated and surrounded by inflammatory cells and astrocytes. Some nerve fibers revealed axonal degeneration and demyelination. Degenerative changes in a moderate number of the neurons were also seen (arrow), H and E, X 200, 400, (9) Muscles revealed minimal interstitial edema with apparently normal morpho-histology of muscle fibers (arrow), H and E, X 200

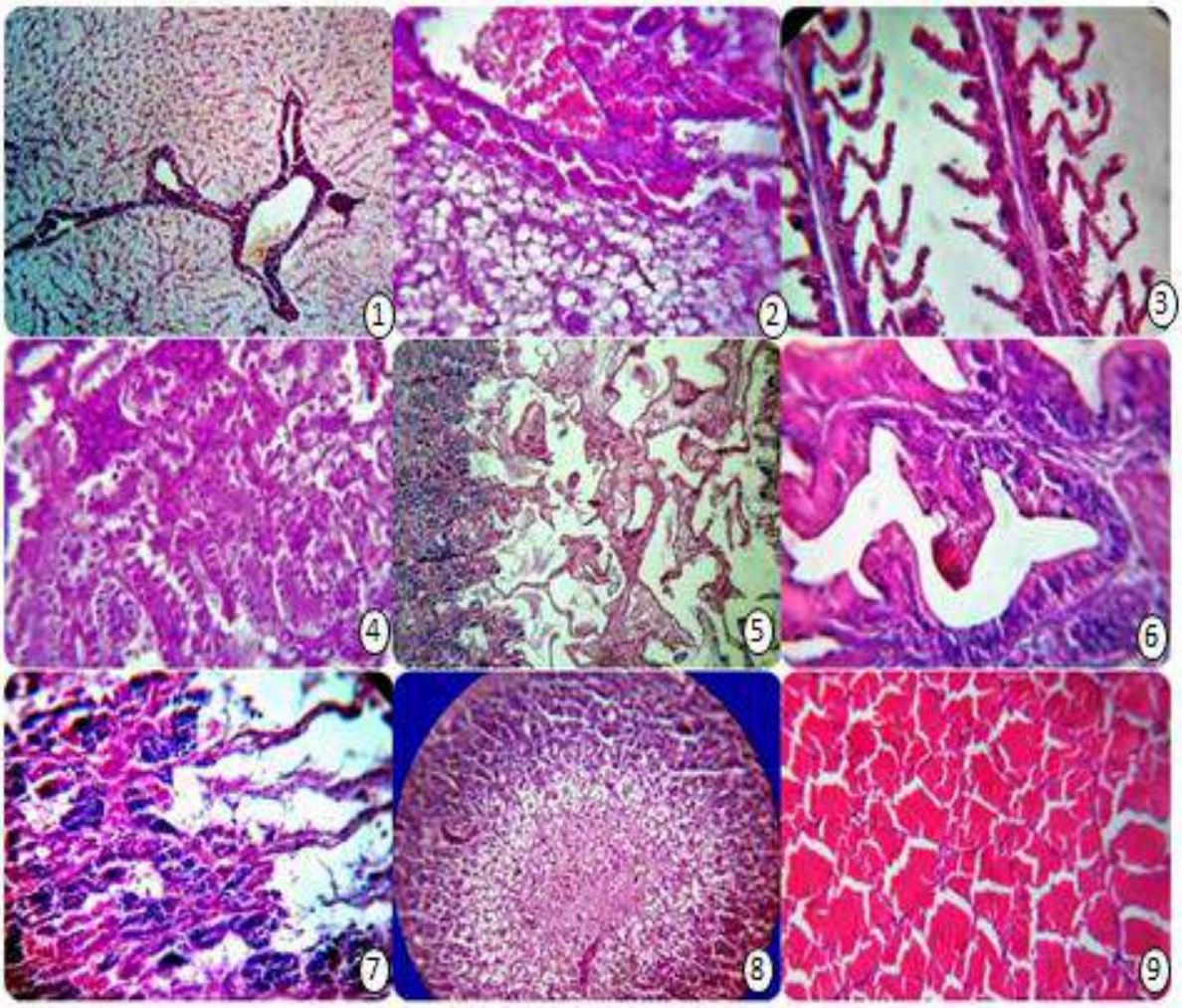

Plate 3. Diazinon-recovery group: hematoxylin and eosin stain of fish group (diazinon-recovery) showing: (1,2) Liver revealed congestion of blood vessels, extensive hydropic degeneration and mildly inflamed hepato-portal pancreas with inactivated pancreatic acini, H and E, X 200, 400 (3) Gills revealed within the normal with minimum degenerative and exfoliative changes, H and E, X 200 (4,5) Testis revealed cystically dilated. The seminephrons tubules showed activated spermatogonia but few numbers of sperms were seen in their lumina H and E, X 200 (6) Intestine revealed mild lymphocytic infiltration in the lamina propria, H and E, X 200 (7) Kidney revealed glomeruli are hemorrhage, perivascular edema and degenerative changes in the renal tubules, H and E, X 200 (8) Brain tissue revealed nodule formed from central caseation followed by vacuolated neuropil and a zone of lymphocytes H and E, X 200 (9) Muscles revealed normal morpho-histology of muscle fibers. H and E, X 200

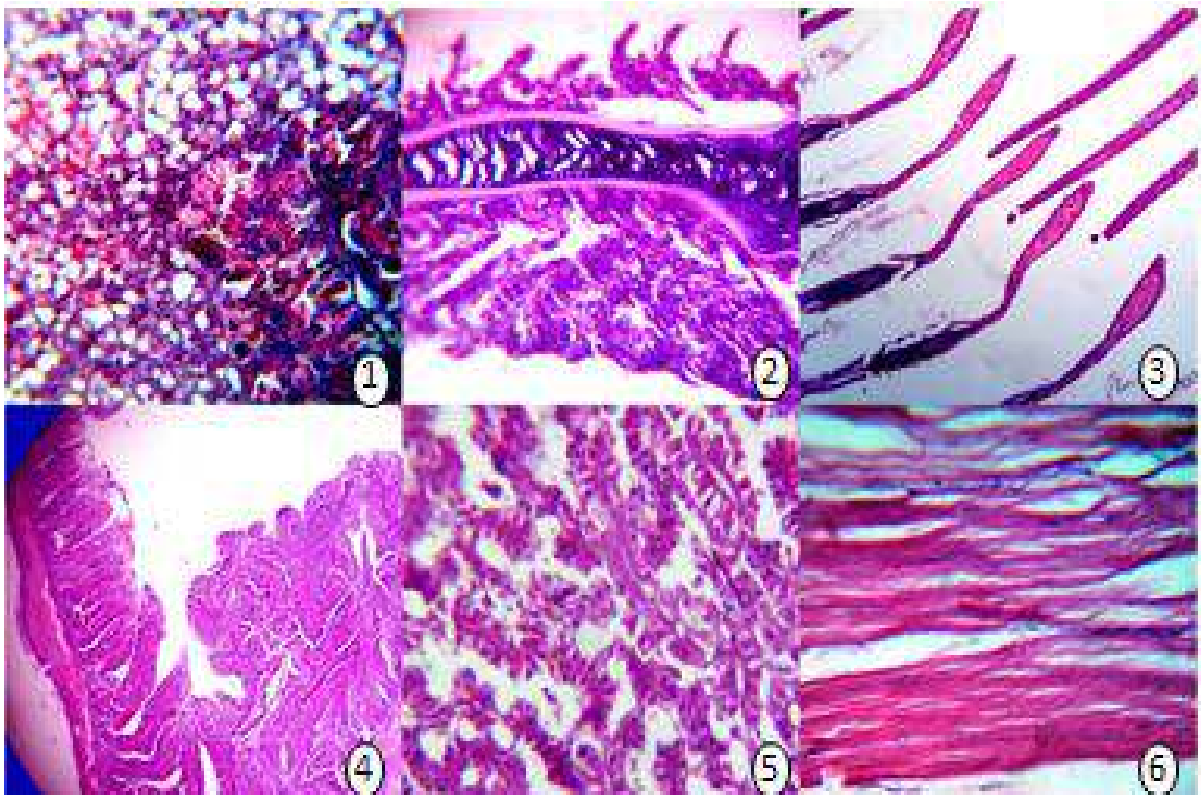

Plate 4. Sodium benzoate: hematoxylin and eosin stain of fish group (soduim benzoate treated) showing: (1) Liver revealed extensive fatty change of a moderate number of hepatocytes. Focal destruction and dissociation of the pancreatic acini was also encountered, H&E X 200 (2,3) Gills revealed focal sloughing and destruction of the secondary filament, H&E X 200 (4) Intestine proliferative changes in the intestinal villus epithelium with a polypoid formation were a characteristic feature, H and E X 200 (5) Kidney revealed degenerative changes and cystic dilatation of some renal tubules together with congetion of renal blood vessels and mild to moderate lymphocytic infiltration were detected. Disorganization (dysplasia) of some tubular epithelium was also recorded, H and E X 200 (6) Muscles revealed hyaline degeneration and interstitial edema were seen, H and E X 400

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التأثيرات التوكسيكولوجية والهستوباثولوجية لمبيد الديازينون وبنزوات الصوديوم في السمك البلطي النيلي *Oreochromis niloticus* L.

رفعت محمود عبدالسميع عبدالمعطي - علي أحمد علي أيوب - أحمد السيد أحمد السبكي- محمد عبدالعال هنداوي

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق - مصر

تم دراسة السمية الحادة وتحت المزمنة لمبيد الديازينون وبنزوات الصوديوم لسمك البلطي النيلي فى وزن ٦٠ جرام، أظهرت النتائج أن قيم التركيز القاتل لنسبة ٥٠ % من الأفراد بعد ٢٤، ٤٨، ٧٢ و٩٦ ساعة بعد المعاملة بمبيد الديازينون هي ٨,٣٧٧، ٦,٨٥٢، ٦,٢٠٠ و٥,٦٧٩ ميللجرام/لتر على التوالي، تبين أن تعرض السمك لتركيزات تحت مميتة من مبيد الديازينون ٠,٥٦ (ميللجرام/لتر) وبنزوات الصوديوم ١٥٠(ميللجرام/لتر) لمدة ٢١ يومًا أحدثت بعض التغيرات البيوكيميائية والهستولوجية لبعض المكونات الموجودة فى الدم وكذلك الأعضاء، أحدث الديازينون زيادة في نشاط انزيمات AST، ALT، الجلوكوز، البروتين الكلي، الألبيومين، الكرياتينين والكولسترول بينما سببت المعاملة بمبيد الديازينون انخفاض في مستويات الأستيل كولين استريز وحمض اليوريك، أحدثت مادة بنزوات الصوديوم زيادة في نشاط AST، ALT، الجلوكوز، البروتين الكلي، الألبيومين، الكرياتينين، وحمض اليوريك والكولسترول، أظهرت الدراسات الهستولوجية ضمور في خلايا الكبد واحتقان بالأوعية الدموية وتغيرات انتكاسية في بعض الأعضاء وأيضًا تضخم كيسي فى بعض الانيبيبات الكلوية المتجمعة مسببة احتقان في الأوعية الدموية بالكلية كما وجد ارتشاح ليمفاوى تراوحت درجتة من طفيفة لمتوسطة مقارنة بالسمك غير المعامل.

المحكمون :

١- أ.د. علي علي علي عبد الهادي أستاذ المبيدات المتفرغ – كلية الزراعة – جامعة المنصورة.

٢- أ.د. عطا علي مرسي شلبي أستاذ المبيدات المتفرغ – كلية الزراعة – جامعة الزقازيق.