Histological Hazards of Chlorpyrifos Usage on Gills and Kidneys of *Tilapia nilotica* and the Role of Vitamin E Supplement in Egypt

Aliaa M. Issa[1], Azza M. Gawish[*1], Gehad M. Esmail[2]

[1]Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt
[2]Central Lab. for Aquaculture Research. Abbasa, Ismailia, Egypt
[*]azzaGawish@ymail.com

Abstract: Fishes had exhibited a time-honored place in the economical nutrition. Chlorpyrifos is a broad-spectrum organophosphate for agriculture. This study aimed to examine pathological changes on gills and kidneys in Nile tilapia and evaluate the protective role of vitamin E supplementation . Fish were exposed to 0, 2.64 and 5.28µg/l lorsban and/or vitamin E. Fishs were divided into six groups (control, 2.64 µg/l lorsban, 5.28µg/l lorsban, vitamin E, vitamin E + 2.64µg/l lorsban vitamin E + 5.28µg/l lorsban treated). Fish behavior was observed. Samples were taken in fixed times for behavioural morphometrical and histopathological studies. The fishes exhibited slowly down swimming, color fading and retardation in opercular movement. The vitamin E + 2.64µg/l lorsban and vitamin E + 5.28µg/l lorsban treated fish showed abnormalities in their behavior. Gills and kidney of the 2.64µg/l lorsban treated group showed several pathological changes throughout the experimental periods. The gills of the vitamin E + 5.28µg/l lorsban fish treated group showed also pathological changes. We may conclude that the effect of lorsban on the fish is well noticed on their behavioral and histopathological aspects of the gills and kidney tissues and vitamin E may be partially able to ameliorate these effects.

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1. Introduction

Pesticides are biologically active chemicals of great value to agriculture (**Khogali *et al.*, 2005**). Lorsban® is a trade name for agricultural-use products of chlorpyrifos (**EPA, 2006**) a broad-spectrum organophosphate used heavily throughout the world for agriculture and domestic purposes (**Ali *et al.*, 2009**).

Nile tilapia, *Oreochromis niloticus* Linnaeus (*O. niloticus* L.), is an African fish species (**Figueredo and Giani, 2005**) of high economic importance which is a benthic omnivorous cichlid commonly found in fish ponds and streams of tropical countries and has much interesting to evolutionary biologists (**Martins *et al.*, 2004**). **Peebua *et al.*(2007)** recorded that, the 96-h LC_{50} values of chlorpyrifos to freshwater fish rainbow trout and fathead minnows were 15µg/l and 0.58µg/l, respectively. The toxicity of chlorpyrifos varies from species to species.

Several authors studied the effect of lorsban on different fish species behavior. (**Dembele *et al.*, 2000, Adeyemo *et al.*, 2004, Chindah *et al.*, 2004 and Rao, *et al.*, 2005**). Histopathological biomarkers in the gills may be valuable as indicators of the general health of the fish and mirror effects of exposure to a variety of anthropogenic pollutants (Wijeyaratne and Pathiratne, 2006). The most common form of gill pathology in juvenile guppies (*Poecilla reticulate*) and (*Oreochromis mossambicus*) treated with chlorpyrifos were short and irregular appearance of gill lamellae, increase vacuolation of gill tissue, mucous mould of secondary lamellae and complete destruction of many lamellae. The pathological effects on the gills of freshwater fish showed necrosis, abnormalities to gill lamellae, extensive fusions of secondary lamellae and a thick coat of mucus on the gill filaments upon the exposure to chlorpyrifose (**De Silva and Samayawardhena 2002, Rao *et al.*, 2003**). In addition, hyperemia of the gill filaments, edema, separation of primary gill lamellae, hemorrhage in the blood vessels, clubbing, fusion of adjacent filaments and hyperplasia in secondary gill lamellae in freshwater fish (*Oreochromis mossambicus*) exposed to chlorpyrifos. Hyperplasia with lamellar fusion, telangiectasia, edema with epithelial lifting and desquamation in the gills of Nile tilapia (*Oreochromis niloticus*) and (*Piaractus mesopotamicus*) observed edema in the secondary lamellae, lamellar fusion, and mild and moderate cell hypertrophy and a marked swelling of blood sinuses in the secondary lamellae (telangiectasia), some foci of sub-epithelial edema, lamellar fusion and foci of blood congestion exposed to organophosphate insecticide trichlorfon and glyphosate (**Jiraungkoorskul *et al.*, 2003, Guimaraes *et al.*, 2007, Kunjamma *et al.*, 2008 and Mataqueiro *et al.*, 2009**).

Kidney plays a vital role in the maintenance of an organism's internal environment, being the key to the regulation of extracellular fluid volume and composition as well as acid–base balance. It is also a target of toxic chemicals, which can disrupt its functions, and cause temporary or permanent derangement of homeostasis. Several authors recorded histopathological changes in the kidney of freshwater fish, *Puntius conchonius* and *Channa punctatus* exposed to organophosphate insecticides diazinon, monocrotophos, dimethoate and elsan, respectively (**Banerjee and Bhattacharya, 1994 and Miller, 2002**). The most histopathological changes in the kidneys of freshwater catfish (*Heteropneutes fossilis*) zebrafish (*Danio rerio*) exposed to organophosphate insecticide chlorpyrifos were shrunken glomeruli, dilated lumina of the renal tubules and vacuolated blood cells in the glomerular tuft declared strong reaction or even destruction, vacuolization, disintegration of epithelia resulting from necrosis and caryolysis in the kidney **(Mataqueiro *et al.*, 2009 and Scheil *et al.*, 2009**).

The purpose of the present work is to determine the half-lethal concentration (96-h LC_{50}) of organophosphate insecticide lorsban to subadults Nile tilapia fish (*O. niloticus* L.). in order to evaluate the effect of its sublethal toxicity on some organs (gills and kidneys) in Nile tilapia as well as the protective role of vitamin E against the different effects of lorsban pesticide.

2. Material and Mehtods

1-Experimental Fish:

Clinically healthy, subadults Nile tilapia (*O. niloticus* L.) of average body weight (18.09±0.3g) and total body length (10.5±0.06cm) were a kind gift of the Arabian Fish Breeding Company in Abbasa, Sharkia Governorate. Fish were transferred alive to the Central Laboratory for Aquaculture Research (CLAR) according to **Selvi *et al.* (2005)**. Fish (30 fish per aquarium) were kept in the maintenance glass aquaria (200 liter capacity) for two weeks to acclimatize to the laboratory environment before transferring them to the test glass aquaria.

2- Test Aquaria:

Test glass aquaria (60 liter capacity) were used for holding fish (10 fish per aquarium) throughout the experimental period. Fish were left (**Monteiro *et al.*, 2006**) to discard the metabolic wastes (**Roy and Bhattacharya, 2006**). A continuous aeration was maintained in each aquarium using electric air pumping compressor. Fish were fed twice daily (at 8:00 a.m. and 5:00 p.m.) with pellets of basal control diet (Fish Nutrition Department, Central Laboratory for Aquaculture Research), at a daily feeding rate 3% of body weight per day. All the aquaria were kept under the same conditions of temperature (27 ±1^{o}C), pH (7.2±0.1), photo period (14 hours light/10 hours' dark) and dissolved oxygen (DO_2: 7.5±0.1mg/l).

3-Diets:

The basal control diet (diet C) was formulated according to **Kim *et al.* (2003)** from practical ingredients to satisfy all known nutrient requirements of Nile tilapia with adequate levels of vitamin E, {α-tochopheryl acetate in diet (α-TA)} (**NRC, 1993**).

4- Insecticide:

An emulsified concentrate of organophosphate insecticide lorsban (0,0-diethyl O-3,5,6-trichloro-2pyridyl phosphorothioate) with commercial name Chlorzane EC® (a.i. chlorpyrifos, 480g/l), a kind gift of the Egyptian Ministry of Agriculture and Land Reclamation was used in the present study (source: ™Kafr El-Ziate for pesticide and chemicals Co., Egypt).

5-Vitamin E:

Vitamin E, as α-**tocopheryl** acetate (α-TA) was purchased from Chemical Industries Development (CID) Company, Al-Haram, Giza, Egypt.

II- Methods

1-Insecticide exposure:

Different concentrations of lorsban insecticide used in the determination of 96-hrLC_{50} and in the sub-lethal study were freshly prepared by diluting the commercial emulsified concentrate of lorsban with aged tap water. The solutions were further diluted to obtain the desired experimental concentrations in the test glass aquaria (**Chandrasekara and Pathiratne, 2007**). Every 24 hours, the test water was renewed to maintain water quality (**Montairo *et al.*, 2006**) and freshly prepared lorsban solutions were added to be maintained at a constant level (**Durmaz *et al.*, 2006**).

2-Morphometric data:

The condition factor (CF) was calculated according to **Teh *et al.* (2005)**, while hepatosomatic index (HSI) and renal somatic index (RSI) were calculated based on the total body weight (TW), total length (L), total liver (LW) and kidney weights (RW) (**Zha *et al.*, 2007**) as the following equations:

Condition factor (CF)=[TW (g) /L (cm)^{3}]X 100.

Renal somatic index (RSI)=[RW (g)/TW g)] X 100.

Where all weights are in grams (g) and lengths are in centimeters (cm)

Histopathological examinations:

Tissues specimens from three fish per treatment were removed and small pieces of several organs (gills and kidneys) were immediately fixed in neutral

buffered formalin 10%, dehydrated in ascending grades of ethanol, embedded in soft paraffin, sectioned at 5µm thickness and stained with hematoxylin and eosin (H&E) Tissue sections were prepared according to **Bancroft and Gamble (2002)**. Condition factor and renosomatic indices were calculated**.**

Experimental Design:

The determined 96-h LC_{50} of lorsban insecticide to subadults *O. niloticus* L. was determined and to be 26.4 µg/l. In the sublethal toxicity test, fish were exposed to 2.64 (1/10 LC50) and 5.28µg/l (1/5 LC50) lorsban and/or vitamin E (0 and 450mg/kg dry diet), divided into six groups (control, 2.64 µg/l lorsban, 5.28µg/l lorsban, vitamin E, vitamin E + 2.64µg/l lorsban vitamin E + 5.28µg/l lorsban treated). Fish behavior was observed. Samples were collected at the end of the 1st, 2nd, 3rd and 4th week at fixed time for behavioural and histopathological studies.

3. Results

Subadults Nile tilapia (*Oreochromis niloticus* L.) were divided into six groups, control, 2.64µg/l (1/10 LC50) lorsban, 5.28µg/l (1/5 LC50) lorsban, vitaminE, vitamin E + 2.64µg/l lorsban and vitamin E + 5.28µg/l lorsban. Fish were sacrificed at the end of the 1st, 2nd, 3rd and 4th week.

1- Behavioral Observations:

Control group of subadults Nile tilapia (*Oreochromis niloticus* L.) showed normal behaviour throughout the sublethal toxicity periods; they exhibited normal swimming activity, normal escape reflex, quick response and normal opercular movement and good appetite.

The subadults Nile tilapia (*O niloticus* L.) exposed to sublethal concentrations (2.64 and 5.28µg/l) of lorsban ($\frac{1}{10}$ LC_{50} and $\frac{1}{5}$ LC_{50}, respectively) exhibited an immediate very fast behavioral changes even at the low concentration. A slow down swimming behavior, than the control group, then staying motionless close to the water surface with loss of escape reflex were observed. Fish showed a colour fading, and retardation in opercular movement. They also lost their feeding appetite. An increase in skin mucus secretion and its accumulation on the gills were also showed. Vitamin E treated fish were behaviourally normal and had good appetite as the control group. Those treated with vitamin E lorsban showed abnormalities in their behavior similar to that mentioned from lorsban treated fish.

2-Morphometrical results

a-Condition factor (CF):

Condition factor is an index of growth rate (**Chuiko *et al*., 2007**). The condition factor (mg/cm^3) of Nile tilapia (*Oreochromis niloticus* L.) of all treated groups were none significantly changed after the end of the 1st, 2nd and 4th week as compared to the control group. Condition factor recorded at the end of the 3rd week was significantly decreased to in the 5.28µg/l lorsban and vitamin E + 5.28µg/l lorsban treated groups, respectively compared to in the control group (Fig. 21).

The results by the three-way ANOVA revealed that, the lorsban concentrations and time significantly affected the CF. Vitamin E none significantly affected the CF, while the interaction between vitamin E and time significantly ($P<0.001$) affected the CF.

b-Renosomatic index (RSI):

The renosomatic index (%) of Nile tilapia *Oreochromis niloticus* L. recorded at the end of 1st week was significantly ($P<0.05$) decreased to (0.236±0.035%) in 5.28µg/l lorsban treated group compared to (0.274± 0.028%) of the control group. At the end of 3rd week, a significant decrease to (0.208±0.015%) in the 5.28µg/l lorsban treated group compared to (0.311±0.025%) of the control group was also recorded (Table, 2 and Fig. 20).

The results of the three-way ANOVA revealed that, the concentrations of lorsban significantly ($P<0.001$) affected the RSI. The interactions between lorsban concentrations and time, and between vitamin E and time significantly ($P<0.01$) affected the RSI. The interaction between lorsban concentrations, vitamin E and time significantly ($P<0.05$) affected.

3-Histopathological studies:

The histopathological alterations of the 2.64µg/l lorsban, 5.28µg/l lorsban, vitaminE, vitamin E+2.64µg/l lorsban and vitamin E+5.28µg/l lorsban Nile tilapia (*Oreochromis niloticus* L.) treated with were demonstrated in the gills and kidneys.

A-Gills:

Gills of control group of Nile tilapia showed a normal structure that persisted through the four weeks of the experimental period (Fig.1).

Gills of the 2.64µg/l lorsban treated group showed several pathological changes throughout the experimental periods. Hemorrhage at the primary lamellae, intraepithelial oedema and lifting up the epithelial cells of secondary lamellae were showed after one week of treatment (Fig.2). At the end of the third week of treatment, epithelial hypertrophy and adhesion of lamellar tips (synechiae) hemorrhage at primary lamellae (Fig.3).

Gills of the 5.28µg/l lorsban treated group showed also histopathological changes throughout the

treatment periods. Epithelial hyperplasia of secondary lamellae and sloughing of secondary lamellae epithelial cells were observed at the end of one week of treatment (Fig. 4). Necrosis and destruction of secondary lamellae architecture appeared after three weeks of treatment; the gill arch appeared hemorrhagic with hyalinization of the adductor muscles, deformation of cartilage core and clavate gill shape were observed after three weeks of treatment (Fig. 5). Complete destruction of the gill architecture; marked necrosis of the epithelial cells of the gill lamellae with obliteration of the apical ends of the gills were noticed at the end of the fourth week (Fig.6).

Gills of the vitamin E treated fish showed normal structure thorough the four weeks of the experimental period (Fig.7).

Gills of the vitamin E+2.64µg/l lorsban treated group showed several pathological changes throughout the experimental periods. Intraepithelial oedma, congestion of the entire secondary lamellae, epithelial hyperplasia (Fig.8) were observed at the end of the first week of treatment. In addition, pronounced epithelial hyperplasia and hyperplasia of epithelial cells of secondary lamellae with fused area (Fig. 9) was noticed at the third week of the treatment.

The gills of the vitamin E + 5.28µg/l lorsban fish treated group showed also pathological changes throughout the experimental periods. Intraepithelial oedema, hyperplasia of epithelial cells of secondary lamellae and lamellar aneurysm were observed after one week of treatment (Fig.10). In addition, hemorrhage at primary lamellae, sloughing of secondary lamellae epithelial cells, necrosis of primary and secondary lamellae (Fig.11) were ecorded at the end of the third week of treatment.

B-Kidney:

The kidney of the control group of subadults Nile tilapia (*O. niloticus* L.) showed normal renal tubules (Fig. 12) and glomeruli inside Bowman's capsules distributed in the renal interstitial hematopoietic tissue.

Kidney of 2.64µg/l lorsban treated group showed several pathological changes throughout the experimental periods. Cloudy swelling of epithelial tubule and fragmentation of glomeruli were observed at the end of first week of treatment (Fig.13). In addition to the previous changes, shrinkage of glomeruli and renal tubule with degenerated epithelial and occlusion lumen was recorded after three weeks of treatment (Fig.14). Renal tubule with degenerated epithelia and dilated lumen, brownish pigments and complete destruction of tubules became pronounced at the end of the experiment (Fig. 15).

The kidney of the 5.28µg/l lorsban treated group showed shrunken and fragmented glomeruli within thickened Bowmen's capsule membrane; occlusion of luminal renal tubule with eosinophilic granules and complete destruction of tubules architecture were noticed after three weeks of treatment. (Fig.16). After four weeks of treatment showing marked collapse of glomerulus (small arrow), shrunken (star) and fragmented glomeruli (arrow) within thickened Bowmen's capsule membrane (arrow head) and increase of Bowmen's capsule space (Fig. 17).

The kidney of the vitamin the E + 2.64µg/l lorsban treated group showed several pathological throughout the experimental periods. Shrunken glomeruli, with appearance of yellow brownish pigments, proliferation of hematoopoietic cells and focal necrosis were observed at the end of the experiment (Fig. 18).

Kidney of the vitamin E + 5.28µg/l lorsban treated group showed also pathological throughout the experimental periods. A vacuolar degeneration of glomeruli, thickened Bowmen's capsule membrane and renal tubule with degenerated cells and narrowed lumen, with fiber formation around sloughed necrotic renal tubule epithelial cells was recorded after two weeks of treatment (Fig. 19).

Table (1): Condition factor (CF) (g/cm^3) of Nile tilapia (*Oreochromis niloticus* L.) daily exposed to the sublethal concentrations of lorsban (0, 2.64 and 5.28µg/l) and/or vitamin E (0, 450mg/Kg dry weight diet) for four weeks.

Treatments	Time intervals			
	1st week	2nd week	3rd week	4th week
Control	1.533±0.14	1.541±0.038	1.555±0.049	1.564±0.032
2.64 µg/l lorsban	1.831±0.058	1.41±0.037	1.434±0.036	1.378±0.067
5.28µg/l lorsban	1.869±0.044	1.49± 0.057	1.368±0.045*	1.462±0.054
Vit. E	1.541±0.087	1.549±0.094	1.625±0.068	1.668±0.055
2.64 µg/l lorsban + Vit. E	1.595±0.07	1.542±0.052	1.525±0.035	1.555±0.11
5.28µg/l lorsban + Vit. E	1.534±0.053	1.452± 0.07	1.387±0.024*	1.41±0.032

Table (2): Renosomatic index (RSI) (%) of Nile tilapia (*Oreochromis niloticus* L.) daily exposed to the sublethal concentrations of lorsban (0, 2.64 and 5.28µg/l) and/or vitamin E (0, 450mg/Kg dry weight diet) for four weeks.

Treatments	Time intervals			
	1st week	2nd week	3rd week	4th week
Control	0.274± 0.028	0.295± 0.017	0.311±0.025	0.292±0.014
2.64 µg/l lorsban	0.266± 0.018	0.274± 0.021	0.279±0.038	0.264±0.034
5.28µg/l lorsban	0.236±0.035*	0.239± 0.014	0.208±0.015*	0.331±0.028
Vit. E	0.29± 0.034	0.302± 0.019	0.304±0.018	0.313±0.025
2.64 µg/l lorsban + Vit.E	0.274±0.021	0.302±0.018	0.252±0.023	0.248±0.015
5.28µg/l lorsban + Vit.E	0.260±0.054	0.2394±0.018	0.234±0.026	0.227±0.012

Data are represented as means ± standard errors of 5 specimens.
*Significant (P<0.05) change compared to the control group of each time interval. Vit. E = Vitamin E

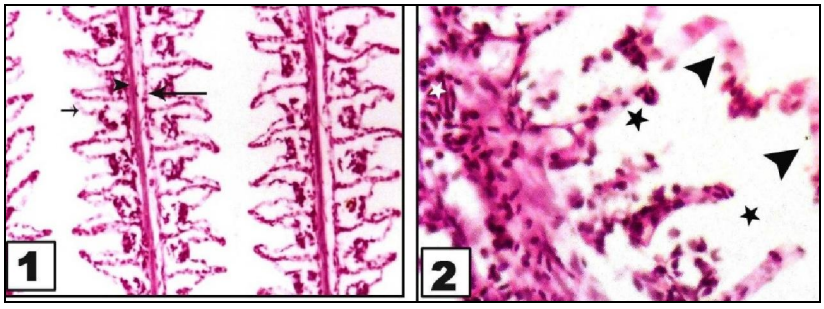

Fig. 1: Photomorphograph of the gill section of control Nile tilapia (*Oreochromis niloticus L.*) showing the primary lamellae (arrow), secondary lamellae (arrow head) and cartilage core (star). H&E. 200 X.

Fig. 2: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with 2.64µg/l lorsban for one week showing hemorrhage at primary lamellae (white star), intraepithelial oedema (black star), hypertrophy (arrow) and lifting up (arrow head) of epithelial cells of secondary lamellae. H&E. X 400.

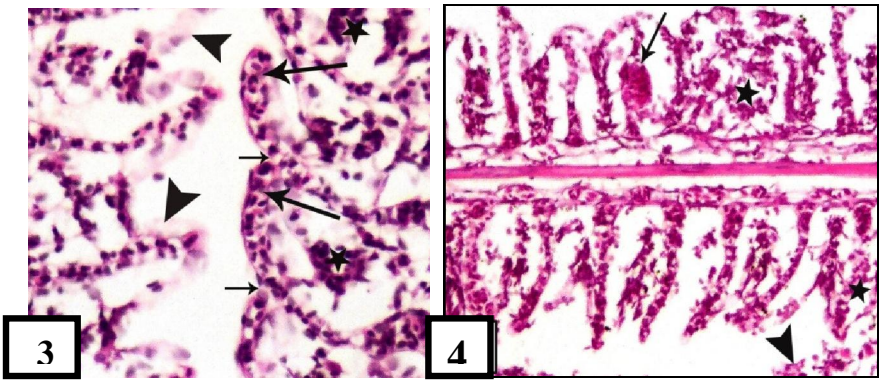

Fig. 3: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with 2.64µg/l lorsban for three weeks showing hypertrophy (arrow head) and hyperplasia (star)of epithelial secondary lamellae, adhesion of lamellar tips synechiae) (small arrow) and congestion in the entire secondary lamellae (arrow) H& E. X 200.

Fig. 4: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with 5.28µg/l lorsban for one week showing epithelial hyperplasia (star) and sloughing of secondary lamellae epithelial cells (arrow head) and lamellar aneurysm (arrow). H & E. X 200.

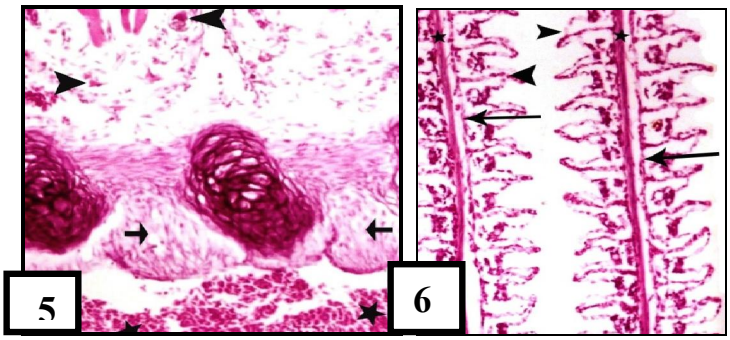

Fig. 5: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with 5.28µg/l lorsban for three weeks showing hemorrhage (star), oedema at the gill arch (small arrow) with leucocytic infiltration (arrow head) and hyalinization of the adductor muscles (arrow), H & E. X 200.

Fig. 6: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with 5.28µg/l lorsban for four weeks showing deformation of the cartilage core (star), epithelial hyperplasia (double arrows), necrosis of the epithelial cells of primary (small arrow) and secondary (arrow) lamellae and complete destruction of the gill; obliteration of normal lamellae architecture affecting the apical distal ends of the gill (arrow head). H & E. X 200.

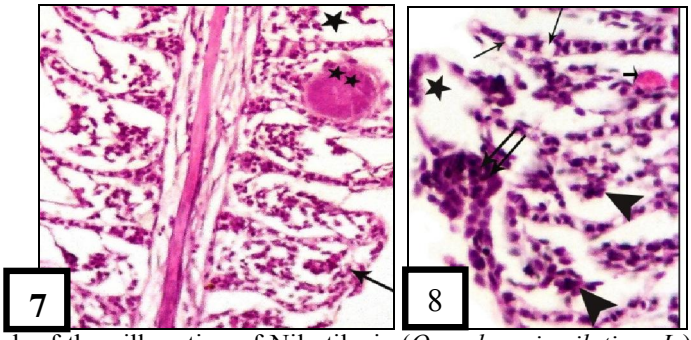

Fig. 7: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*) treated with vitamin E for one week showing the primary lamellae (arrow), secondary lamellae (arrow head) and cartilage core (star). H&E. 200 X.

Fig. 8: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*), treated with vitamin E + 2.64µg/l lorsban for one week showing intraepithelial oedma (star), congestion of entire secondary lamellae (arrow head), epithelial hyperplasia (arrow). H & E. X 400.

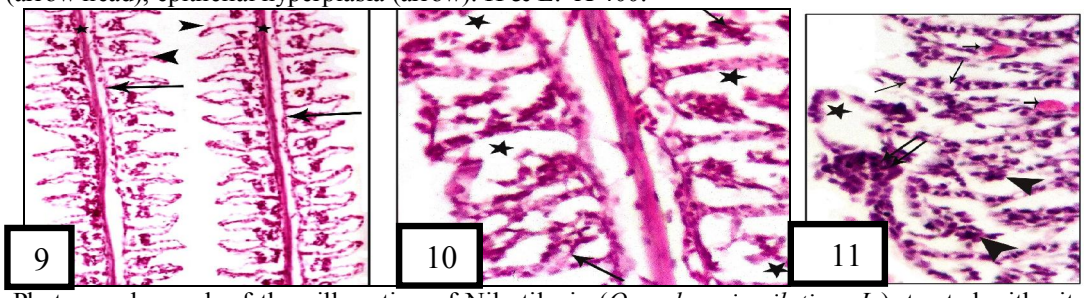

Fig. 9: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus L.*), treated with vitamin E + 2.64µg/l lorsban for three weeks showing intraepithelial oedema (star), vacuolar degeneration of pillar cells (arrow), marked epithelial hyperplasia (arrow head) with leucocytic infiltration (small arrow) and lamellar synechiae (double arrow). H& E. X 400.

Fig. 10: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus* L.), treated with vitamin E + 5.28µg/l lorsban for one week showing intraepithelial oedema (star), hyperplasia of epithelial cells of secondary lamellae (arrow) and aneurysm (double stars). H& E. X 200.

Fig. 11: Photomorphograph of the gill section of Nile tilapia (*Oreochromis niloticus* L.), treated with vitamin E + 5.28µg/l lorsban for three weeks showing intraepithelial oedema (star), lifting of secondary lamellae epithelial cells (arrow head) and quite frequent epithelial hyperplasia (arrow) forming fused area. H & E. X 200.

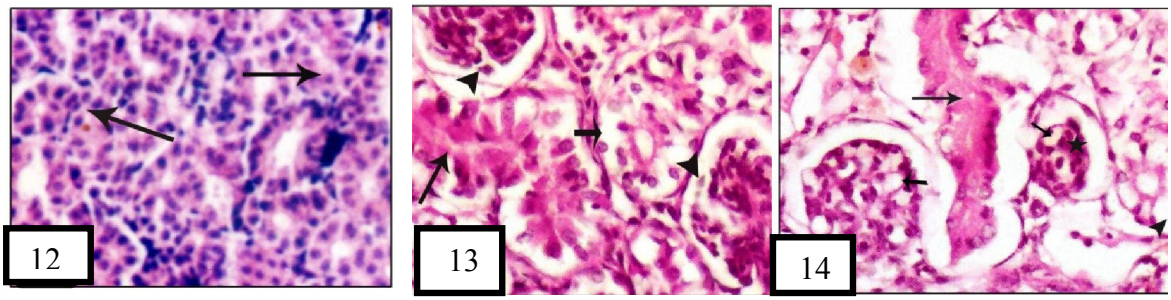

Fig. 12: Photomorphograph of the kidney section of control Nile tilapia (*Oreochromis niloticus* L.) showing normal architecture; renal tubule (arrow). H & E. X 200.

Fig. 13: Photomorphograph of the kidney section of Nile tilapia *Oreochromis niloticus* treated with 2.64µg/l lorsban for one week showing cloudy swelling of epithelial cells of renal tubule (small arrow), renal tubule with dilated lumen (star) and occlusion lumen (star) and fragmentation of glomeruli (arrow head). H & E. X 400.

Fig. 14: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with 2.64µg/l lorsban for three weeks showing shrinkage (star) and vacuolar degeneration of glumeruli (small arrow), cloudy swelling of epithelial cell of renal tubule with narrowing lumen (arrow head), renal tubule with degenerated epithelia and occlusion lumen (arrow). H & E. X 400.

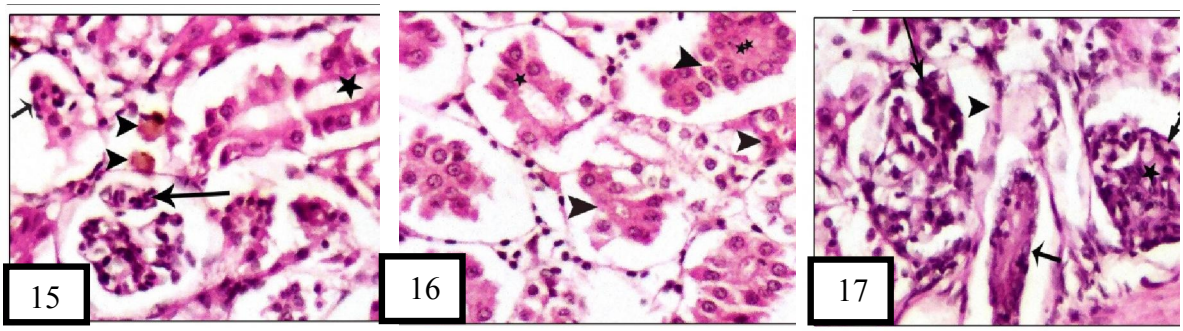

Fig. 15: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with 2.64µg/l lorsban for four weeks showing renal tubule with degenerated epithelial cells and dilated lumen (star), complete destruction of tubule architecture (small arrow), fragmentation of glumerulus (arrow) and brownish pigments (arrow head). H & E. X 400.

Fig. 16: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with 5.28µg/l lorsban for three weeks showing degenerated tubules obstructed with eosinophilic granules (arrow head), with complete occlusion lumen (double stars) and with dilated lumen (star). H& E. X 400.

Fig. 17: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with 5.28µg/l lorsban for four weeks showing marked collapse of glomerulus (small arrow), shrunken (star) and fragmented glomeruli (arrow) within thickened Bowmen's capsule membrane (arrow head) and increase of Bowmen's capsule space (double heads arrow). H& E. X 400.

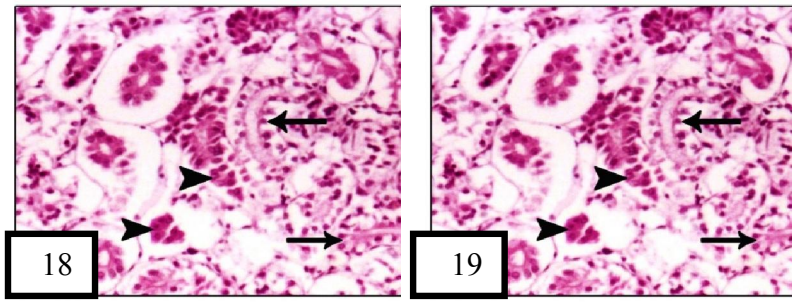

Fig. 18: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with vitamin E + 2.64µg/l lorsban for four weeks showing yellow brownish pigments (arrow head), degenerated renal tubule with complete occlusion lumen (small arrow), proliferation of hematopoietic cells (arrow) and focal necrosis (star). H & E. X 400.

Fig. 19: Photomorphograph of the kidney section of Nile tilapia (*Oreochromis niloticus* L.) treated with vitamin E + 5.28µg/l lorsban for two weeks showing marked degeneration of epithelial tubule (arrow) and destruction of renal tubule (arrow head). H & E. X 200.

4. Discussion

Chlorpyrifos forms the active ingredient in Dursban™ and Lorsban™ insecticides (**Kienle *et al.*, 2009**). Acute toxicity tests with lorsban on different fish species, at different life stages and under different environmental conditions were studies (**Karen *et al.*, 1998 and 2001**).

Behaviour integrates responses to internal (physiological) and external (environmental, social) factors represent a sensitive method to detect the effects of contaminants (**Dell'Omo, 2002**). In the present study, Nile tilapia (*O. niloticus* L.) treated with lorsban exhibited behavioral changes; such as slow down, swimming and a less general activity than the control group, as well as staying motionless close to the water surface and losses of escape reflex. Fish color fading, retardation in opercula movement were also noticed. Moreover, loss of the fish (feeding) appetite, increase skin mucus secretion and accumulation on the gills were observed.

Vitamin E was not able to prevent these behavioural changes in lorsban treated fish. Increased frequency of fish surfacing may be due to the deficiency in respiratory exchange, as a result of severe disruption of gill membranes and deposition of mucus on gills (**Velmurugan *et al.*, 2007**). This was histopathologically confirmed in the present study by the noticed marked gill lesions. The mucus deposition on the gills and damage caused to gill lamellae by the toxicant would reduce gaseous exchange across them (**Al-Ghanim *et al.*, 2008**). The decline in the opercular movement observed in the present study is in agreement with **Chindah *et al.* (2004)**, who exposed another tilapia species (*Tilapia guineensis*) to chlorpyrifos. This could be explained by an adaptive response of the fish to minimize the intake of toxicant by reducing the frequency of opercular beating (**Pandey *et al.*, 2008**).

The observed increase of mucus secretion and accumulation on skin and gills gills recorded by (**Rao *et al.*, 2005**). Histopathologically this was by the proliferation of mucus cells and epithelial hyperplasia of gills. This could be explained by the probability of coating the body so as to reduce contact with the toxic environment and get relief from the pollutant irritation (**Al-Ghanim *et al.*, 2008**)

The loss of fish appetite was observed in the present study. This was may be due to a decrease in the amount of food consumed in (*Abramis brama*) fish and attributred that to the cholinergic system in fish brain and the inhibition of acetylcholine esterase enzyme the feeding behavior in fish (**Pathiratne, 1999**)

Histopathological results of the present study indicated that, gills of Nile tilapia (*O. niloticus* L.) were the primary target tissue affected by lorsban and vitamin E not able to prevent these effects. Many lesions in the gills were recorded in the present study as hemorrhage at the primary lamellae, intraepithelial edema with lifting up and sloughing of epithelial cells of the secondary lamellae. Hypertrophy and hyperplasia of the epithelial cells of the secondary lamellae, vacuolar degeneration of pillar cells and lamellar aneurysms were also noticed. At the end of the experiment, necrosis with leucocytic infiltration and deformities of cartilage were obtained.

The different concentrations of lorsban used in the present study as well as the different exposure periods showed different degrees of pathological changes. Similar results were recorded in the freshwater fish (*Puntius gonionotus*), *Oreochromis niloticus*), (*Gambusia affinis*) and (*Corydoras paleatus*) exposed to pesticides paraquat, and dimethoate (**Elezaby *et al.*, 2001, Cengiz and Ünlü, 2003, Fanta *et al.*, 2003 and Jiraungkoorskul *et al.*, 2003**) respectively.

In Sri Lanka, gills of (*Rasbora caverii*) collected from canals near rice fields, covering pesticide application periods during rice cultivation season showed also similar changes (**De Silva and Samayawardhena, 2002; Wijeyaratne and Pathiratne, 2006**) and juvenile guppies (*Poecilla reticulate* Peters) and (*Oreochromis mossambicus*) exposed to sublethal concentration of chlorpyrifos (**Rao *et al.*, 2003 and Kunjamma *et al.*, 2008**)

The histopathological results observed in present study as gill epithelial necrosis was considered a direct response of lorsban, while excessive mucus secretion, the epithelium lifting up, lamellar fusion and clavate lamella were defense responses (**Richmonds, and Dutta, 1989**). Epithelium lifting increases the distance through which the toxicant has to travel to reach the blood stream and lamellar fusion could be protective as it diminishes the amount of vulnerable gill surface area (**Ortiz *et al.*, 2003**). These epithelial lifting reactions could result in dysfunctional or even non-functional gills, and eventually asphyxiate the fish. This epithelial lifting could be due to edema following the exposure to the used chemical of the lamellar tissue pollutant (**Morrison *et al.*, 2001**).

Roberts (2001) recorded that, if the irritant stimulus is more severe, it will have three different responses depending on the toxicant; these are lamellar edema; lamellar hyperplasia; and lamellar fusion. In the present study, the epithelial hyperplasia could be a consequence of the epithelial detachment (**Machado and Fanta, 2003**) and lamellar fusion could be a result of both hyperplasia of epithelial cells and the adhesion of the lamellar tips, seen as synechiae (**Morrison *et al.*, 2001**).

Lesions associated with the disturbance of blood flow in the gills (lamellar aneurysm) were prevalent in the present study and could be due to the effect of lorsban. According to **Campagna *et al.* (2007)** hyperplasia, total fusion of the secondary lamellae, dilation of capillaries of secondary lamellae and lifting up the gill epithelium in the respiratory area observed in the present study, were considered to be of the first degree of gill lesions while lamellar aneurysms It was that extensive lamellar aneurysm (telagiectasis) takes considerably longer time to resolve than the hyperplastic lesions of the gill (**Roberts, 2001**).

The appearance of leucocytic infiltration in the gills of the present study was also noticed by **Neskovic *et al.* (1996)** in carp fish *Cyprinus carpio* treated with glyphosate. They explained that, the leucocytic infiltration in the gills supports the inflammatory reaction indicated by hyperplasia in the freshwater environment. Thus, fish have to fight constantly against the osmotic influx of water that occurs across the gills during respiration.

The kidney plays the major role in this fight, producing large quantities of diluted urine. Although the kidney does not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in the liver have been shown to occur in the kidney (**Mohssen, 2001**). It receives the bulk of the post branchial blood flow; kidney tissue is of importance in the detoxification and elimination of aquatic contaminants in fish (**Durmaz *et al.*, 2006**).

The kidney appears to be particularly sensitive to a variety of toxins due to the high renal blood flow, the ability to concentrate substances, and the biotransformation of the parent compound to a toxic metabolite (**Mohssen, 2001**).

The present work revealed that, Nile tilapia fish *(O. niloticus)* treated with lorsban showed several histological alterations in the kidney, such as vacuolar degeneration of glomerular tuft, shrinkage of some glomeruli and dilatation of others, increase Bowmen's capsule space. Moreover, cloudy swelling of some epithelial tubules, degeneration of others, and dilatation of tubules lumens and obstruction of others were also observed. At the end of the experiment, focal necrosis with leucocytic infiltration was noticed. These results are in agreement with the changes in the kidney of zebrafish (*Danio rerio*), exposed to sublethal concentration of chlorpyrifos (**Scheil *et al.*, 2009**) and on freshwater fish (*Piaractus mesopotamicus*) exposed to organophosphate insecticide (**Mataqueiro *et al.*, 2009**). The shrinkage in renal corpuscles clearly indicates that treated fish adopt some other routes of nitrogen excretion while the dilation of the renal corpuscles may be due to an increase in the filtration rate and consequently in urine volume, which may be a mechanism used by fish to overcome the toxic effect of the pesticide (**Roy and Bhattacharya, 2006**).

The decreases in the tubular lumen may be due to the cloudy swelling of the epithelial cells of the renal tubules, which could be a reversible change Also, the dilation in the tubules lumen may be due to the marked decrease in the length of the epithelial cells as a result of epithelial tubules degeneration while in the present study, the recognized homogenous eosinophilic deposits within tubular lumen could be attributed to the protein leakage into the filtrate due to the glomerular disease (**Roberts, 2001**).

Lorsban used in the present research caused some histopathological changes in the kidney tissues and the used vitamin E was not able to prevent these changes. Organophosphate insecticide chlorpyrifos caused kidney damage, and a combination of vitamins E and C reduced partially this damage. On a relative basis, lorsban appears to be capable of producing a wider spectrum of significant histopathologic impairments in fish with even sub lethal concentrations and should be categorized as an important pollutant of the aquatic environment (**Oncu *et al.*, 2002**).

The morphometric study included condition factor and renosomatic indices. The condition factor (mg/cm^3) of Nile tilapia (*O. niloticus* L.) of all treated groups showed very minor change; significant decrease in the condition factor of 5.28µg/l lorsban at the end of the 3rd week compared to the control group and vitamin E couldn't prevent this decrease. The significant lower condition factor was recorded by **Teh *et al.* (2005)** in (*Pogonichthys macrolepidotus*) exposed to sublethal concentrations of diazinon. The few changes in condition factor through the present experimental periods could be attributed to that, this factor could not be enough a sensitive biomarker to measure the environmental stress in natural environments (**Wijeyaratne and Pathiratne, 2006**)

The recorded non significant effect of vitamin E on the condition factor throughout the experimental periods was also recorded in the freshwater fish rainbow trout (*Oncorhynchus mykiss*) (**Chaiyapechara *et al.*,2003**). This indicates that, the addition of α-tocopherol to the diet did not significantly alter the palatability of the diet, its nutrient content, and the caloric values (**Al- Juary *et al.*, 2006**).

Tissue somatic indices, such as the renosomatic index are general measurement of the overall condition of fish or growth status of a specific tissue (**West, 1990**). The minor changes was recorded in the present study in the renosomatic index of fish treated with lorsban. A significant decrease in the

renosomatic index of fish treated with the high sublethal concentration of lorsban was recorded at the end of the 1st and 3rd weeks. While non significant changes were observed in the vitamin E and the vitamin E + lorsban treated groups throughout the experimental periods. Absolute kidney weight and relative kidney weight were decreased in methyl parathion treated groups after 4and 7 weeks of treatment, while did not show any significant changes in vitamin E and C treated groups during experimental periods compared to the control group (**Kalender, 2007**). We can concluded from the present study that the toxic effect of lorsban on fish, is clear on their behavioral and histopathological aspects of gills and kidney tissues while vitamin E has a fair amelioration effects on these parameters.

Corresponding author
Azza M. Gawish
Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt
azzaGawish@ymail.com

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