Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide.

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Abstract: Nile tilapia is the main cultured species in Egypt; the Egyptian fish farms are irrigated with agricultural drainage which contains pesticides residues or their metabolites which may affect fish. This study concerned with the pathologic and clinicopathologic findings due to chronic exposure to the organophosphate fungicide edifenphos on Nile tilapia *Oreochromis niloticus*. Eight weeks exposure to 1/10 96 hours LC_{50} (0.1 ppm) led to adverse effect on some serum parameters including AST, ALT, ALP, cholinesterase activity, total protein, blood urea nitrogen and creatinine. Also some of hematological parameters such as RBCs count, Hb content and blood indices were affected negatively. Histopathological investigations revealed various degrees of pathological lesions in different organs like gills, hepatopancreas, spleen, kidney, brain, and others. From this study it was obvious that edifenphos caused harmful effects on Nile tilapia fish. [Journal of American Science 2010;6(10):542-551]. (ISSN: 1545-1003).

Keywords: Edifenphos, Hinosan, *Oreochromis niloticus*, tilapia, organophosphate, histopathology.

1. Introduction

Chemical pesticides are well recognized as an economic approach to control pests, at the same time such chemicals are highly toxic to other species in the environment. Now there is growing concern worldwide over the indiscriminate use of such chemicals, which result in environmental pollution and toxicity risk to nontarget organisms.

Most of pesticides find their way into rivers, lakes and pond, and have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes (Anees, 1975).

The organophosphate fungicide edifenphos (Hinosan) is cutinase inhibitors and displays a specific antipenetrant action, but in practice its therapeutic activity may also involve direct fungitoxicity (Sisler, 1986). As organophosphate pesticide, it causes irreversible inhibition of the cholinesterase enzymes (Haddad and Winchester, 1983). A lot of fish toxicity studies; including histopathological and clinicopathological studies; were conducted upon organophosphate pesticides.

For instance (Jauch 1980) confirmed gill pathology in *Herotilapia multispinosa* and *Tilapia leucostica* ranging from hyperplasia and separation of the respiratory epithelium in the secondary lamellae with congestion and numerous telangiectasis, while studying the toxic effects of Lebaycid R on fish gills. Also (Joshi and Desai 1981) assessed that exposure of *O. mossambica* to monocrotophos increased activity of acid and alkaline phosphatase and correlatedthis result with necrosis of hepatic and renal tissues. Meanwhile (Prasada and Ramana 1984) stated the increase of activity levels of (AST), (ALT) and (ATPase) in muscle, gill, hepatopancreas and brain tissues of methyl parathion exposed fish. While (El-Zahaby 1986) stated that acid and alkaline phosphatase activities showed a slight elevation in mucosal intestinal epithelial cells of cyolane-injected *Clarias Lazera*. (El-Sheikh *et al.* 1990) assessed the biochemical changes of (AST) and (ALT) and acid and alkaline phosphatase in *Oreochromis niloticus* fingerlings exposed to a nonlethal concentration of Ofunac and Sumithion for a period of 28 days. Also (El-Aulaimi *et al.* 1994) recorded abnormalities in some hematological parameters; (RBCs) count, (Hb) content, (Ht) value and (MCV) in *Sarotherodon*

galilaeus exposed to dimethoate. The data obtained here revealed marked reduction in (RBCs) count, (Hb) contents and (Ht) values in fish exposed to dimethoate. While (MCV) displayed significant increase in its values. Also (Sherif and Eisa 1994) exposed *Tilapia zillii* to sublethal concentrations of chlorpyrifos and recorded histopathological changes in the gills such as, dilation of the blood vessels, tilangectasis in the secondary lamellae with slight proliferative changes of the epithelial covering, and hepatopancreas showed vacuolar degeneration and necrosis of hepatocytes together with activation of pancreatic acini.

While somewhat few studies are conducted using edifenphos, (El-Gendy *et al.* 1996) stated the depressive effect of edifenphos on the activities of acetylcholinesterase (AChE), adenosine triphosphatase (ATPase) and glutathion-S-transferase (GST) with the elevation of catalase activity in tissues of *O. niloticus*. Also (Aly 1996) found the same results in addition to decrease of serum protein and reduced response of splenocytes to mitogens. In the same year (Ramadhan 1996) investigated the probable genotoxic effects of edifenphos on common carp fish and found a significant changes in the relative proportions of protein fractions in the electrophoretic patterns of hepatopancreas and brain esterase isozyme of the fish.

On the same way (Rezq-Allah *et al.* 1997) revealed the same results with edifenphos on the electropherograms of sarcoplasmic proteins of common carp (*Cyprinus carpio*). Again (El-Gendy *et al.* 1998) investigated the effects of $^{1}/_{1000}$ field recommended concentration of edifenphos on the immune response and protein contents of *O. niloticus* and found that The cell mediated immune response assessed by proliferative response of splenocytes to mitogens; phytohemagglutinin (PHA) and concanavalin A (Con A) for T cell and lipopolysaccharide (LPS) for B cell decreased significantly in terms of the level of stimulation index in the treated fish and reached maximal depression after 4 weeks.

Humoral immunity assessed as splenic antibody plaque forming cells (PFC) measured after 5 days in vitro immunization to sheep erythrocytes (SRBC's) were suppressed in a concentration dependent pattern of the used pesticide.

2. Material and Methods

Edifenphos is presented as 50% solution (Hinosan EC 50® Bayer). The acute 96 hours LC_{50} value of edifenphos in Nile tilapia was 1 ppm (Gaafar 2005).

The levels of serum acetyl cholinesterase activity, alkaline phosphatase, transaminases, total protein, total serum bilirubin, blood urea nitrogen and serum creatinine were estimated by using kits obtained from BioMérieux – France.

A total No. of 150 fish (80±20 g. B.W); were divided into 2 groups. The 1st group was 100 fish exposed to $^{1}/_{10}$ LC_{50} of edifenphos (0.1 mg/ℓ) were divided into four (50 liters). The 2nd (control group) was 50 fish placed in two (50 liters) aquaria. The experiment lasted for 8 weeks, collection of blood samples and scarification of fish were done on weekly interval.

Blood samples were collected from the caudal vein of each fish, 1 ml of each blood sample was mixed wis anticoagulant for estimation of the blood parameters: Total erythrocytic count (Kanaev, 1985), Hemoglobin content, Hematocrit value (PCV) (Tietz, 1976), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) (Hrubec *et al.*, 2000). Also another one ml of blood was left to coagulate, centrifuged and serum was collected to evaluate the following biochemical parameters: Serum cholinesterase activity, alkaline phosphatase, transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total serum protein, total serum bilirubin, blood urea Nitrogen and creatinine, according to the methods mentioned by the Kits manufacturer.

One ml citrated blood was collected to perform the following haematological parameters:
Following necropsy, the specimens were collected from sacrificed fish. Tissue specimen from the skin with the underlying musculature, gills, liver, spleen, intestine, brain, heart and kidneys were taken weekly from 5 fish of treated and 3 of control group.

Tissue specimens were rapidly fixed in 10% neutral buffered formalin. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared, from which 5 microns thick sections were obtained. These sections were stained by Hematoxyline and Eosin (H&E) according to the method described by Culling (1983).

Statistical analysis

Statistical analysis of the obtained data was by using the SPSS 11 computer program (SPSS Inc. Chicago, Illinois, USA). Using analysis of variance (One-way ANOVA).

3. Results and Discussion:

Clinical signs:

Respiratory distress after 3 days, nervous

manifestations, including hyperexcitability by erratic movements appeared after 6 days. Paleness of the gills appeared after 1 week. After 12 days there was paleness of whole body surface (Fig.1) with slimness. Severe convulsive reflexes upon stimulation were abundant after 3 weeks. After 5 weeks the fish shows lethargy and slowing of all movements.

Biochemical parameters:

Biochemical analysis of fish revealed that AST level was significantly increased during the 1st and 4th week with a significant decrease during the 2nd week. While ALT showed only a significant increase on the 1st week. ALP level also was significantly increased during the 1st and 3rd weeks then showed a significant decrease from the 4th week till the end of the experiment.

Regarding total protein showed only a significant increase on the 4th and 5th weeks with general significant decrease during the rest of the experimental period.

Creatinine also showed a significant decrease only on the 3rd week and a significant increase on the 5th week. On the other hand blood urea nitrogen levels was significantly decreased on the 1st ,4th and 6th weeks and increased significantly on the 2nd ,5th and 7th week respectively.

The activity of cholinesterase was significantly decreased during the entire period.
All of these results are included in **(Table 1)**.

Table (1): Effect of chronic exposure of *Oreochromis niloticus* to 1/10 LC50 of edifenphos on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, creatinine and blood urea nitrogen and serum cholinesterase for 8 weeks.

Parameter	AST **(IU/l)**		**ALT** (IU/l)		ALP **(IU/l)**		Total protein **(g/dl)**		**Creatinine** (mg/dl)		Blood Urea Nitrogen (BUN) **(mg/dl)**		**Cholinesterase (U/l)**	
Treatment	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED
Time (Weeks)														
1	33.9 ^{ei} ± 0.66	42.55 ^{l} ± 1.06	82.24 ^{ac} ± 0.66	140.88 ^{i} ± 7.17	25.43 ^{j} ± 0.66	45.06 ^{k} ± 0.73	4 ^{mo} ± 0.01	2.85 ^{dh} ± 0.32	0.2 ^{eg} ± 0.007	0.2 ^{eg} ± 0.016	47.98 ^{dh} ± 0.66	43.88 ^{bc} ± 8.57	57023 ^{l} ± 529.52	44353 ^{i} ± 14.65
2	38.39 ^{k} ± 0.66	30.05 ^{bd} ± 0.69	92.68 ^{ae} ± 0.66	100.63 ^{cf} ± 5.5	16.62 ^{ef} ± 0.66	17.35 ^{fg} ± 0.73	3.45 ^{im} ± 0.01	2.85 ^{dh} ± 0.26	0.21 ^{fh} ± 0.007	0.19 ^{cf} ± 0.002	40.13 ^{a} ± 0.66	45.17 ^{cd} ± 0.99	53826 ^{k} ± 129.47	52351 ^{k} ± 404.95
3	28.13 ^{ab} ± 0.66	31.01 ^{be} ± 0.29	86.71 ^{ad} ± 0.66	93.54 ^{ae} ± 10.42	22.36 ^{i} ± 0.66	24.63 ^{j} ± 0.73	3.33 ^{hl} ± 0.01	2.65 ^{dg} ± 0.14	0.24 ^{hi} ± 0.007	0.19 ^{df} ± 0.002	48.74 ^{eh} ± 0.66	50.53 ^{gj} ± 0.58	48598 ^{j} ± 561.31	33883 ^{ef} ± 311.6
4	25.89 ^{a} ± 0.66	29.49 ^{bc} ± 0.3	79.63 ^{ab} ± 0.66	87.58 ^{ad} ± 3.09	22.36 ^{i} ± 0.66	12.17 ^{b} ± 0.73	2.92 ^{ei} ± 0.01	4.1 ^{no} ± 0.32	0.29 ^{kl} ± 0.007	0.28 ^{jk} ± 0.011	52.67 ^{j} ± 0.66	47.43 ^{dg} ± 0.74	53014 ^{k} ± 940.67	29108 ^{c} ± 332.58
5	34.71 ^{fi} ± 0.66	32.29 ^{cg} ± 0.38	77.02 ^{a} ± 0.66	93.54 ^{ae} ± 3.76	16.48 ^{df} ± 0.66	17.35 ^{fg} ± 0.73	2.29 ^{bd} ± 0.01	3.54 ^{jn} ± 0.09	0.11 ^{a} ± 0.007	0.16 ^{bd} ± 0.009	40.43 ^{a} ± 0.66	47.28 ^{df} ± 1.12	47095 ^{j} ± 865.07	25410 ^{b} ± 231.27
6	35.03 ^{gj} ± 0.66	32.69 ^{dh} ± 0.26	107.58 ^{eg} ± 0.66	97.64 ^{bf} ± 1.52	19.42 ^{gh} ± 0.66	13.01 ^{bc} ± 0.73	3.33 ^{hi} ± 0.01	2.5 ^{cf} ± 0.18	0.18 ^{cf} ± 0.007	0.15 ^{bc} ± 0.016	52.36 ^{ij} ± 0.66	45.02 ^{cd} ± 0.68	41638 h ± 327.39	33614 ^{df} ± 43.63
7	38.23 ^{jk} ± 0.66	38.14 ^{jk} ± 0.65	107.21 ^{eg} ± 0.66	98.76 ^{bf} ± 2.35	17.04 ^{f} ± 0.66	14.41 ^{cd} ± 0.73	3.73 ^{lo} ± 0.01	2.99 ^{fj} ± 0.14	0.15 ^{bc} ± 0.007	0.16 ^{bd} ± 0.003	41.34 ^{ab} ± 0.66	50.98 ^{hj} ± 1.9	53135 ^{k} ± 819.87	33549 ^{df} ± 25.16
8	36.47 ^{ik} ± 0.66	35.82 ^{hk} ± 1.66	104.6 ^{dg} ± 0.66	90.94 ^{ae} ± 1.19	12.98 ^{bc} ± 0.66	6.68 ^{a} ± 0.73	3.63 ^{ko} ± 0.01	1.38 ^{a} ± 0.05	0.18 ^{cf} ± 0.007	0.17 ^{be} ± 0.002	47.38 ^{dg} ± 0.66	47.81 ^{dh} ± 0.31	53890 ^{k} ± 1263.85	21977 ^{a} ± 395.16

- *Ctrl = Control fish
-**ED = Edifenphos treated fish.
- Means with the same letter(s) of the same parameter are not significantly different at $p \geq 0.05$.
- Data are represented as Mean ± SE SE = Standard error.
- Number of observation in each mean =5

Hematological parameters:

Hematological parameters of fish revealed that PCV and RBCs count showed a significant decrease mostly during the entire period except the 2nd week in PCV and the 5th week in RBCs count. While Hb showed a significant decrease only on the 3rd ,5th and 6th weeks respectively. Blood indices such as MCV showed a significant increase during 1st ,2nd ,4th and 7th weeks. While MCH showed a significant increase during the entire period except the 5th week, MCHC showed a significant decrease on the 1st ,3rd and 4th weeks with a significant increase on the 2nd ,5th and 8th weeks **(Table 2)**.

Table (2): Effect of chronic exposure of *Oreochromis niloticus* to 1/10 LC_{50} of Edifenphos (ED) on some blood parameters for 8 weeks.

Parameter	PCV%		Hb (g/dl)		RBCs x10^6		MCV (fl)		MCH (pg)		MCHC (g/dl)	
Treatment	Ctrl*	ED**	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED	Ctrl	ED
Time (Weeks)												
1	7.36 ^{j} ± 0.13	5.30 ^{bc} ± 0.07	7.36 ^{bc} ± 0.13	5.30 ^{ce} ± 0.07	5.20 ^{m} ± 0.01	2.20 ^{eg} ± 0.10	36.48 ^{a} ± 0.16	88.51 ^{gh} ± 5.51	14.15 ^{a} ± 0.22	24.37 ^{cf} ± 1.34	38.78 ^{ce} ± 0.43	27.63 ^{ab} ± 0.74
2	6.76 ^{hi} ± 0.13	6.70 ^{hi} ± 0.07	6.76 ^{hi} ± 0.13	6.70 ^{hi} ± 0.07	3.50 ^{l} ± 0.01	2.11 ^{df} ± 0.07	62.62 ^{bf} ± 0.14	85.14 ^{gh} ± 1.40	19.25 ^{be} ± 0.30	31.78 ^{h} ± 0.76	30.74 ^{ad} ± 0.41	37.36 ^{ce} ± 0.99
3	7.56 ^{j} ± 0.13	5.90 ^{ef} ± 0.34	7.56 ^{ef} ± 0.13	5.90 ^{gh} ± 0.34	3.50 ^{l} ± 0.01	2.06 ^{ce} ± 0.03	54.06 ^{bd} ± 0.17	72.94 ^{dg} ± 2.65	21.54 ^{bd} ± 0.30	28.72 ^{fh} ± 1.68	39.83 ^{de} ± 0.42	39.33 ^{de} ± 1.48
4	6.56 ^{gh} ± 0.13	5.50 ^{ce} ± 0.07	6.56 ^{ce} ± 0.13	5.50 ^{ef} ± 0.07	3.20 ^{jl} ± 0.01	1.91 ^{be} ± 0.03	62.44 ^{bf} ± 0.15	96.49 ^{h} ± 2.71	20.50 ^{bc} ± 0.33	28.73 ^{fh} ± 0.22	32.82 ^{ae} ± 0.44	29.89 ^{ac} ± 1.06
5	7.46 ^{j} ± 0.13	6.10 ^{f} ± 0.11	7.46 ^{f} ± 0.13	6.10 ^{a} ± 0.11	2.61 ^{gi} ± 0.01	2.17 ^{dg} ± 0.05	72.46 ^{dg} ± 0.14	76.00 ^{fg} ± 3.65	28.48 ^{fh} ± 0.36	28.06 ^{fh} ± 0.21	39.30 ^{de} ± 0.42	37.31 ^{ce} ± 2.05
6	6.76 ^{hi} ± 0.13	4.60 ^{a} ± 0.07	6.76 ^{a} ± 0.13	4.60 ^{cd} ± 0.07	3.00 ^{ik} ± 0.01	1.62 ^{ac} ± 0.10	59.73 ^{bf} ± 0.18	71.21 ^{cg} ± 6.75	22.46 ^{be} ± 0.34	28.88 ^{fh} ± 1.31	37.59 ^{ce} ± 0.46	41.55 ^{e} ± 2.89
7	7.16 ^{ij} ± 0.13	5.80 ^{df} ± 0.07	7.16 ^{df} ± 0.13	5.80 ^{fg} ± 0.07	3.40 ^{kl} ± 0.01	1.23 ^{ac} ± 0.05	55.82 ^{be} ± 0.17	117.96 ^{i} ± 9.56	21.06 ^{bc} ± 0.31	47.43 ^{i} ± 2.63	37.72 ^{ce} ± 0.43	41.09 ^{e} ± 3.35
8	36.47 ^{ik} ± 0.66	5.10 ^{bc} ± 0.11	7.16 ^{bc} ± 0.13	5.10 ^{ab} ± 0.11	3.54 ^{l} ± 0.02	1.70 ^{bd} ± 0.12	56.27 ^{be} ± 0.16	52.66 ^{ac} ± 5.95	20.16 ^{bc} ± 0.30	30.52 ^{gh} ± 1.52	35.83 ^{be} ± 0.42	60.94 ^{g} ± 7.21

- *Ctrl = Control fish
-**ED = Edifenphos treated fish.
- Means with the same letter(s) of the same parameter are not significantly different at $p \geq 0.05$.
- Data are represented as Mean ± SE SE = Standard error.
- Number of observation in each mean =5

Histopathological changes:

Hepatopancreas:

After the 1st week of treatment till the end of the study, the detectable lesions were congestion of hepatic sinusoids and diffuse vacuolar degeneration of the hepatocytes with necrotic focal areas with some presence of Esinophilic granular (EG) cells (Fig. 3) with progression of the severity of the lesions with the progression of the experimental period.

Gills:

After the 1st week till the end of the 8th weeks of treatment, the mostly recorded lesions were congestion, separation between surface epithelium and capillary beds, and telangiectasis (Fig. 4, 5). Then after the 2nd week filamentous clubbing of tips of primary gill filaments due to hyperplasia and fusion of the secondary lamellae (Fig. 6) and edema and epithelial hyperplasia at the base of secondary lamellae were noticed. After the 3rd week congestion and edema in the

gill arch were observed. The before-mentioned lesions continued till the end of the experiment.

Kidneys:

Alterations exhibited in the posterior kidney of *Oreochromis niloticus* during the 8 weeks of the experiment and directly after the 1st week were congestion, diffuse cloudy swelling and hyaline droplet degeneration of renal tubules with depletion of interstitial hemopoietic tissue and activation of melanomacrophage centers (Fig. 7,8). Moreover, the anterior kidney revealed severe activation of melanomacrophage centers with necrosis and depletion of the haemopoietic tissues.

Spleen:

The histopathologic examination from the beginning of the 1st till the end of the 8th week revealed activation of melanomacrophage centers with diffuse reduction of the spleenic haemopoietic tissues. From the 2nd week hyperplasia of spleenic ellipsoid and multifocal necrotic areas surrounded by the activated melanomacrophage centers were common (Fig. 9).

Brain:

The brain revealed that there was severe congestion of cerebral blood vessels after the 2nd week till the end of the experimental period, with concurrent congestion of meningeal blood vessels (Fig. 10) with neuronal degeneration after the 3rd week there were pyknotic Purkinje cells and necrotic areas in the inner granular layer of the cerebellum (Fig. 11).

Skin:

After the 2nd week it showed mild vacuolar degeneration of epidermal cells, proliferation of club cell and hyperactivation of the melanophores (Fig. 12) from then till the end of experimental period.

Intestine:

After the 1st week of treatment, the histopathologic examination revealed that there was esinophilic granular (EG) cell infiltration in the submucosa then after the 2nd week epithelial degeneration and submucosal edema were observed till the end of the 8 weeks.

In this study, the fishes were exposed to edifenphos concentration equal to $^{1}/_{10}$ 96 hours LC_{50} (0.1 ppm), the clinical signs and post mortem changes of edifenphos were in the form of nervous manifestations. Respiratory distress reflected by congestion then paleness of the gills, chocolate discolouration of most internal organs and severe distention of gall bladder, cachexia with prominent paleness of whole body surface with slimness. These results supports that edifenphos has the same nervous toxic effect of its chemical group the organophosphate as described by **Jauch (1980)** and **Joshi and Desai (1981)** with unique anemic action leading to cachexia with prominent paleness of whole body surface as observed by **El-Aulaimi *et al.* (1994)** while investigating dimethoate.

The results of serum biochemistry revealed a significant increase in serum ALT and AST and ALP at the beginning of the experiment, these findings supported the hypothesis that the increased serum transaminases (ALT and AST) may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation **(Daabees *et al*., 1992).** Then after that, the enzymes suffered from a significant decrease in their levels. ALP level also showed significant increase then significant decrease towards the end of the experiment. The decrease in activity of AST, ALT and ALP in fish exposed to pesticides was also reported by different authors **(El-Boushy, 1994 and Begum, 2004)**. **Agius and Coushman (1986)** linked the increased activity of ALP in fish to the increased catabolic tissue breakdown in melanomacrophage centers.

Saeed (1983) attributed the decrease in liver transaminases activity to the decrease in protein content in serum and tissues due to the resultant hepatic necrosis of fish exposed to pesticides. Also the severe hepatic necrosis leads to lack of cells from which the enzymes are produced**.** These findings indicated that decrease in liver function was occurred which may lead to major dangerous sequelae in body metabolism.

Total serum proteins showed general significant decrease after exposure to edifenphos. This may be due to liver damage where most of plasma protein synthesis usually occurs in the liver, this result agreed with that of Singh *et al.* (1998).

Creatinine also showed some sort of significant increase after the 5th week. Also blood urea nitrogen levels generally showed significant increase. These results supported that edifenphos exerts harmfull effects on kidney tissue.

Acetyle cholinesterase (ACHase) Showed total decrease in activity, this agreed with many authors (Gosselin, 1984). This may

Fig. (1): An *Oreochromis niloticus* in the glass aquaria after 1week of edifenphos toxicosis showing paleness of the skin and fins.

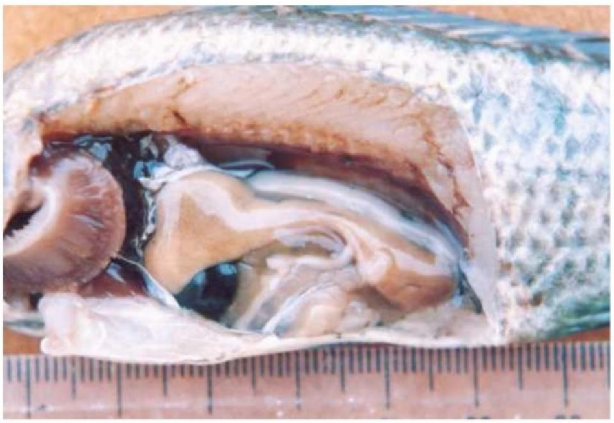

Fig. (2): An *Oreochromis niloticus* after 1week of edifenphos toxicosis showing chocolate discoloration of gills and kidneys, distended gallbladder and paleness of the hepatopancreas in addition to presence of yellowish intestinal content.

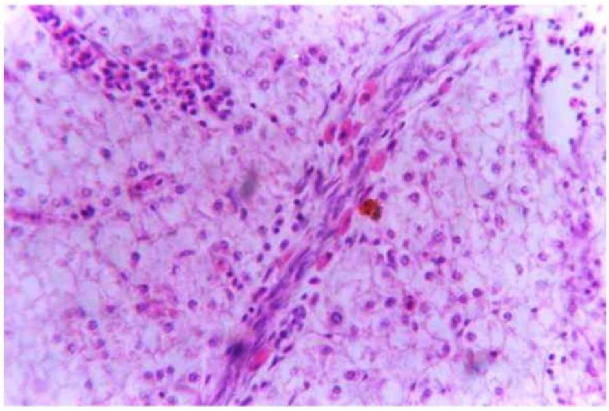

Fig. (3): Hepatopancreas of *Oreochromis niloticus* after 1week of edifenphos toxicosis showing diffuse vaculation of hepatocytes and congestion of hepatic sinusoids with the presence of few EGCs (arrow). H&E. (X 400).

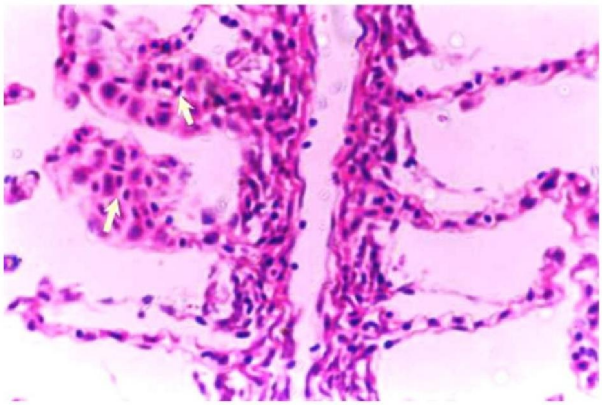

Fig. (4): Gills of *Oreochromis niloticus* after 1week edifenphos toxicosis showing telangiectasis (arrows) and separation of surface epithelium from capillary beds of some secondary lamellae. H&E. (X 400).

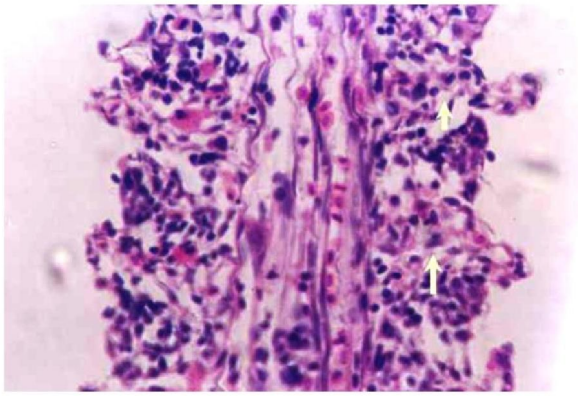

Fig. (5): Gills of *Oreochromis niloticus* after 3 weeks of edifenphos toxicosis showing severe epithelial hyperplasia particularly at the tips of gill filaments and diffuse filamentous clubbing due to fusion of the secondary lamellae. H&E. (X 160).

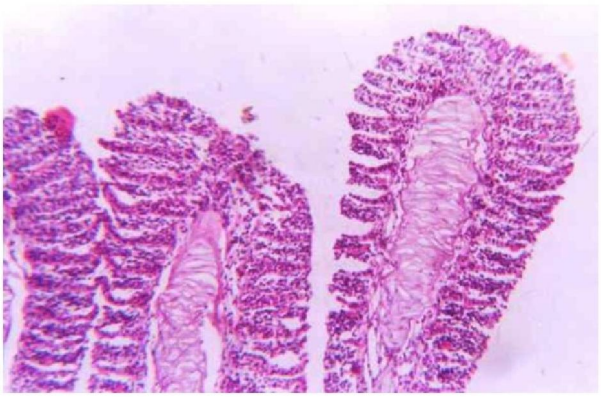

Fig. (6): Gills of *Oreochromis niloticus* after 7 weeks of edifenphos toxicosis showing severe epithelial hyperplasia particularly at the tips of gill filaments and diffuse filamentous clubbing due to fusion of the secondary lamellae. H&E. (X 160).

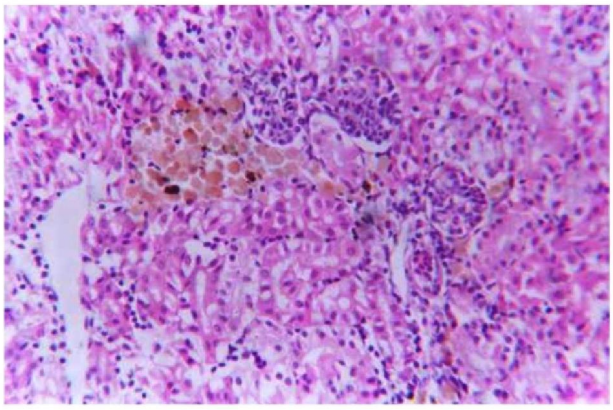

Fig. (7): posterior kidney of *Oreochromis niloticus* after 5 weeks of edifenphos toxicosis showing diffuse tubular vacuolar degeneration and focal necrosis with activation of the melanomacrophage centers. H&E. (X 400).

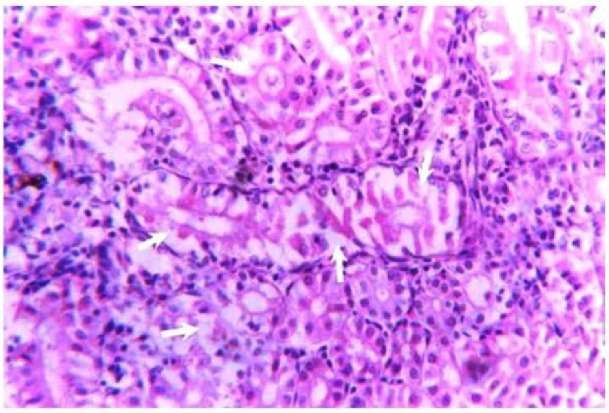

Fig. (8): Posterior kidney of *Oreochromis niloticus* after 6 weeks of edifenphos toxicosis showing vacuolation of the tubular epithelium (arrows). H&E. (X 400).

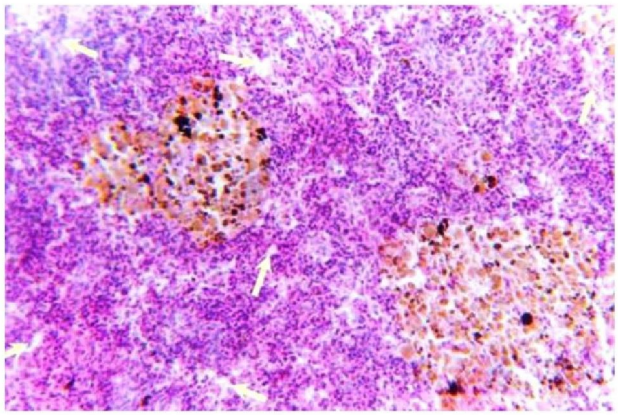

Fig. (9): Spleen of *Oreochromis niloticus* after 3 weeks of edifenphos toxicosis showing marked hyperactivation of the melanomacrophage centers and slight lymphocytic cell depletion (arrows). H&E. (X 250).

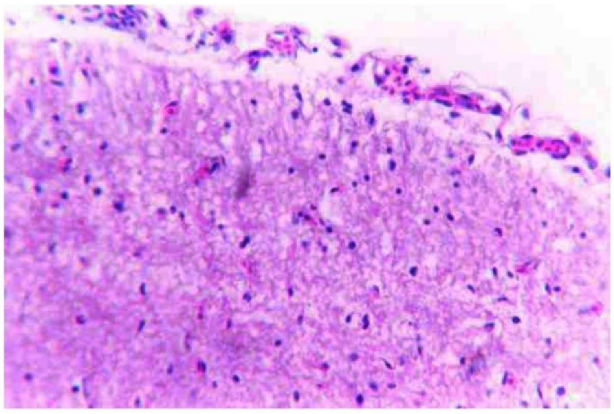

Fig. (10): Brain of *Oreochromis niloticus* after 3 weeks of edifenphos toxicosis showing congestion of meningeal blood vessels. H&E. (X 400).

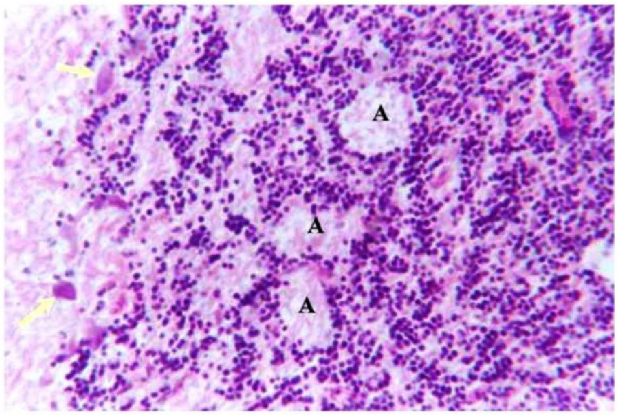

Fig. (11): Cerebellum of *Oreochromis niloticus* after 4 weeks of chronic edifenphos toxicosis showing pyknotic Purkinje cells (arrows) and necrotic areas (**A**) in the inner granular layer.H&E. (X 250).

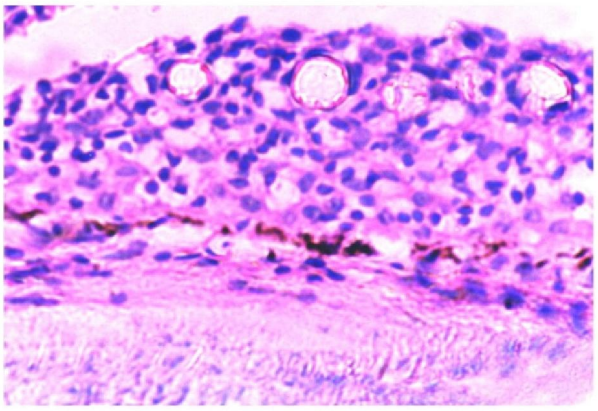

Fig. (12): Skin of *Oreochromis niloticus* after 5 weeks of edifenphos toxicosis showing hyperactivation of the epidermal alarm and mucous cells and mild vacuolar degeneration in epidermal cells. H&E. (X 400).

be attributed to that edifenphos is a potent anticholinesterase which results in accumulation of acetylecholine at the synapsis of neurons leading to nervous manifestations. Decreased ACHase activity may be used as boindicator of pollution by such pesticides in the environment.

The hematological changes during the chronic toxicosis of edifenphos showed a significant decrease mostly during the entire period in Hb, RBCs count and PCV, this reveals the prominent anemic effect of edifenphos which is confirmed by the results of the blood indices. Which in turns revealed the hemolytic effect of edifenphos, and may explain the chocolate discoloration of parynchymatus organs, as hemoglobin may be converted into methemoglobin with resultant hemolysis and reduced blood oxygen carrying capacity which accumulates with the irritant effect of edifenphos causing respiratory distress to the fish? The severity of anemia also is magnified by the hypoproteinemic effect showed by edifenphos.
The haemolytic and destructive effects of the pesticides on blood cells was supported by El-Boushy (1994) and Robert (2001).

The marked decrease in RBCs count was in agreement with those reported by other workers, Rani *et al.* (1987) and Venkateshwarthlu *et al.* (1990) proved these changes in blood parameters in catfish intoxicated with the organophosphate pesticide dichlorvos.

The histopathological changes during the chronic toxicosis of edifenphos were various. The hepatic tissue showed congestion with various degrees of degenerative changes starting firstly with granular degeneration then vacuolar degeneration with progression towards hepatic cell necrosis after 1 week of exposure. These changes may be attributed to direct toxic effect of edifenphos on hepatocytes since the hepatopancreas is the site of detoxification of all types of toxins and chemicals (Robert, 2001).

Congestion and various degrees of pathological harm in gills were evident. The firstly observed lesion was lamellar edema which is frequent following exposure to chemical pollutants. Complete edematous separation of the respiratory epithelium of primary and secondary lamellae with necrosis of lamellar epithelial cells and severe, often lethal, respiratory and osmoregulatory distress may supervene (Yang and Albright, 1992).

Also severe epithelial proliferation of secondary gill lamellae, which resulted as a response of the malpighian cells to chemical irritation, as they migrate distally, often in the early stages, resulting in an accumulation of cells at the leading edge of the secondary lamella, progression of this migration leads to lamellar fusion and terminal lamellar clubbing (Robert, 2001). This may be attributed to that edifenphos has a direct effect on gill filaments as cytotoxic and irritating substance which resulted in proliferation and fusion of secondary lamellae. Moreover, gills are important not only for gaseous exchange but also for osmoregulation and excretion of toxic waste products (Robert, 2001), thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes . Hyperplasia may in some situations represent an adaptation by the organism to protect underlying tissues from any irritant. However, increased thickness of the epithelial layers including mucous cell hyperplasia and fusion of adjacent secondary lamellae as the result of hyperplasia will not only decrease the surface area available for oxygen extraction but also will increase the oxygen diffusion distance between water and blood (Kumaraguru *et al.*, 1982). Also exposure to pollutants, including pesticides can cause rupture of the retaining pillar, or pilaster cells, which normally join the dorsal surface of secondary lamellae to the ventral one. The result will be dilation of the lamellar capillary and pooling of the blood, thrombosis and eventually fibrosis. Fusion with adjacent lamella, leads to the telangiectasis which is a characteristic pathological change of the gill associated with physical or chemical trauma (Robert, 2001).

The skin of the fish showed varied degrees of vacuolar degeneration of epidermal cells, proliferation of club cell and hyperactivation of the melanophores this may be attributed to that being in contact with edifenphos causes direct cytotoxic and irritating effect on dermal cells

The renal tissue of posterior kidney exhibited congestion, diffuse granular and vacuolar degenerative changes and focal hyaline droplet degeneration after 1 week of exposure and the marked depletion in haemopoietic elements which was evident in spleen and anterior and posterior kidney were probably caused by direct cytotoxic effect of edifenphos.

As neurotoxin, edifenphos caused degenerative effect on the brain tissue as revealed in this study appeared as severe congestion of cerebral blood vessels with neuronal degeneration led to necrotic areas in the brain tissue.

The activation of melanomacrophage centers either in spleen, hepatopancreas, or anterior and posterior kidney was a prominent and constant lesion. It is quite known as an unusual sequel to infection or irritation in fish belonging to fish immune response (Robert, 2001).

From the results of the present work, it can be concluded that:

Edifenphos should be listed under the highly toxic pollutants to *Oreochromis niloticus* fish even at sublethal dose (1 ppm) where it may cause toxicity or death, not only to rice fungi but also to fish.

Prolonged exposure of *Oreochromis niloticus* to low **doses** of edifenphos pesticide (0.1 ppm) caused damage in kidney, liver, spleen and gills tissues.

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