SOME STUDIES ON CATFISH EXPOSED TO THE HERBICIDE ATRAZINE

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ABSTRACT

This study was achieved to study the effects of atrazine on some selected blood hematological, serum components and associated histopathological studies in catfish. Investigations of acute and long term toxicity of atrazine in catfish (Clarias gariepinus) were carried out. Acute toxicity was applied in a semi-static test during a 96-hr exposition. Mortality was recorded after 24, 48, 72 and 96 hrs. Lethal concentration inducing 50% mortality (LC-50) was calculated. Long term toxicity was investigated on 70 Catfish divided randomly into two groups of 35 each .The first group was kept as control (normal water), the second was exposed to 4.5 mg/L atrazine for four weeks. Sampling was carried out at 0, 7, 14 and 28 days. Biochemical, hematological and histopathological changes in certain organs and tissues were investigated. The results showed that, the estimated LC-50 value was 18.1 mg/1. Hematological results showed significant decrease of RBCs, hemoglobin and haematocrit value and changes of MCV, MCH and MCHC as compared with the control groups. Biochemical changes revealed significant reduction in levels of glucose, total protein, cholesterol, total lipids and triglycerides than the control ones .Most prominent enzymatic changes were in the alkaline phosphatase, transaminases, and acetylcholine activities whereas the most severe histopathological changes were observed in the fish gills followed by liver, kidney and intestine. With increase of the time of exposure to atrazine, gill lesions and other lesions of the different organs increased in severity.

INTRODUCTION

Despite some pollutants have been already banned in many countries, they are still found in the environment *(Stevens et al., 2003 and Oliveira Ribeiro et al., 2005; 2007)*. Aquatic herbicides are widely used for controlling undesirable weeds *(Maroni and Bersani, 1994)*. The use of herbicides to control aquatic weeds has applied in fish management where they used in aquatic habitats especially rice fields and some fish farms. Atrazine (2-chlor-4-ethylamino-6-isopropyl-amino-S-triazine) is considered the main aquatic herbicide used *(Brusick, 1994)*. It is one of the most significant water pollutants in rain, fresh, marine and ground waters *(Tasli et al., 1996)*. It enters the aquatic system through run-off from agricultural fields or directly through careless application. It has been found display in different sources: up to 3.5 µg/L in rainwater, up to 1.25 µg/L in surface waters, more than 0.5 µg/L in ground water, exceeding of 0.1 µg/L for drinking water in Germany while in USA was 88.4 µg/L, and up to 69.4 µg/L in surface water. Moreover, the high concentrations normally occur in the environment for a short period after accidents *(Fischer-Scherl et al., 1991)*. Third world countries have the problem of adverse effects of these chemical compounds in ecosystem on fish production *(Parkinson and Agius, 1988)*.

Similar to other herbicides, atrazine is a potential contaminant of surface and ground water; it is known to have high mobility through soil, so atrazine may easily find a way to the water *(Marchini et al., 1988)*. In water, this chemical is practically unaffected by microbial or hydrolytic degradation processes *(Gamble et al., 1983)*. Thus any data concerning their toxic effects on aquatic organisms are very helpful for the assessment of the dangers. A chemical toxicity stress leads to a depletion

of energy reserves, resulting in reduced growth and or reproduction *(De Coen and Janssen, 2003)*. Herbicide exposure produces oxidative stress in fish hemopoietic organs *(Fatima et al., 2007)*.

The application of herbicides has hazard effects on the fish beside the change of available plankton food *(Mason, 1991)*. The effects of insecticides on fish are well documented *(Hurlbert et al., 1970 and Carter and Graves, 1973)*; however, the effects of herbicides on fish as well as other non-target aquatic organisms are not as well documented. Atrazine persists particularly in anaerobic or denitrified soils *(Jones et al., 1982; Topp et al., 1995 and Tasli et al., 1996)* and in some aquatic systems at biologically effective levels for several weeks *(De Noyles et al., 1982 and Pratt et al., 1997)*. Consequently, it is likely to cause chronic effects due to lifelong exposures of non-target organisms *(Hussein et al., 1996)*. Atrazine affects the hydromineral balance and gill function in crabs *(Prasad et al., 1995 and Silvestre et al., 2002)*, as well as affecting the haematology *(Hussein et al., 1996)*; metabolism *(Srinivas et al., 1991 and Prasad et al., 1995)*; Physiological processes of fish. *(Waring and Moore, 2004)*. Animals have been found to accumulate it in a variety of tissues *(Gunkel and Streite, 1980 and Du Preez and Van Vuren 1992)*.

Hematological tests and analysis of serum constituents have proved to be useful in the detection of the stress and metabolic disturbances. Atrazine effects could be evaluated through hematological tests since RBCs, hemoglobin and haematocrit were significantly affected by the exposure of fish to atrazine *(Prasad et al., 1991)*. *Egaas et al. (1993)* declared that the inhibition of AChE activity is a useful indicator of fish exposure to atrazine *(Chaturvedi, 1993)*. Moreover, atrazine plays an important role in osmoregulation since it control urinary excretion and stress on the gills and kidney *(Catenacci et al., 1993)*.

Atrazine provokes certain histopathological changes in some organs and tissues of fish. These changes vary depending on the organ, the parameter tested, time of exposure and atrazine concentration *(Neskovic et al., 1993)*.

There are little data on atrazine effects on fresh water fish although it's widely used. Therefore, the objective of this study was to achieve atrazine effects on some selected serum components and associated histopathological studies in catfish.

MATERIAL AND METHODS

Chemicals:

Atrazine (2-chlor-4-ethyl-amino-6-isopropyl-amino-s-triazine) as powder ingredient (96% purity) was obtained from Plant Protection Dept., Faculty of agriculture, Suez Canal University. It was dissolved in distilled water to a saturated mixture, filtered and added constantly to aquarium at rate of 3 and 6 mg atrazine/L water according to *Pluta, (1989)*. The test water was replaced daily along with the required amount of stock solution to prevent deterioration of water quality and to replenish atrazine levels.

Fish:

Catfish (*Clarias gariepinus*) with an average body weight 90-100 g/fish were used in this study. Fish were reared in aerated glass aquarium (40 x 50 x 70 cm) and acclimatized for two weeks in dechlorinated tap water (pH 6.95, DO 5.5 mg/L, 150 mg/L as CaCo3) before being used in the experiment. Fish were fed with commercial pellets.

Experimental design:

Acute toxicity:

Acute toxicity was tested according to the OECD procedures *(OECD guidelines. No. 203, 1992)* semi static test. Fish were exposed to different concentrations of atrazine for 96 hrs. Mortality was recorded after 24, 48, 72 and 96 hrs. Lethal concentration inducing 50% mortality (LC-50) was calculated by *Stephan C.E. (1977).*

Long term toxicity:

Seventy apparently health fish were divided randomly into two groups of 35 each .The first group was kept as control (normal water), the second was exposed to 4.5 mg/L atrazine for four wk according to *(OECD guidelines. No. 204, 1992)*. Five fish samples from both the experimental and control groups were sacrificed at 0, 7, 14 and 28 days. Toxicoclinical signs as well as changes in fish behavior were observed and recorded.

Blood samples:

Blood samples were taken from the caudal vein of live fish per each group in a clean dry vials, a portion (1 ml) was mixed well in a clean dry vials contained EDTA as an anticoagulant (1.5 mg/ml) according to *McKnight, (1966)*. The remaining blood was transferred to plastic vials. The blood left to clot then centrifuged at 3000 r.p.m for 15 minutes. The separated serum samples were stored at - 20°C until analysis for biochemical analysis.

Hematological analysis:

Total red blood cells were counted *(Natt and Herrich, 1952).* Hemoglobin concentration and packed cell volume (PCV) were estim-

ated *(Wintrobe, 1967)*. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to *Coles, (1986)*.

Serum Biochemical analysis:

Serum total protein was determined *(Doumas, 1981)*. Fractionation of plasma proteins was performed *(Laemmli, 1970)*. Determination of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were done *(Reitman and Frankle, 1957)*. Serum urea and creatinine was determined *(Chancy and Marbach, 1962* and *Husdan and Rapoport, 1968)*. Glucose was determined according to *Trinder, (1969)*, Triglycerides *(Rice, 1970)*, alkaline phosphatase *(Bessey et al., 1946)* and cholesterol *(Pearson et al., 1953)*.

Gross pathology and histopathology:

All selected fish were examined immediately after sacrificing. The abdominal and thoracic walls were incised, macroscopical pathology was evaluated and specimens of gills, livers, kidneys, intestine, spleen, heart, skin and muscles were taken. Tissue specimens were immediately immersed in 10% phosphate buffer formalin at room temperature and fixed for at least 48 h before processing. Fixed specimens were dehydrated through graded alcohols and embedded in paraffin wax; 3-5 µm thick sections were cut, stained with haematoxylin and eosin (H&E) according to *Bancroft et al. (1996)* and then examined with a light microscope.

Statistical analysis:

Data were statistically analyzed using *SAS, (1989)*.

RESULTS

The results of acute toxicity testing of the herbicide atrazine on the catfish (*Clarias gariepinus*) are presented in Table (1). Acute toxicity was examined in a semi-static test for 24, 48, and 96 hours. Acute toxicity at 24 and 48 hrs of exposure was the same with LC-50 values of 39.6 mg/1. After 96 hr, however, it was considerably greater, with an LC-50 of 18.1 mg/1.

Fish exposed to atrazine in concentrations of 4.5 mg/L exhibited change in colour from pinkish grey to dark grey. A rapid respiration and increased rate of gill cover movements, slow-down of reflexes and impairment of swimming performance , sinking to the bottom of aquaria were evident 14 days post exposure. Reduction in the feeding activities was also observable in exposed fish. Before death, fish floated at the surface of water gasping for more oxygen, very rapid movements in various directions. About 25% of the treated fish had ascites in the two groups at 14, 28 days exposure. The mortality rate was 10%. The RBCs, hemoglobin concentration and haematocrit percentage of catfish which exposed to 4.5 mg/L atrazine were recorded in Table (2). Significant (p<0.01) decrease of RBCs, hemoglobin and haematocrit as compared with the control group. There were significant (P<0.01) changes of MCV, MCH and MCHC. Results of some serum components for catfish after the exposure to atrazine were illustrated in Table (3); there were significant reduction in levels of glucose, total protein, cholesterol, total lipids and triglycerides than the control ones. Urea level was significantly increased than control.

Changes in the activity of certain enzymes in fish exposed to atrazine concentrations were recorded (Table 4). Most pronounced changes were in the AP activity. In all serum examinations, AP demonstrated a statistically significant increase (P < 0.01) in relation to

the control. The gross pathology revealed congestion in the kidney, spleen and the liver during first week post exposure. The liver was friable and yellowish in colour at 14 and 28 days post exposure.

Histopathological results:

Acute toxicity:

Histopathological examination has shown that, all the changes found in the internal organs of catfish (*Clarias gariepinus*) following acute exposure to atrazine could be considered to be moderate pathological responses.

Long term toxicity:

Among the examined organs the greatest variety of changes was found in gills followed by liver, kidney and intestine. The gills 7th day post exposure (PE) showed congestion and hyperplasia of the epithelial cells causing thickening of the lamellar epithelial cells (Fig. 1). Others showed edema, telangectasis, desquamation and sloughing of some lamellar epithelium as well as shortening and thickening of some secondary lamellae (Fig. 2). On 14th day PE, gills showed cellular vaccuolation of secondary lamellar epithelium (Fig. 3). Others suffered congestion, leucocytic inflammatory cells infiltration in its arch and lamellae with partial fusion of secondary lamellae (Fig. 4). The gills 28th day PE revealed severe destructive changes as severe congestion, telangectasis, epithelial cell vacuolation, subepithelial edema, fusion of secondary lamellae as well as sloughing and necrosis of lamellar epithelium (Fig. 5).

The liver 7th day PE revealed congestion, edema and vacuolar degeneration (Fig. 6). On 14th day PE, it showed severe dilatation of the central vein, severe congestion, necrobiotic changes of hepatocytes and

some mononuclear cells infiltration around the blood vessels and central vein (Fig. 7), as well as hyperplasia of the pancreatic cells (Fig. 8). On 28th day PE, the liver exhibited edema, coagulative necrosis of the hepatocytes, mild portal fibrosis and perivascular inflammatory cells aggregation (Fig. 9).

The kidney of catfish 7th day PE exhibited tubular nephrosis, atrophy of the glomeruli and renal tubules, mononuclear cells infiltrations in the interstitium and severe congestion of the renal blood vessels (Fig. 10), as well as interstitial nephritis, distention of Bowman's space and vacuolar degeneration of the renal tubules (Fig. 11). On 14th day PE, it showed hyper cellularity of the glomeruli, vacuolar degeneration and various necrobiotic changes of the renal tubular epithelium as well as active proliferation of melanomacrophages (Fig. 12). On 28th day PE the kidney displayed severe congestion, edema, fibrous tissue proliferation around the blood vessels, mononuclear cells infiltrations in the interstium as well as severe necrosis and degenerative changes of the renal tubules and glomeruli (Fig. 13).

The intestine 7th day PE displayed mucinus degeneration, edema, congestion in lamina propria, sloughing and necrosis of some mucosal epithelium (Fig. 14 & 15). At 14th day PE, it revealed hyperplasia of the intestinal villar epithelium with goblet cells proliferation, edema and severe congestion of the intestinal blood vessels in lamina propria and submucosa (Fig. 16). 28th day PE, it showed some mononuclear cells infiltrations in lamina propria, severe necrosis of the villar epithelium as well as edema and necrosis of the muscular layer (Fig. 17).

In the heart, 7th day PE, congestion and edema between the myofibers were observed (Fig. 18). 14th day PE, it showed edema, myocardial necrosis and pericarditis (Fig. 19). Zenker's necrosis, hyaline degeneration and severe edema were seen at 28th day PE.

Spleen suffered different degrees of histopathological lesions along the experimental period as congestion, edema and lymphcytic depletion.

Skin exhibited edema and some leukocytic cells infiltrations in the dermis 7th day PE in addition to epidermal sloughing 14th day PE (Fig. 20). Activation of melanomacrophages was observed 28th day PE (Fig. 21). The muscles of catfish that exposed to atrazine suffered focal granular cells infiltrations in between the muscular bundles 28th day PE (Fig. 22).

Table (1): acute toxicity (LC-50) of the herbicide atrazine to Catfish (*Clarias gariepinus*).

Herbicide	Duration of exposure (hr)	LC-50 (mg/1)
Atrazine	24	39.6 (36.2-43.0)*
	48	39.6 (35.5-43.7)
	96	18.1 (16.7-19.5)

Table (2): Means of hematological components for Catfish (*Clarias gariepinus*) after the exposure to atrazine.

Haematology	Control			Atrazine 4.5 mg/l		
	7 days	14 days	28 days	7 days	14 days	28 days
RBCs	1390000	1460000	1380000	820000	720000	600000
Haemoglobin (g/L)	6.02± 2.56	6.34± 2..34	6.01± 2.16	4.44± 0..76**	3.18± 1.06**	2.84± 0..56**
Haematocrit (%)	30.5± 3.76	35±3.25	31± 3.16	22± 1.46**	20± 1.76**	18± 1.06**
MCV (fL)	218.4± 14.56	237.55± 14.76	224.6± 11..56	268.31± 13.71**	277. 86± 12.46**	300.0± 15.22**
MCH (pg)	43.21± 3.25	44.53± 3.25	43.5± 3.25	53.73± 2.20**	44.26± 1.95**	47.31± 2.15**
MCHC (%)	19.72± 1.25	18.66± 1.25	19.43± 1.25	20.0± 1.00**	15.92± 0.95**	15.84± 0.9**

** P <0.01

Table (3): Means of serum components for Catfish (*Clarias gariepinus*) after the exposure to atrazine.

Haematology	Control			Atrazine 4.5 mg/l		
	7 days	14 days	28 days	7 days	14 days	28 days
Glucose (mg/dL)	51.63± 2.77	52.40± 2.79	51.37± 2.65	32.79± 1.45**	30.64± 1.45**	28.05± 1.55**
T.protein (g/100 mL)	3.89± 0.06	3.85 ± 0.08	3.42± 0.07	2.66± 0.08*	1.83± 0.03**	1.37± 0.02**
Albumin (g/100 mL)	0.97± 0.13	0.76± 0.08	0.80± 0.11	0.57± 0.07**	0.60± 0.09**	0.30± 0.06**
Globulin (g/100 mL)	2.92± 0.09	3.09± 0.10	2.62± 0.14	2.09± 0.05*	1.23± 0.08**	1.07± 0.03**
A/G ratio	0.33	0.24	0.31	0.27	0.49	0.28
Cholesterol (mg/dL)	161.34± 14.22	221.73± 11.27	235.74± 19.12	178.7± 10.22*	191.3± 9.52**	182.6± 9.98**
Triglycerides (mg/100 mL)	209± 11.22	323.76± 14.56	437.76± 19.32	142.9± 10.22**	20.52± 0..82**	37.24± 0..89**
T.lipids (g/L)	27.83± 2.82	26.98± 1..77	27.0± 3..51	18.86± 0..92**	7.91± 0..12**	6.92± 0..09**
Urea (mg/100 mL)	8.88± 0.74	7.30± 0.75	7.86± 0.82	11.58± 0.35**	9.6± 0.56**	14.20± 0.45**

* P <0.05   **** P <0.01**

Table (4): Enzyme activities in serum in Catfish (*Clarias gariepinus*) after the exposure to atrazine.

Haematology	Control			Atrazine 4.5 mg/l		
	7 days	14 days	28 days	7 days	14 days	28 days
ALT U/L	48.45± 2.5	47.85 ± 2.28	48.15± 2.11	65.40± 1.5**	79.39± 1.78**	89.11± 2.7**
AST U/L	130.78± 2.21	132.02± 1.98	131.05± 2.39	158.25± 2.56**	181.44± 2.88**	192.56± 3.19**
♠Serum AChE (IU)	456.50± 14.56	423± 13.32	536.2± 19.32	451.7± 12.32**	220.8± 10.02**	170.0± 9..32**
Alkaline phosphatase U/L	26.0 ± 1.5	26.05 ± 1.2	27.0 ± 2.03	45.5 ± 3.0**	51.9 ± 2.9**	52.0± 3.1**

♠ Serum AChE = serum Acetylcholinestrase   **Mean ± SE of 10 fish**   **** P <0.01**

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Fig. (1): Gills of catfish 7th day PE to atrazine showing congestion and hyperplasia of the lamellar epithelial cells. H&E stain. X 250.

Fig. (2): Gills of catfish 7th day PE showing edema, telangectasis, congestion, shortening and thickening of some lamellae, desquamation and sloughing of some lamellar epithelium. H&E stain. X 250.

Fig. (3): Gills of catfish 14th day PE showing vaccuolation of secondary lamellar epithelial cells. H&E stain. X 400.

Fig. (4): Gills of catfish 14th day PE showing congestion, leucocytic inflammatory cells infiltration with partial fusion of secondary lamellae. H&E stain. X 400.

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Fig. (8): liver of catfish 14th day PE showing sever congestion and hyperplasia of pancreatic cells. H&E stain. X 250.

Fig. (9): liver of catfish 28th day PE showing edema, coagulative necrosis, mild portal fibrosis and perivascular inflammatory cells aggregation. H&E stain. X 400.

Fig. (10): kidney of *Clarias gariepinus* 7th day PE showing tubular nephrosis, atrophy of the glomeruli and renal tubules, mononuclear cells infiltrations in the interstitium and severe congestion of the renal blood vessels. H&E stain. X 100.

Fig. (11): kidney of catfish 7th day PE showing interstitial nephritis, distention of Bowman's space and vacuolar degeneration of the renal tubules. H&E stain. X 250.

Fig. (12): kidney of catfish 14th day PE showing hyper cellularity of the glomeruli, vacuolar degeneration, various necrobiotic changes of the renal tubular epithelium and active proliferation of melanomacrophages. H&E stain. X 400.

Fig. (13): kidney of catfish 28th day PE showing severe congestion, edema, fibrous tissue proliferation around the blood vessels, mononuclear cells infiltrations in the interstitium and severe necrosis of the renal tubules and glomeruli. H&E stain. X 250.

Fig. (14): Intestine of catfish 7th day PE to atrazine showing mucinus degeneration, edema, congestion, sloughing and necrosis of some mucosal epithelium. H&E stain. X 100.

Fig. (15): Higher magnification of figure (13). H&E stain. X 400.

Fig. (16): Intestine of catfish 14th day PE showing hyperplasia of the intestinal villar epithelium with goblet cells proliferation, edema and severe congestion of the intestinal blood vessels in lamina propria and submucosa. H&E stain. X 250.

Fig. (17): Intestine of catfish 28th day PE showing congestion, mononuclear cells infiltrations in lamina propria, severe necrosis of the villar epithelium as well as edema and necrosis of the muscular layer. H&E stain. X 250.

Fig. (18): Heart of catfish 7th day PE to atrazine showing congestion and edema between the myofibers. H&E stain. X 250.

Fig. (19): Heart of catfish 14th day PE showing edema, myocardial necrosis and pericarditis. H&E. stain. X 250.

Fig. (20): Skin of catfish 14th day PE showing edema, some leukocytic cells infiltrations in the dermis and epidermal sloughing. H&E stain. X 250.
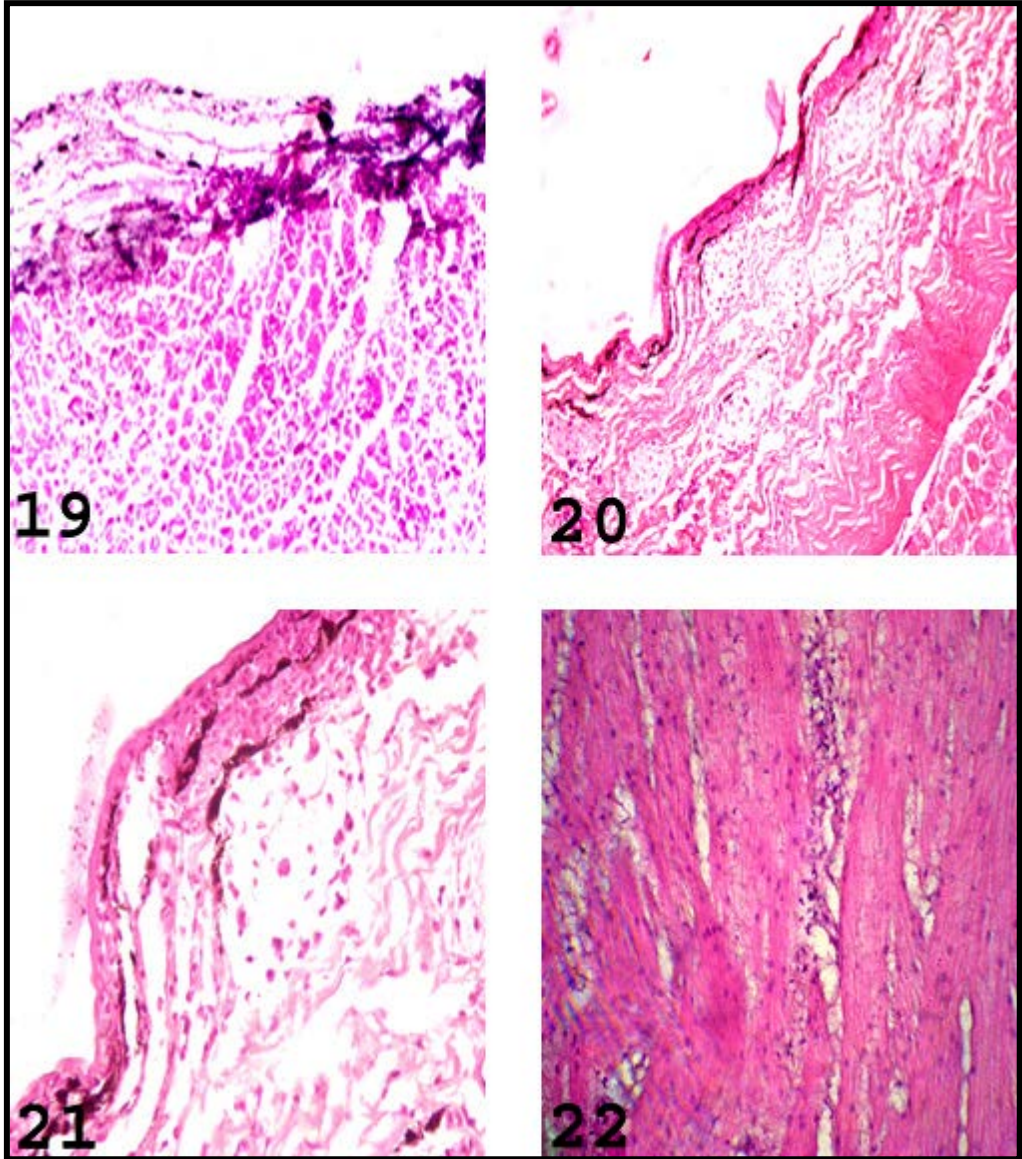
Fig. (21): Skin of catfish 28th day PE to atrazine showing activation of melanomacrophages. H&E stain. X 400.

Fig. (22): Muscles of catfish 28th day PE showing focal granular cells infiltrations in between the muscular bundles. H&E stain. X 250.
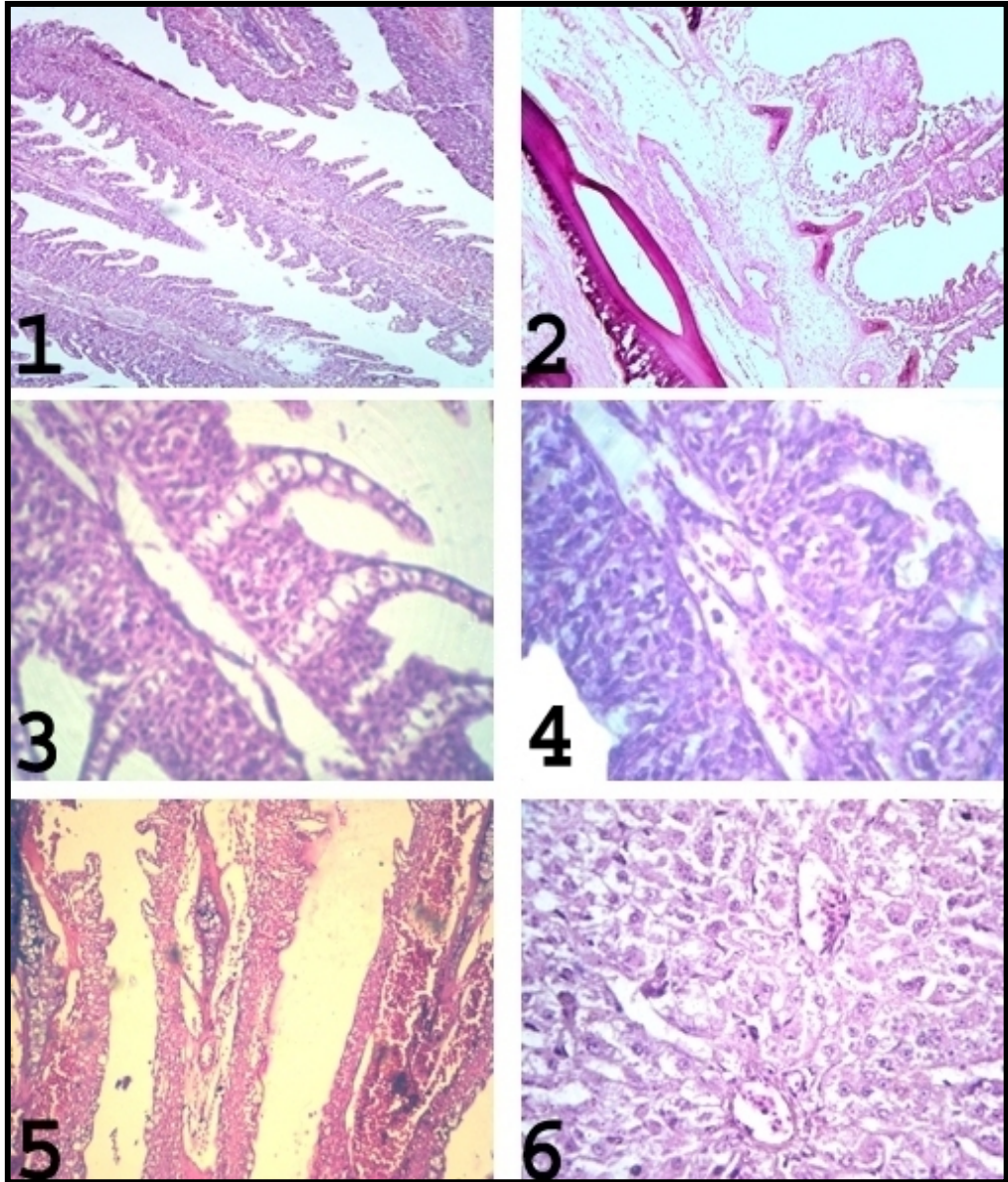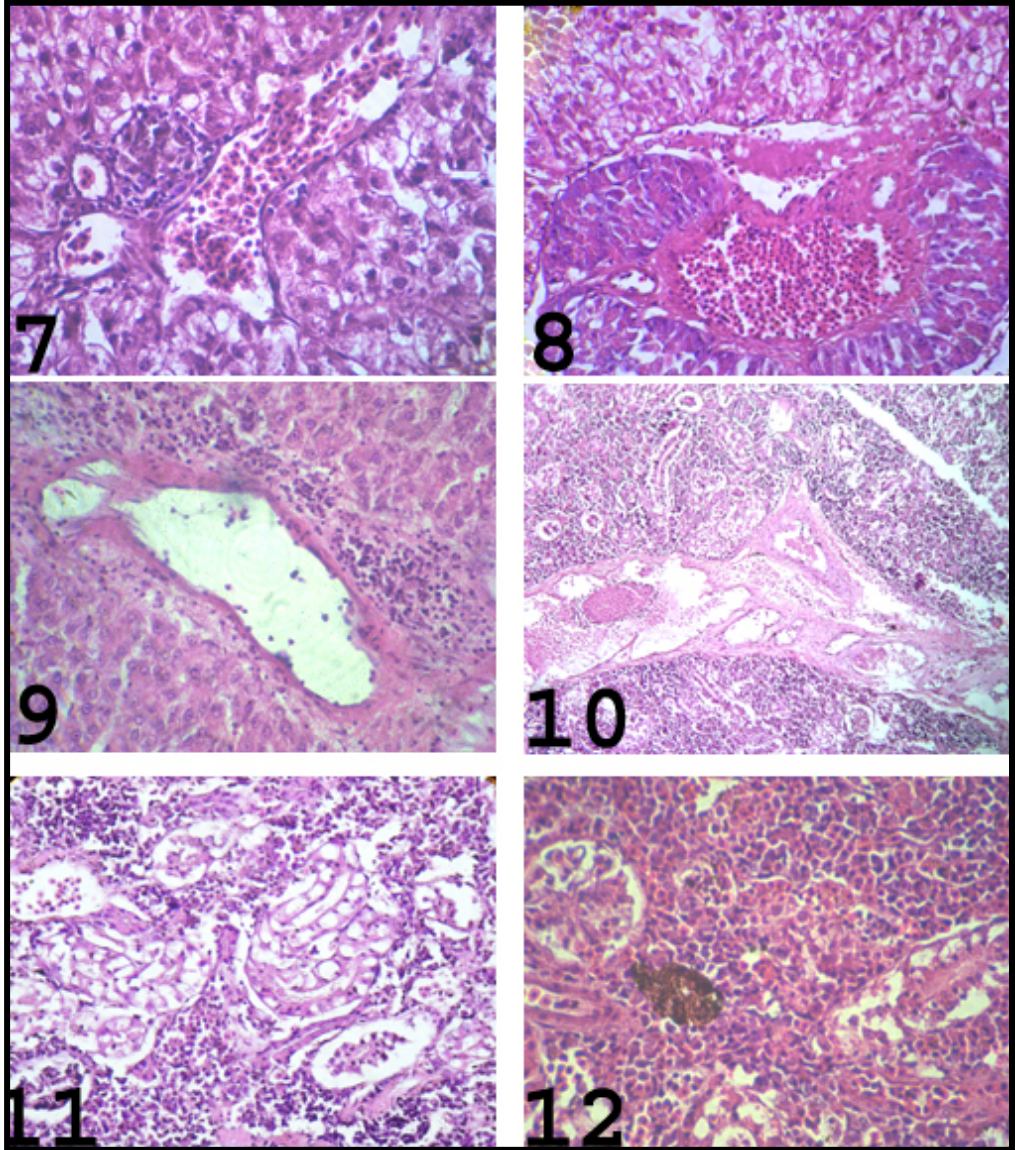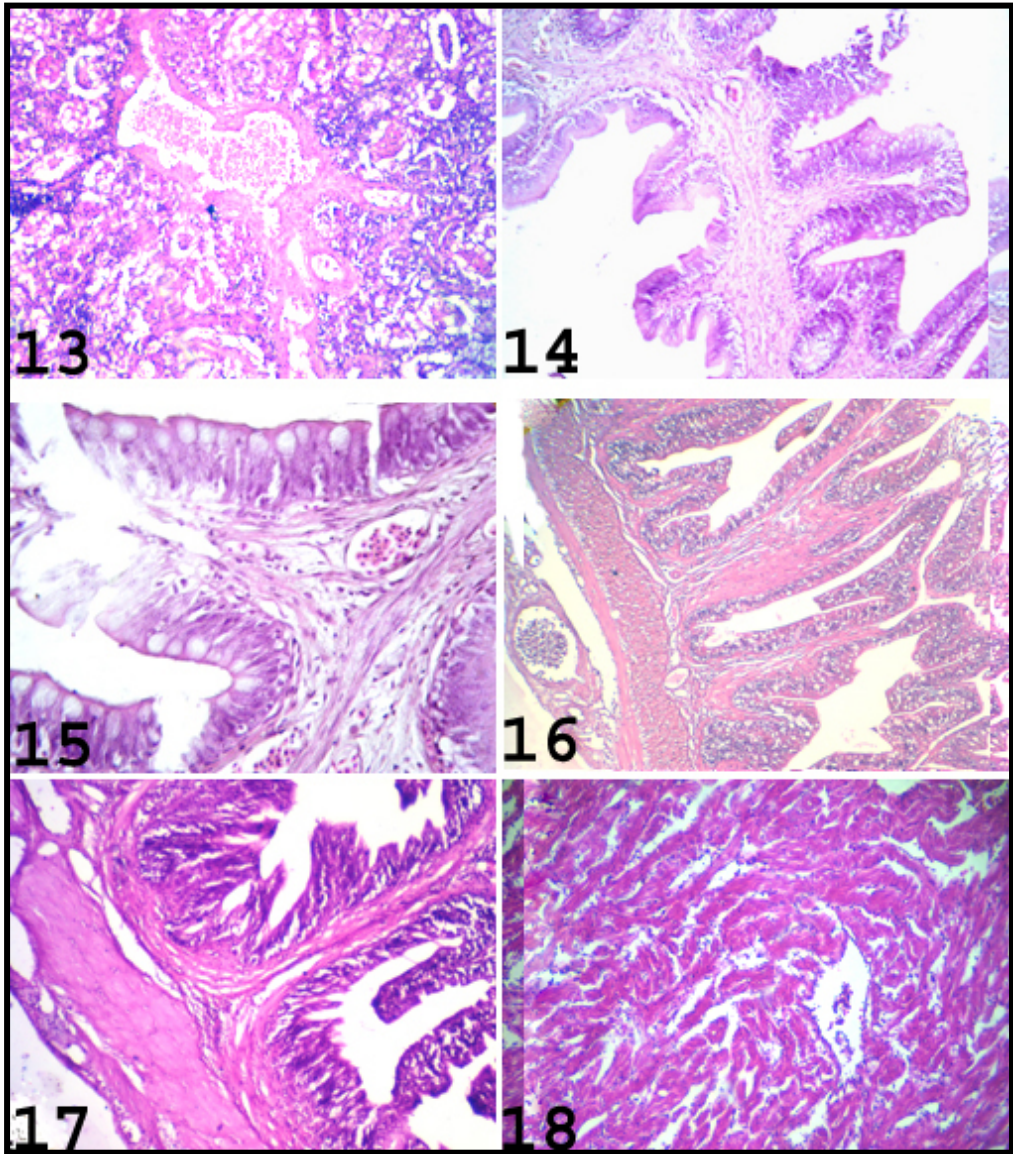
DISCUSSION

Fish are susceptible to pesticides dissolved in water and can be exposed to these chemicals through gills and skin and by contaminated food *(Bisson and Hontela, 2002)*.

The results of investigations on acute toxicity of atrazine to fish found that LC-50 for catfish (*Clarias gariepinus*) was 18.1 mg/l. This obtained result agreed with the LC50 value obtained by *Neskovic et al. (1993)* (18.8 mg/L) and *Phyu et al. (2006)* (14.8 mg/l), and much less with that recorded by *Hussein et al., (1996)*. Where the LC50 of atrazine was 9.37 and 6.37 mg/L for *Oreochromis niloticus* and *Chrysichthyes auratus*. *While Tomlin, (2000)* found that LC50 ranged from 4.5-11 mg/l for *rainbow trout*. This difference may be due to the difference in water temperature since the present results obtained under about 15°C but *Hussein et al. (1996)* results obtained at about 22°C and **Neskovic's** results obtained at about 15°C. The difference in LC50 may be due to the fish species. Where, *Hussein et al. (1996)* applied the experiment on two fish species *Oreochromis niloticus* and *Chrysichthyes auratus* which differ from that used in this study. Moreover, the difference in LC50 may be explained by the fish lipid content. The increase of lipid content of the fish may be the reason of mortality due to stress action *(Pluta, 1989)*.

Concerning with long term toxicity, fish exhibited change in color from pinkish grey to dark grey, rapid respiration and increased rate of gill cover movements, slow-down of reflexes and impairment of swimming performance, sinking to the bottom of aquaria were evident 14 days post exposure. Reduction in the feeding activities was also observable in exposed fish. *De Coen and Janssen, (2003)* stated that, a chemical toxicity stress leads to a depletion of energy reserves resulting

in reduced growth and or reproduction. Before death, fish floated at the surface of water gasping for more oxygen, very rapid movements in various directions. Similar signs were observed by ***Hussein et al. (1996).*** These observed signs could attribute to the increase of norephinepherne and dopamine in brain ***(Bretaud et al., 2002),*** or the decrease of acetylcholine activity ***(Katherine et al., 2007)*** which goes in accordance with decreased serum acetylcholine activity in this study.

Haematological examination revealed that, exposure of fish to 4.5 mg/L atrazine resulted in significant decrease of RBCs, hemoglobin and haematocrit as compared with the control group. These results agreed with ***Hussein et al. (1996)***. However, it is obvious that the significant decrease in RBCs, hemoglobin and haematocrit in exposed fish to atrazine may be attributed to the hemolytic and destructive effect of atrazine on erythrocytes. The histopathology confirmed these findings. Moreover, atrazine may lower the water oxygen contents. It was reported that the oxygen tension in the water is decreased in presence of herbicide ***(Olli Ojala, 1966).*** It is well known that reduced oxygen tension lead to anoxia and mortality in fish. Also, ***Prasad et al. (1991)*** showed significant changes in hematological constituents such as RBCs, Hb, PCV, MCV, MCH and MCHC of fish exposed to atrazine.

Guthmann, (1989) reported that hematological parameters for *rainbow trout* exposed to atrazine decreased significantly in the total number of erythrocytes and haematocrit. This can be explained by the morphological alterations and signs just before death. Serum glucose level, was significantly decreased ($P<0.05$). This decrease could be attributed to the toxic effect of atrazine on the liver ***(Braunbeck et al., 1992).*** Reduction in food intake of the treated fish where, the diet is the main source of energy.

Serum total protein and globulin tended to decrease significantly (P<0.05). The decrease of total protein was mainly due to globulin. This means that atrazine has toxic effects on immune system in these fishes. Also there was significant decrease (P<0.01) in albumen. These changes in total protein, albumin and globulin as affected by atrazine exposure can be explained by different theories. **Santa Maria et al. (1986)** showed that atrazine exposure led to increase of protein amount in the urine. Moreover, atrazine has harmful effects on spleen, liver and anterior kidney *(Rehwoldt, 1978)*.

Katherine et al. (2007) reported that, atrazine cause hyperplasia and fusion of the secondary lamellae in the gill epithelium of carp that, in turn, affects osmoregulation in addition impair kidney function by causing degeneration of the proximal tubules which may increase the renal excretion of ions and protein. Moreover, the exposure of *Cyprinus carpio* to 1.5, 3.0 and 6.0 mg/L atrazine for 14 days resulted in biochemical changes such as alkaline phosphatase, and transaminase activities *(Neskovic et al., 1993),* all of these opinions' goes parallel with our result.

Serum cholesterol decreased significantly in exposed groups. Both triglycerides and total lipids were significantly decreased in fish exposed to atrazine over the experimental duration period. This result agreed with **Hussein et al. (1996)** and **Katherine et al. (2007).** This reduction could be due to decreased glucose availability in exposed fish. Glucose is essential for triglycerides synthesis because it forms alpha glycerophosphate which is the specific precursor of glycerol with which fatty acids are esterified for triglycerides formation **(Bergman, 1983).** In addition glucose furnishes NADPH which is required as a reducing agent in the synthesis of long chain fatty acids. In general, the decrease in serum levels of both glucose and triglycerides indicate that the exposed fish to atrazine were in worse condition compared with control fish.

The significant increase of urea nitrogen in exposed catfish to atrazine may be due to necrosis of endothelial cells and renal hemopoietic tissue (necrobiotic changes). This opinion supported by the histopathological results and agreed with that reported by *Fischer-Scherl et al. (1991)*. Also*, Wotton and Freem, (1982)* reported that the herbicides led experimentally to degeneration of kidneys in fish. *Gunkel and Streit, (1980)* indicated that atrazine was accumulated via the gills and blood during its exposure phase. This accumulation of atrazine in the gills caused their dysfunction and resulted in kidney stress which leads to increase of urea in the blood. While, investigating biochemical effects of different herbicides on fish, several authors *(Nemcsok et al., 1985, 1987; Nemcsok and Hughes, 1988 and Singh and Reddy, 1990)* have also reported changes in activity of some fish enzymes, primarily transaminases. Such effects vary and depend on the type of compound, its concentration, and the length of exposure.

Acetylcholine estrase (AChE) activity obtained in the present study showed significant decrease after exposure to atrazine along the whole experimental period. Inhibition of AChE activity of fish may cause it to exhibit a number of symptoms; these symptoms include a reduction in the ability of the fish to tolerate reduced oxygen tension, a slow-down of its reflexes and swimming movements and a reduction in the feeding activities of the fish. So, inhibition of AChE activity is generally regarded as a useful indicator of exposing the fish to hazard compounds. The obtained results on inhibition of AChE activity are in agreement with findings of *Hussein et al. (1996), Forget et al. (2003)*. Also *key et al., (2003)* found that monitoring organisms for AChE levels can be a means of detecting exposure to pesticide contamination.

The gross pathology revealed congestion in the kidney, spleen and the liver during first week post exposure. The liver was friable and yellowish in colour 14th and 28th days post exposure. Similar lesions were reported by *Hussein et al., 1996*.

A histopathological study of the target tissues is the standerd method largely used in biomonitoring programs *(Au, 2004)*, which might indicate acute or chronic exposure to contaminants. The greatest variety of changes was found in gills, this unique organ of fish is one of the most sensitive organs because of the intimate and continuous contact with the water *(Mallat, 1985)*. In the present study with the increase of the time of exposure to atrazin, gill lesions and other lesions of the different organs increased in severity.

The histopathological changes in fish gills found in the present study are: congestion, hyperplasia, vaccuolation, necrosis, desquamation and sloughing of the lamellar epithelium as well as leucocytic inflammatory cells infiltrations and telangectasis. Such changes act as a defense response to pollutants which leads to an increase of pollutant-blood diffusion distance but reduces the respiratory surface and capacity *(Mallt, 1985; Ebele et al., 1990; Black and McCarthy 1990; Ibrahim, 1996; Cengiz and Unlu, 2003; Oropesa-Jimenez et al., 2005)*. These structural changes agreed with that reported by *Neskovic et al. (1993) and Alzemi et al. (1996).*

Gunkel and Streit, (1980) indicated that, atrazine was accumulated via the gills and blood during its exposure phase and resulted in gills dysfunction and kidney stress which lead to increasing of urea in blood. *Prasad et al., (1991)* found that, damage of gill lamellae causes decreased respiratory capacity in tilapia. *Phyu et al. (2006)* stated that atrazine causes damage to the gill epithelium and physiological disturbance including disarrangement of osmoregulation and increased respiration.

The liver showed congestion, edema, fatty change, vacuolar degeneration, perivascular inflammatory cells aggregation, mild portal fibrosis, hyperplasia of pancreatic cells **and** necrosis. These results agreed with *Neskovic et al. (1993); Ibrahim, (1996); Li et al. (2003) and Miranda et al. (2008)*. Fatty change and vaccular degeneration are the

most commonly encountered liver lesions reported following pesticide exposure *(**Ribelin and Migaki, 1975**)*. On the other hand, possible focal fibriosis and even necrosis of hepatocytes recorded in the liver of fish exposed to atrazine are progressive types of damage **(Couch, 1975).** Changes that have been noted in the liver because it is the first organ to be exposed by the portal circulation, after enteric uptake *(**Wester, 1988).**

Miranda et al. (2008) stated that**,** triazine herbicides as atrazin are suspected to cause hepatotoxicity, immune suppression and carcinogeni-city. Some hepatic lesions as necrobiotic changes and necrosis in liver, are generally typical in multiple contaminant exposure as described by other researchers in this field as ***Bondy et al. (2003); Au (2004); Oliveira Ribeiro et al. (2005) and Rabitto et al. (2005).*** According to *Avci et al. (2005),* necrosis is strongly associated with oxidative stress. **Li et al. (2003)** reported that, atrazin induced free-radical generation and antioxidant depletion which might cause oxidative stress and lipid peroxidation in the liver of *Crucian carp,* leading to high incidence of necrosis and other degenerative lesions found in the liver. These lesions disturb liver function as manifested by changes of the enzymatic activity (AP, GOT.GPT).

In the present study, atrazin causes sever degenerative changes in kidney tissue as congestion, edema, vacuolar degeneration, various necrobiotic changes of the renal tubular epithelium and glomerulei. These changes are considerly agreed with ***Fisher-Scherl et al. (1991); Neskovic et al. (1993); Oulmi et al. (1995)*** and ***Ibrahim, (1996)*** and may increase the renal excretion of ions and proteins**.** ***Wotton and Freem, (1982)*** reported that the herbicides led experimentally to degeneration of kidney in fish.

The intestine suffered mucinus degeneration, edema, congestion, mononuclear cells infiltrations, sloughing and necrosis of villar epithelium. The type of changes found in the intestine of catfish were nearly similar to those which reported by ***Neskovic et al. (1993).***

The spleen suffered congestion, edema and lymphcytic depletion wheares atrazin has harmful effects on kidney, liver and spleen *(Jackim et al., 1970 and Rehwoldt, 1978)*. Lymphoid depletion in spleen is subsequent to immunological events arising because of the effects of the toxin *(Vall, 1993).*

Skin exhibited edema leukocytic cells infiltration, epidermal sloughing and activation of melanomacrophage cells. *Miranda et al. (2008)* stated that the contamination of fresh water and the accumulation of the toxicant in aquatic organisms through bioconcentration by the direct uptake from water through gills and skin.

Activation of melanomacrophages was noticed in the liver as agreed with *Miranda et al. (2008)* as well as in the skin tissue. The incidence or absence of melanomacrophages was reported as a disorder of the immunological system and it is hence, used as a biomarker of environmental degradation and pollution *(Wolk, 1992; Rabitto et al., 2005 and Mela, et al., 2007). Walsh and Ribelin, (1975)* confirmed that all changes observed in fish exposed to atrazine appear to be related to tissue accumulation.

In our study, the heart displayed congestion, edema between the myofibers, Zenker's necrosis, hyaline degeneration and pericarditis. On the other hand, the muscles of catfish that exposed to atrazine suffered focal granular cells infiltrations in between the muscular bundles. Cereal degeneration of small number of muscular fibers arose from the toxogenic effects of atrazine *(Van Vleet and Ferrans, 1986).*

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بعض الدراسات على أسماك القراميط المعرضة لمبيد الاترازين

لئ حب لشت غبل ئلخ قظ   ڪ ب لشت عروطحتتظ

معهد بحوث صحة الحيوان – الإسماعيلية

قسم الهستوباثولوجى– الفارماكولوجى

هذه الدراسةِ أجريت لاستبيان التأثير المحتمل لمبيد الاترازين على مكونات الدمِّ و بعض مكوّناتَ المصلِ فى اسماك القراميط والتغيرات الباثولوجية المصاحبة. وقد تم دراسة التأثير السمى الحاد ودراسة تأثير تعاطي المبيد لفترات طويلة. و عند دراسة التأثير الحاد لسمية الاترازين لمدة 96 ساعة تم تسجيل النفوق فى 24–48–72–96 ساعة وكذلك تم تحديد نصف الجرعة الحادة المميتة للأسماك.

وعند دراسة تأثير تعرض الأسماك للاترازين لفترة طويلة حيث قسمت 70 سمكة بشكل عشوائى الى مجموعتين الاولى ضابطة والثانية تم تعريض الأسماك للاترازين بمعدل 4.5 ملجم/لتر لمدة 4 أسابيع. وتم تجميع عينات من الأسماك عند 0–7–14–28 يوم من التجربة وذلك لدراسة التغيرات فى مكونات الدم والمصل والأنسجة.وقد اظهرت النتائج ان نصف الجرعة الحادة المميتة للأسماك هو 18.1 ملجم /لتر .

وقد أظهرت النتائج نقصان هامّ فى عدد كرات الدم الحمراء والهيموجلوبين والهيماتوكريت وباقى مكونات الدم بنسب مختلفة تبعا لمدة التعرض للمبيد. بينما كانت التغييرات الكيميائية الحيوية عبارة عن انخفاض معنوي في مستويات الجلوكوز، البروتين الكليّ، الكولوستيرول، الدهون الكلية والتراى جليسرايد وكانت ابرز التغييراتِ فى أنزيمات الكبد الترانس امينيز والالكلين فوسفاتيز وكذلك فى نشاطات إنزيم الاستيل كولين. وان معظم التغيرات الباثولوجية قد لوحظتْ في خياشيمِ السمكَ ثَلثها الكبدِ والكلي والأمعاء .وبزيادةِ وقتِ التعرّضِ إلى الاترازين ذادت الآفات الباثولوجية المصاحبة فى الخياشيمِ و الأعضاء الأخرى بدرجات متفاوتة.