*Egypt. J. Aquatic Biology & Fisheries; Vol. 11, No.3:709 - 720 (2007) ISSN 1110 – 6131*

# Influence of phenol pollution on Nile tilapia (*Oreochromis niloticus*)

**Anisa, M. Moustafa.[1]; Abd-El-Menem, A. Aly[1]; Salah Mesalhy [2]; Hussein, A. Elghobashy[3];Samya, I. Hasanin[3]and Atef, E. Ibrahim[3]**

*1- Pathology Dept., Faculty of Vet. Medicine, Zagazig University, Egypt.*
*2- World Fish Center, Abbassa, Egypt.*
*3- Central Lab for Aquaculture Research, Agricultural Research Center, Egypt.*

## ABSTRACT

In this study a field investigation was done by collection of water and Nile tilapia (*Oreochromis niloticus*) from Helwan (branch of Nile River), El-Abbassa earthen ponds and Bahr El-Baqar fish ponds every 4 months during the year to determine the residues of phenol in fish and water. Also experimental laboratory studies were done by using apparently healthy Nile tilapia for determination of $LC_{50}$ for 72hr. to phenol and evaluate the effect of long term exposure to phenol 1/10 $LC_{50}$ for 12 weeks.

The levels of phenol in water samples from Helwan were higher than in Bahr-El-Baqar. While the residues of phenol in fish muscle samples were lower than permissible limit in the investigated localities. Long term exposure of Nile tilapia to 1/10 $LC_{50}$ revealed nervous manifestations and respiratory signs with mortality rate of 8 %. Macroscopically, erosion of fins and tail, pale gills and liver were seen. Microscopically, desquamated and hyperplastic gill lamellae, neuronal degeneration, hyperplasia of epidermis and Zenker's necrosis of muscles were seen. Degeneration with necrosis in liver, kidneys, spleen and gonads were observed. The residual levels of phenol in experimented fish muscles were 0.07, 0.25 and 1.15 ppm after 4, 8 and 12 weeks, respectively.

The results clearly indicated that phenol affects on fish quality by induction of gills, internal organs and brain lesions with residues in muscles.

**Key words**: Nile tilapia, phenol, histopathology, residues

## INTRODUCTION

In Egypt, the recently rapid development of chemical industry, has created the environmental pollution. The presence of these pollutants caused many serious hygienic and economic problems. The most important pollutant is phenol produced from petroleum processing, coal or wood distillation and many chemical industries (Mohamed *et al*, 2002). Moreover, Aboul-Dahab (1996) determined the phenolic compounds in sea water from Alexandria and Doha coastal environments. Phenolic residues in the fish flesh may lead to a risk of stomach cancer for the consumers (Ohshima *et al.*, 1989). The sublethal dose of phenol revealed nervous and respiratory signs, and excess mucus on the skin and gills in exposed fish (El-Manakhly and Soliman, 1993). Phenol

induced extensive circulatory disturbances, degeneration and necrosis in the gill lamellae, liver, spleen, brain and kidneys in the experimental fish (Bucher and Hofer, 1993; El-Tabakh, 1999, Mohamed *et al.*, 2002).

The present study was undertaken to determine the levels of phenol in the water and the muscles of Nile tilapia (*Oreochromis niloticus*) collected from different localities together with experimental pathological investigation to the effect of long term exposure to phenol on Nile tilapia and the associated residues in their muscles.

## MATERIALS AND METHODS

**Materials:**

**Water and fish samples**: Twelve water samples and one hundred and twenty Nile tilapia fish samples (40 fish from each region) were taken from three localities: Helwan (branch of River Nile), El-Abbassa earthen ponds (receive their water supply from Ismailia canal) and Bahr El-Baquar fish ponds (receive water supply from Bahr El-Baquar drain). Samples were taken from each locality every 4 months along 2004 and analyzed for the phenol using Spectrophotometer was done according to APHA (1985). .

**Experimental Fish:** A total number of 233 apparently healthy Nile tilapia (*Oreochromis niloticus*) sexually mature were used in this study, with an average body weight of 90 ±10 g. Fish were transferred a live from the ponds of Central Laboratory for Aquaculture Research to the laboratory. Fish were held in full glass aquaria containing dechlorinated tap water and acclimatized for two weeks prior to the experiment.

**Ten glass aquaria:** Each of 40 x 60 x 75 cm with 180 liters capacity supplied with dechlorinated tap water were prepared. Continuous aeration and filtration were maintained in each glass aquarium and the water temperature was adjusted at (23±2 ºC) by a thermostatic heater. Locally prepared fish diet containing 30% protein was provided once daily as 3% of body weights according to Eurell *et al.* (1978).

**Pollutants:** Pure phenol was obtained from ADWIA.

**Methods:**

**Experimental laboratory studies**:

A total number of 133 fish were used for determination of $LC_{50}$/72hr for phenol according to Behrens and Karbar (1953).

**Long term exposure:** Chronic toxicity was performed to study the toxic effect of low concentration of phenol for long exposure. A total number of 100 fish were used. 50 fish exposed to 1/10 of their $LC_{50}$/72 hr (2.5 mg/L) of phenol for 12 weeks. Another 50 fish were used as non- exposed control group. Fish samples were collected at the end of 1$^{st}$ week, 2$^{nd}$ week, 4$^{th}$ week, 8$^{th}$ week and 12$^{th}$ week PE. Postmortem examination was done for Nile tilapia as described by Schäperclaus *et al.* (1992). Specimens from different organs were taken for pathological studies and muscles samples used for determination of residues.

**Histopathological examinations:** Tissue specimens from different organs of experimented fish were trimmed and fixed in 10% phosphate buffer formalin. Paraffin sections of 5 microns thickness were prepared and stained with hematoxylin and eosin (H.&E.) (Bancroft *et al*., 1996).

**Residual analysis:** Samples from the muscles of both natural and experimented Nile tilapia were taken after 4, 8 and 12 weeks of experiment for determination of phenol residues. The samples analyzed according to procedures recommended by AOAC (2002**)** using atomic absorption spectrophotometer.

**Statistical analysis:** Data were analyzed using Analysis of Variance (ANOVA) and means were separated by Duncan at a probability level of < 0.05 (SAS, 2000).

## RESULTS AND DISCUSSION

**Field investigation**:

**The residue of phenol in water and fish muscles from investigated localities:**

The present results indicated that, there were detectable levels of pollution in some industrial or agricultural localities in Egypt as Helwan and Bahr-El-Baqar. The level of phenol in El-Abbassa water samples was not detectable but the maximum level of phenol in water samples was 0.42 in Helwan. Our results were in agreement with EPA (1979) which recorded the phenol level in lower Mississbi River and Detroit River. No residues of phenol in fish muscles obtained from El-Abbassa were detected. Phenol in fish samples from Helwan was 0.009 ppm whereas phenol in Bahr-El-Baquar was 0.003 ppm (Table1). Belfroid *et al*. (2002) recorded phenolic compounds (bisphenol) in water and fish muscles from Netherland. FAO (1983) mentioned that, the permissible level of phenolic compounds in fish is 0.01ppm.

**Table (**1**)**: Residual analysis of phenol (ppm) in water samples and muscles of fish from investigated localities.

| Locality | Helwan | Bahr-El-Baquar | El-Abbassa |
|---|---|---|---|
| **Water** | $0.420 \pm 0.060^{a}$ | $0.100 \pm 0.030^{b}$ | ND |
| **Muscles** | $0.009 \pm 0.001^{a}$ | $0.003 \pm 0.001^{b}$ | ND |

[a-b] Means within a row with the different superscript are significantly different ($p<0.05$).
Values are expressed as Mean ± SE. ND = Not Detectable

In the present study, phenol $LC_{50}$ and mortalities were determined in Nile tilapia after exposure to different concentrations of phenol for 72 hr. $LC_{50}$ for phenol was 25.0 mg/L. Several investigations were carried out regarding the estimation of $LC_{50}$ of phenol. In Zebrafish it was 24.9 ppm for 96 hr by Razani *et al*. (1986). The difference between the results presented in this work in comparison with the previous data could be attributed to the differences in the body weight, age of fish and period of exposure. Moreover Post (1987) mentioned that, the toxicity of phenol varies between fish species and under

varying environmental conditions. Also $LC_{50}$ of fish was 7.5 to 56 mg/ L for phenol.

In the present study, the clinical signs of Nile tilapia exposed to phenol 1/10 $LC_{50}$ (2.5 mg/L), showed nervous and respiratory manifestations then loss of appetite and depression. The mortality rate reached 8 % after 12 weeks. These signs could be attributed to the metabolic inhibitors and toxic effects of phenol on the nervous system with damage in the gill filaments. Krajnovic & Ozretic (1988) and El-Tabakh (1999) reported similar findings. Moreover, Alabaster and Lioyd (1982) mentioned that, fish surviving the long-term exposure to low concentration of phenol showed wide spread inflammation and necrosis of all tissues, possibly because of irreversible changes in the protein metabolism.

In the present study, the macroscopic changes in Nile tilapia during the 1st week of exposure, to 1/10 $LC_{50}$ of phenol were congestion in the gills and the meninges with erosion of fins and tail. After 2 weeks of exposure, excess mucus was seen covering the skin and gills. After 8 weeks of exposure, pale gills, kidneys and liver were observed. At end of 12 weeks of exposure the fish showed severe loss of weight and focal skin discoloration compared with control (Figs.1&2). Similar lesions were mentioned by Waluga (1966-a) and Mohamed *et al.* (2002).

Microscopically;

**The gills** showed congestion in the blood vessels with hemorrhage. The secondary lamellae were desquamated or hyperplastic along the 1st two weeks PE. From the 4th week till the end of the experiment, mild edema in primary lamellae and fusion in secondary lamellae were observed. Hemosiderosis in the venus sinuses was seen (Fig.3). The proliferative changes in the gills of exposed fish due to direct contact with phenol causing its severe irritation. These results are in agreement with Mohamed *et al.* (2002**)** in Nile tilapia.

**The brain** revealed congestion of cerebral and meningeal blood vessels, perivascular edema and melanomacrophages aggregation after 1-2 weeks PE. After 4 weeks, the brain showed submeningeal hemorrhage with diffuse gliosis and neuronal degeneration. After 8-12 weeks PE, the brain exhibited diffuse malacia with satellitosis as well as neuronophagia (Fig.4). These findings were attributed to phenol neurotoxicity (Viccellio, 1993) and were in agreement with Devi and Sastry (1987) in Java tilapia

**The skin** showed hyperactivation of the mucous cells, hyperplasia and spongiosis of epidermis and mild dermal edema (Fig.5). After 4-12 weeks of exposure, the dermis revealed focal proliferation of melanomacrophages.

**The muscles** showed focal hyaline degeneration or Zenker's necrosis, edema with few leukocytic infiltration mainly lymphocytes (Fig.6). These findings were attributed to the defensive tissue reaction against the irritants. Similar lesions were previously mentioned by Alabaster and Lioyd (1982) and El-Tabakh (1999).

**The heart** showed hemorrhage in the pericardium and in the cardiac muscles as well as edema. After 4 weeks, the heart exhibited focal hyaline degeneration and/ or Zenker's necrosis and infiltration of mononuclear leukocytic cells. Hemorrhage and hemosiderin deposits were observed from 8 weeks till the end of the experiment (Fig.7). These findings were attributed to phenol toxicity that cause extensive tissue necrosis and act as hemolysing agents of the erythrocytes (Viccellio, 1993).These results were in agreement with El-Manakhly and Soliman (1993).

**The liver** showed congestion of the hepatic and pancreatic blood vessels with focal vacuolar degeneration in the hepatocytes after 1-2 weeks PE. Pancreatic acinar cells exhibited degeneration and necrosis with focal proliferation of melanomacrophages. Hemosiderosis and hyperplasia in bile ducts were seen. After 8 weeks till the end of the experiment, the hepatocytes and pancreatic cells suffered advanced coagulative necrosis and periductular fibrosis of the bile ducts (Fig.8). These findings were attributed to liver detoxification of phenol where its metabolites were detected mainly in the bile. Phenol causes hypoxia to the red blood cells and hemolysis then hemosiderin pigment release which stimulates fibrous tissues formation around the bile ducts with hyperplasia of epithelium. These results were in agreement with Owen and Rosso (1981); Krajnovic and Ozretic (1988) Vogelbein *et al.* (1990) and Bucher and Hofer (1993).

**The spleen** exhibited mild activation of hemopoietic tissues and focal necrosis within the lymph follicles after one week PE. After two weeks of exposure, the melanomacrophage centers showed activated cells. From 4-12 weeks, the spleen showed depletion of hemopoietic tissues, necrosis of most lymph follicles and melanomacrophage cells with hemosiderin deposits (Fig.9). These results were attributed to the direct cytotoxic effects of phenol and hypoxic conditions. Similar lesions were mentioned by Cooper *et al.* (1984); El-Manakhly and Soliman (1993) and Fournie *et al.* (2001).

**The kidneys** exhibited congestion and vacuolar degeneration in the epithelium lining the renal tubules after one week. After 2 week PE, the kidneys revealed congestion, coagulative necrosis in the renal tubules and peritubular fibrosis (Fig.10). Periglomerular edema and focal hemorrhages were noticed. For weeks PE, the hemopoietic tissues and melanomacrophages revealed necrosis. From 8 week till the end of experiment, severe tubular nephrosis with atrophy and necrosis of most glomerulei were seen. These results were attributed to the direct cytotoxic effects of phenol which acts as a general protoplasmic poison. These results were in agreement with Gupta and Dalela (1987); El-Manakhly and Soliman (1993) and Mohamed *et al.* (2002).

**The intestine** revealed congestion in the intestinal blood vessels, mucinous degeneration and hyperplasia in the goblet cells with mononuclear leukocytic infiltration. Focal epithelial desquamation in the intestinal villi was evident (Fig.11). The lamina propria and submucosa were edematous and the

serosa was infiltrated by melanomacrophages during 1-12 weeks PE. At the end of 12 weeks, mild fibroblast cells proliferation in the lamina propria was seen. These results were in agreement with El-Tabakh (1999) in Common carp.

**In the ovaries**, the majority of oocytes were atretic. The ovarian stroma showed hemorrhage, hemosiderin deposits and mild edema. After 4 weeks, degeneration and necrosis of follicles with hemorrhages were observed. After 8 weeks till the end of experiment**,** extensive degeneration to both mature and immature follicles were observed (Fig.12). These results are in agreement with Post (1987).

**The testes** revealed congestion of blood vessels, mild edema and few mononuclear cells infiltration following 1-2 weeks PE. After 4 weeks till the end of experiment**,** most of the seminiferous tubules lacked spermatozoa, others contained few number of sperms and few peritubular fibroblast cells were seen (Fig.13). These results were in agreement with Zaroogian *et al.* (2001) and Weber *et al.* (2002).

The present results showed that, phenol was accumulated in the muscles of the experimental Nile tilapia after 4, 8 and 12 weeks (0.07, 0.25 and 1.15 ppm, respectively**) (**Table 2**)**. The possible explanation for its low level through 4 weeks could be due to phenol detoxification by the liver through glucuronides and sulfate conjugation (Hickman and Trump, 1969) or through an enzyme related eliminations (Owen and Rosso 1981). Long exposure lead to degeneration and necrosis in the liver and kidneys followed by high accumulation of phenol in the muscles after 12 weeks. Pedersen and Hill (2002) determined major residue of phenolic compound in the muscles.

From the results obtained in the present study, it could be concluded that, severe lesions in the gills, liver, kidney, gonads and brain and residues in the muscles of the experimental fish after 4, 8 and 12 weeks were recorded due to phenol toxicities, so it may affect on fish quality and the health of consumers. Therefore, the governmental protective measures should be done for industrial and agricultural hygienic drainages.

Table (2): Residual analysis of phenol in experimented Nile tilapia muscles after 4, 8 and 12 weeks of experiment**.**

| Type of pollutant | Time of sampling (weeks) | | |
|---|---|---|---|
| | $4^{th}$ | $8^{th}$ | $12^{th}$ |
| Control | ND | ND | ND |
| Phenol (ppm) | $0.070 \pm 0.003^{c}$ | $0.250 \pm 0.004^{b}$ | $1.150 \pm 0.006^{a}$ |

$^{a-c}$ Means within a row with the different superscript are significantly different ($p<0.05$).
Values are expressed as Mean ± SE. ND = Not Detectable

## REFERENCES

Aboul-Dahab, O. (1996). Phenolic compounds in two different coastal environments. Fresenius Environ. Bull., 5 (5-6) 264-269.

Alabaster, J.S. and Lioyd; R. (1982). Water quality criteria for Fresh Water Fish. 2nd .Ed. Butter worth, London, Boston: pp. 103-125.

AOAC (2002). Official method of analysis of association of official analytical chemists. No. 968.08 chapter 4 P. 40 vol. 1, 17 edition.

APHA (American Public Health Associetion) (1985). Standard methods for the examination of water and wastewater, 16th edition American Public Health Association Washington.

Bancroft, G.D.; Stevens, A. and Turner, D.R. (1996). Theory and Practice of Histopathological Techniques. 4th edition, Churchill Livingstone Edinburgh, London, San Francisca and New York.

Behrens, B. and Karbar, G. (1953). Wie sind reihenversuch fur biologische Auswertungen Am zweckma Bigstm Anzuordnen. Arch. Fur. Exp. Path. Und Pharm., 177: 379- 388.

Belfroid, A.; Velzen; M. Horst, B. and Vethaak D. (2002). Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. Chemosphere, 49 (1): 97-103.

Bucher, F. and Hofer, R. (1993). Histopathological effects of sublethal exposure to phenol on two variously pre-stressed populations of Bullhead (*Cottus gobio L.*) Bull. Environ. Contam. Toxicol. 51: 309 - 316.

Cooper, K.; V. Kindt and Snyder, R. (1984). Correlation of benzene metabolism and histological lesions in rainbow trout (*Salmo gairdneri*). Drug Metab Rev., 15 (4): 673-696.

Devi, C.R. and Sastry, C.A. (1987). Pathological changes due to O-cresol, resorcinol and 2;5-xylenol to the brain (optic tectum) of a teleost *Sarotherodon mossambicus* (peters ). J. Environ. Biol. 8 (4) 307- 314.

El-Manakhly, E.M. and M.K. Soliman (1993). Pathologic studied on the sublethal effects of phenol on Grass carp (*Ctenopharyngon idella*). Alex. J. Vet. Science, 9(1): 83-87.

El-Tabakh, M.H. (1999). Pathology of the toxic effect of some phenolic compounds on carp fish. M. V. Sc. Thesis Dept. Pathology and Parasitology, Fac. of Vet. Med. Alex. Univ.

EPA (Environmental Protection Agency) (1979). Phenol ambient water quality criteria. PB296-787. Office of water planning and standards, EPA Washington, D.C.

Eurell, T.E.; S.D. Lewis and L.H. Grumbles (1978). Comparison of selected diagnostic tests for detection of motile *Aeromonas septicaemia* in fish. Am. J. vol. Rs., 39 (8): 1384-1386.

Fournie, J.W.; J.K. Summers; L.A. Courtney; V.D. Engle and V.S. Blazer (2001). Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. J. of Aquatic Animal Health, 13 (2): 105-116.

Gupta, S. and R. Dalela (1987). Kidney damage in *Notopterus notopterus* (Pallas) following exposure to phenolic compounds. J. Environ. Biol. 8 (2): 167-172.

Hickman, C.P. and B.J. Trump (1969). The kidney. In Fish Physiology (Ed. By W.S. Hoar and D.J. Randall), Vol. 1, London, Academic press: 201.

Krajnovic, M. and Ozretic, B. (1988). Toxic effects of phenol on grey mullet, *Mugil auratus* Risso. Bull. Environ. Contam. Toxicol.; 40: 1.

Mohamed, S.G.; R.H. Khalil; I.A. Eassa; A.F. Badran and E.A. Wassef (2002). Drastic effect of phenol pollution on *Oreochromis niloticus*. Procceeding of the 4$^{th}$ international conference on recirculating aquaculture cooperative extension service Virginia polytechnic institute and state. University

Ohshima, H.; M. Friesen; B. Malaveillec; A. Hautefeuille and H. Bartsch (1989). Formation of direct acting genotoxic substances in nitrostated smoked fish and meet products, identification of simple phenolic precursors and phenoly diezonium inos as reactive products. Feed Chem. Toxicol., 27 (3): 93-203.

Owen, J.W. and S.W. Rosso (1981). Effects of sublethal concentration of pentachlorophenol on the liver of Bluegill Sunfish (*Lepomis macrochirus*). Bull. Environm. Contam. Toxicol. 26: 594-600.

Pedersen, R.T. and E.M. Hill (2002). Tissue distribution and depuration of 4-tert.-octylphenol residues in the cyprinid fish, *Scardinius erythrophthalmus.* Environ. Sci. Technol. 1; 36 (15): 3275- 3283.

Post, G. (1987). Textbook of fish health. 2nd Ed. TFH Publ. Inc. 259 pp.

Razani, H.; K. Nanba and S. Murachi (1986). Acute toxic effect of phenol on zebrafish *Brachydanio rerio.* Bull. Jap. Soc. Sci. Fish Nissuishi, 52 (9): 1547- 1557.

SAS. (2000). SAS User's Guide: statistics, SAS Institute INC., Cary, NC.

Schäperclaus W.; H. Knlow and K. Schrecerback (1992). Fish Diseases, vol. I. A.A. Balkema / Rotterdam. ISBN, 90 6191 951 7.

Vogelbein, W.K.; J.W. Fourine; P.A. Veld and R.J. Huggett (1990). Hepatic neoplasms in the mummichog *(Fundulus heteroclitus)* from a creosote-contaminated site. Cancer Res., 15: 50 (18): 5978-5986.

Viccellio, M.D. (1993). Handbook of Medical Toxicology. United states of America, pp.265-269.

Waluga, D. (1966-a). Phenol effects on the anatomo-histopathological changes in bream (*Abramis brama L*). Acta Hydrobiol., 8 (1): 55-78.

Weber, L.P.; Y. Kiparissis; G.S. Hwang; A.J. Niimi; D.M. Janz and C.D. Metcalfe (2002). Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (*Oryzias latipes*). Comparative Biochemistry and Physiology Part 131 (C): 51-59.

Zaroogian, G.; G. Gardner; H. Borsay; G. Gutjahr; R. Haebler and L. Mills (2001). Effect of 17B-estradiol, O,P-DDT, Octylphenol and P,P-DDE on gonadal development and liver and kidney pathology in juvenile male summer flounder (*Paralichthys dentatus*). Aquatic toxicol., 54: 101-112.

**Fig. (1):** Nile tilapia (control)

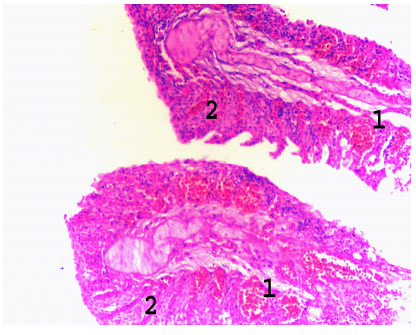


Fig. (2): Nile tilapia, exposed to 1/10 $LC_{50}$ phenol after 12 weeks, showing loss of weight compared to control, mild congested gills[2] and focal skin discoloration[3].

Fig. (3): Gill, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 12 weeks, showing congestion in lamellar blood vessels[1], hyperplasia and fusion in the epithelium of the secondary gill lamellae[2] (H.&E., X 250).

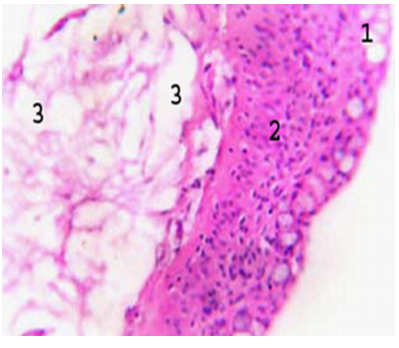


Fig. (4): Brain telencephalon of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 12 weeks, showing satellitosis[1] and neuronophagia[2] (H.&E., X 100).

Fig. (5): Skin, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 2 weeks, showing hyperactivation of the mucous cells[1] with hyperplasia and spongiosis of epidermis[2] as well as dermal edema[3] (H.&E., X 250).

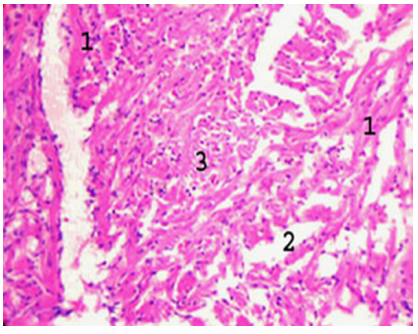


Fig. (6): muscles, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 12 weeks, showing hyaline degeneration[1], Zenker's necrosis[2], edema[3] and leukocytic infiltrations[4] (H.&E., X 250).

Fig. (7): Heart, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 4 weeks, showing focal hyalinization[1] and edema among degenerated myocardial muscle fibers[2] (H.&E., X 250).

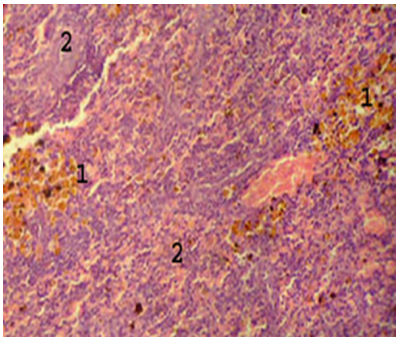


Fig. (8): Liver, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 8 weeks, showing periductular fibrosis of bile duct[1], necrosis of pancreatic cells and hepatocytes[2] as well as intracytoplasmic brown granules[3] (H.&E., X 250).

Fig. (9): Spleen, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 2 weeks, showing activated melanomacrophage cells[1] beside necrosis with depletion of hemopoietic and lymphoid tissues[2] (H.&E., X 250).

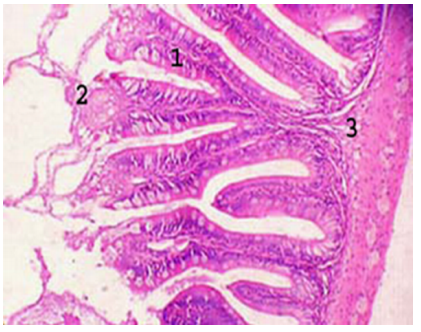


Fig. (10): Kidney, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 2 weeks, showing congestion[1] with peritubular fibrosis[2] and coagulative necrosis in renal tubules[3] (H.&E., X 250).

Fig. (11): Intestine, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 8 weeks showing, mucinous degeneration[1], desquamation of epithelial cells[2] and mild fibroblast cells in lamina propria[3] (H.&E., X 250).

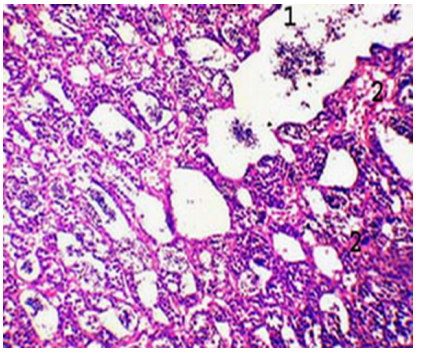


Fig. (12): Ovary, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 4 weeks, showing hemorrhage[1], congestion[2] and edema[3] with degeneration and necrosis of follicles[4] (H.&E., X 250).

Fig. (13): Testis, of Nile tilapia exposed to 1/10 $LC_{50}$ phenol after 8 weeks, showing few number of sperms[1] and peritubular few fibroblast cells proliferation[2] (H.&E., X 250).