Research Article

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Pathological changes in Liver Structure and Function of Oreochromis Niloticus experimentally exposed to Escherichia Coli

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Abstract:

Fish of Oreochromis niloticus (Linnaeus, 1758) were treated with different concentrations of Enterotoxigenic Escherichia coli (ETEC) as (103-105, 106-107 and 109-1010 CFU/ml water). The investigation had conducted to detect experimentally the impact of E. coli toxins on Oreochromis niloticus fish throughout physiological liver function and histopathological changes of liver. The values of GPT, GOT and alkaline Phosphatase ranged between (905.90 IU/L - 75.40 IU/L), (4827.7 IU/L - 385.50 IU/L) and (51.30 IU/L - 5.70 IU/L) for the three liver enzymes respectively. The liver of Oreochromis niloticus treated with Escherichia coli demonstrated fatty degeneration, ballooning degeneration, pyknotic nuclei of hepatocytes, necrosis of hepatocytes and pancreatic acini, hemorrhage, inflammatory infiltration of WBCs and accumulation of hemosiderin pigment. The results of the experiment revealed that the liver of O. niloticus was highly susceptible to E. coli infection inducing elevated liver enzymes and reversible and irreversible liver damage and finally caused mortality in the highest concentration

Keywords: Oreochromis Niloticus, Escherichia Coli, Gpt, Got, Alkaline Phosphatase, Liver Histopathology

Introduction:

Fish are susceptible to wide variety of bacterial pathogens especially when the fishes are physiologically unbalanced or nutritionally deficient, or subjected to stressors, i.e. poor water quality and overstocking. Infectious diseases are the main cause of economic losses in aquaculture industry which is negatively impacted by various pathogenic micro-organisms (MOs) such as Escherichia coli (E. coli) (Plumb, 1997). Escherichia coli is G-negative rods within the family Enterobacteriaceae, and represents a part of the normal micro-flora of the intestinal tract of human and warm-blooded animals. Due to their high prevalence in the gut,

E. coli is used as the preferable indicator to detect and measure fecal contaminate in the assessment of food and water safety. Pathogenic E. coli strains are distinguished by their ability to cause serious illness as a result of their genetic elements for toxins production, adhesion and invasion of the host cells, interference with cell metabolism and tissue destruction (Borgatta et al., 2012).

Historically, cultured fish were not considered important factor

of human pathogens. This situation is changing due to increasing awareness by health care providers of pathogens in aquatic species that may results in human illness (Greenlees et al., 1998). Bacterial hemorrhagic septicemia of ciprinides is caused by Aeromonas sp. bacteria, in association sometimes to Pseudomonas sp. bacteria. Histological examination of liver revealed dystrophy of the hepatocytes. Hepatic congestion and interstitial edema were noticed. In some cases, interstitial edema was located exclusively at the periphery of the bile ducts (Lazar et al., 2011).

Authman et al. (2013) noticed increase in glutamate-oxaloacetate transaminase (GOT) and glutamate- pyruvate transaminase (GPT) activities and different biochemical parameters of clarias gariepinus living in El-Rahawy drain. Also, histological study cleared that liver of C. gariepinus living in El-Rahawy drain suffered from several pathological alterations. All these findings were discussed and referred to the input of agricultural, industrial and sewage water into El-Rahawy drain.

Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) belongs to the family of Cichlidae and is known to the ancient Egyptian for more than five thousand years ago. It was common in natural fisheries in Africa, such as Nile River and freshwater lakes. The original home of tilapia is the content of Africa. Because of its importance, it has been introduced and spread in the world where there is warm water appropriate for their growth and reproduction especially in Southeast Asian countries (Dey et al., 2000). Most tilapia species are suitable for aquaculture. The growth rate varies depending on the nature of the food. It feed mainly on phytoplankton, algae, aquatic plants, remaining of decaying organic material and insect larvae (Tharwat, 2013). *Oreochromis niloticus* has been responsible for the significant increase in global tilapia production from freshwater aquaculture and accounted for about 83% of total tilapias produced worldwide (Food and Agriculture Organization, FAO 2002).

Fish is a good source of proteins for human. Untreated sewage water contains intestinal pathogens as *E. coli*. Some fish breeders use untreated sewage water for growing fish. The presence of fish in untreated sewage water lead to extremely hazardous affectation externally and internally, also, the microbial toxins may transfer pathogens to human. So this research had conducted to detect experimentally the impact of *E. coli* toxins on *Oreochromis niloticus* fish understudy throughout physiological liver function through blood and histopathological changes of liver and consequence impact to human health.

MATERIAL AND METHODS:

- o *O. niloticus* fish were bought from a fish farm and transferred to water aquaria for acclimatization. Weights of tilapia fish were (200-250 gm.).
- o Tap water was used in the experimental aquaria. The physicochemical characters of tap water were measured. The results revealed that tap water was free of bacterial pollution, nitrite and ammonia (Table 1).
- o Gotten isolation of ETEC (Enterotoxigenic *Escherichia coli*) from authorized laboratory and prepared the concentration for experiment, and exposed the fish to different concentrations (McFarland, 2009).
- o All sinks were filled with de-chlorinated water at room temperature. *O. niloticus* were exposed to control and three consecutive doses of ETEC (ETEC concentrations were 103- 105/ml water, 106 -107/ml water and 109-1010/ml).
- o Samples of fish and water on control and consecutive periods were collected according to plan at (1st, 3rd, 5th, 7th, and 9th days) of experiment.
- o ETEC were isolated and classified from the blood and liver of under experiment fish and water (Cline. Lab., 2011; CDC, 2013).
- o The collected data was subjected to statistical analysis using one- way Analysis of Variance (ANOVA).

Table 1: Physicochemical characters and microbial analysis of tap water where fish survived in the bioassay aquaria

Parameters	Concentra-tions
<i>Temperature</i>	25 °C
<i>pH</i>	7.9
<i>Total dissolved solids</i>	95.7 mg/L
<i>Ca⁺⁺</i>	16.00
<i>Mg⁺⁺</i>	0.96
<i>Chloride Cl⁻</i>	6.7
<i>Ammonia NH3</i>	0.0
<i>Nitrate NO3</i>	1.1 mg/L
<i>Nitrite NO2</i>	0.0
<i>Total Bacteria Count/100ml</i>	0.0
<i>E. coli/100ml</i>	0.0
<i>Fungi</i>	0.0
<i>Dissolved O²</i>	2.36 mg/L
<i>Na⁺</i>	110
<i>K⁺</i>	3.40
<i>HCo3</i>	190
<i>PO4</i>	0.47

Physiological methods:

o Blood samples were taken from the caudal vein of the fish using a heparinized syringe and collected into small sterilized plastic tubes.

o Blood samples were left to coagulate for 15-20 min. at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate serum.

o Serum samples were used to estimate calorimetrically the liver enzymes (GOT, GPT and Alkaline Phosphatase) by the method of Dumas and Biggs (1972).

Histological methods

Nile tilapia (*O. niloticus*) fish were dissected and specimens of liver were removed, washed in saline solution and prepared for histological study. Liver specimens were fixed in 10% formalin for 48 hrs.

o After fixation, fish liver was dehydrated in ascending grades of ethanol, cleared in pure xylene then embedded in paraffin wax.

o The paraffin wax blocks were serially sectioned with microtome at 5 micrometers.

o Finally, the sections were mounted on glass slides, stained with hematoxylin and eosin (Bernet et al., 1999).

o The stained sections were examined and photographed with OMAX light microscope with USB digital build in camera.

RESULTS:

Table (2) showed the incidence of re-isolation of ETEC during the experiments phases, ETEC were in 00% from control during all the experiment. At 1st phase ETEC re-isolation were from water as (25, 25, 50, 75 and 75%) and from liver as (00, 25, 25, 25 and 50%) at 1st, 3rd, 5th, 7th and 9th days respectively. At 2nd phase the ETEC re-isolation from water as (25, 50, 50, 75 and 100%) and liver were (00, 50, 50, 75 and 75%) at 1st, 3rd, 5th, 7th and 9th days respectively. At 3rd phase, the fish did not survive i.e. all fish died with ETEC concentration after the 5th day

of the phase. The ETEC re-isolation from water as (25, 50 and 75%) and liver were (50, 75 and 100%) at 1st, 3rd and 5th days respectively. ETEC were zero in blood in all phases of experiment.

Table 2: Incidence of re-isolation of ETEC during the experiment phases

Days	ETEC *Conc.	10 ³ -10 ⁵ *CFU/ml 1 st phase					10 ⁶ -10 ⁷ *CFU/ml 2 nd phase					10 ⁹ -10 ¹⁰ *CFU/ml 3 rd phase		
	Control	1 st	3 rd	5 th	7 th	9 th	1 st	3 rd	5 th	7 th	9 th	1 st	3 rd	5 th
Water	00%	25	25	50	75	75	25	50	50	75%	100	25	50	75
		%	%	%	%	%	%	%	%		%	%	%	%
Liver	00%	00	25	25	25	50	00	50	50	75%	75%	50	75	100
		%	%	%	%	%	%	%	%			%	%	%
Blood	00%	00	00	00	00	00	00	00	00	00%	00%	00	00	00
		%	%	%	%	%	%	%	%			%	%	%

Table (3) showed that the mean $\pm$ standard deviation was 408.63 $\pm$ 438.87. The values of GPT concentration in the experiment ranged from the highest value 905.90 IU/L in the 5th day in concentration (109- 1010 CFU /ml) and the lowest value 75.40 IU/L in the 3rd day in the same concentration. The control was 1577.50 IU/L.

(Table and diaphragm 3) showing GPT concentration (IU/L) in blood of Oreochromis niloticus treated with different concentrations of ETEC.

Days/ concentra- tion	1	3	5	7	9	Mean ± STD
10³-10⁵/ ml	802.8	91.80	734.10	128.6	106.2	358.3±377.39
10⁶-10⁷/ ml	238.70	195.70	358.70	146.60	329.50	253.84±89.20
10⁹-10¹⁰/ ml	244.60	75.40	905.90	-	-	408.63±438.8 7
Mean ± STD	428.7± 323.9	96.96± 89.9	666.23± 279.8	137.6± 12.7	217.85± 157.8	/

IU= International Unit, L=Liter

Table 4 showed that the mean ± standard deviation was 2156.2±2354.11. The values of GOT concentration in the experiment ranged from the highest value 4827.7 IU/L in the 5th day in concentration (109- 1010 CFU /ml) and the lowest value 385.50 IU/L in the 3rd day in the same concentration. The control was 5072.80 IU/L.

Table and diaphragm 4 showed GOT concentration (IU/L) in blood of Oreochromis niloticus treated with different concentration of ETEC

Days/ concentratio n	1	3	5	7	9	Mean ± STD
10^3 - 10^5 /ml	1500.4	770.50	3374.9	1012.8	481.7	1428.06±1150.6
10^6 - 10^7 /ml	986.60	892.70	2077.9	783.8	1425.90	1233.38±5351.3
10^9 - 10^{10} /ml	1255.4	385.50	4827.7	-	-	2156.2±2354.1
Mean ± STD	253.8± 89.2	682.9± 264.7	3426.8± 1375.6	898.3± 161.9	953.8± 667.6	/

(Table 5) showed that the mean ± standard deviation was 16.06±4.71. The values of Alkaline Phosphatase concentration in the experiment ranged from the highest value 51.30 IU/L in the 7th day in concentration (10^6 - 10^7 CFU /ml) and the lowest value 5.70 IU/L in the 5th day in concentration (10^3 - 10^5 CFU / ml). The control was 18.10 IU/L.

(Table and diaphragm 5) showing Alkaline Phosphatase concentration (IU/L) in blood of Oreochromis niloticus treated with different concentration of ETEC

Days/ concentration	1	3	5	7	9	Mean ± STD
10^3 - 10^5 /ml	17.20	13.80	5.70	30	24.0	18.14±9.34
10^6 - 10^7 /ml	18.90	24.50	25.60	51.30	40.60	32.18±13.37
10^9 - 10^{10} /ml	21.00	11.60	15.60	-	-	16.06±4.71
Mean ± STD	19.03± 1.95	16.63± 6.90	15.63± 9.95	40.65± 15.06	32.3± 11.73	/

The structure of normal liver is illustrated in (Fig.1).The liver of Oreochromis niloticus treated with concentration (103-105 CFU /ml) of Escherichia coli demonstrated fatty degeneration of hepatocytes in the 1st day and 5th day (Figs.2 and 5). In the 3rd day, 7th day and 9th day degeneration appeared in hepatocytes and pancreatic acini in Figs.3, 4, 6 and 8 , in the 7th day, showed sever necrosis in hepatocytes (Fig. 7).

The liver of Oreochromis niloticus treated with concentration (106-107 CFU /ml) of Escherichia coli demonstrated necrosis of pancreatic acini in the 1st day, 3rd day and 7th day(Fig.9,12 and16).In the 1st day showing necrotic area occupied by stagnant blood (Fig.10). In the 3rd day of exposure the liver demonstrated accumulation of hemosiderin pigment (Fig.11).In the 3rd day and

7th day of exposure necrosis and inflammatory infiltration of WBCs appeared(Fig. 12 and16).In the 5th day, microscopic observations showed stagnant blood(Fig.13), ballooning degeneration (Fig.14) and fatty degeneration (Fig.15).

The liver of Oreochromis niloticus treated with concentration (106-107 CFU /ml) of Escherichia coli demonstrated infiltration of WBCs in the 9th day and necrosis of hepatocytes (Fig. 17 and18).

In the concentration (109-1010 CFU /ml), pyknosis of nuclei appeared in the 1st day (Fig.19), in the 3rd day and 5th day, fatty degeneration of hepatocytes (Fig.20 and 23), hemorrhage in Fig. 20 and in the 3rd day, degeneration of pancreatic acini in Fig.21. In the 5th

day, necrosis of pancreatic acini was observed in Fig. 22 and 24.

***(Fig.1):** Normal structure of liver of Oreochromis niloticus fish from the control group, showing bile ductile (BD), hepatic artery (HA) and hepatoportal vein (HPV).

***(Fig.2):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 1st day of exposure, showing fatty degeneration of hepatocytes (F).

***(Fig.1):** Normal structure of liver of Oreochromis niloticus fish from the control group, showing bile ductile (BD), hepatic artery (HA) and hepatoportal vein (HPV).

***(Fig.3):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 3rd day of exposure, showing degeneration (D) and rupture of hepatocytes.

***(Fig.4):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 3rd day of exposure, showing degeneration of pancreatic acini (PA).

***(Fig.5):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 5th day of exposure , showing fatty degeneration of hepatocytes (F) and rupture of cellular walls.

***(Fig.6):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 7th day of exposure, showing degeneration (D) and necrosis of hepatocytes.

***(Fig.7):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 7th day of exposure, showing severe necrosis (N) of hepatocytes. The liver tissues lost its normal architecture.

***(Fig.8):** Section of Oreochromis niloticus liver treated with concentration (103_ 105 CFU / ml) of ETEC in the 9th day of exposure, showing degeneration (D) and fragmentation of hepatocytes.

*(Fig.9): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 1st day of exposure, showing necrosis of pancreatic acini(N).

*(Fig.10): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 1st day of exposure, showing necrotic area occupied by hemolysed blood (B)

*(Fig.11): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 3rd day of exposure, showing accumulation of hemosiderin pigment (He)

*(Fig.12): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 3rd day of exposure, showing necrosis and inflammatory infiltration of WBCs (arrow)

*(Fig.13): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 5th day of exposure, showing stagnant blood (SB).

*(Fig.14): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 5th day of exposure, showing ballooning degeneration (BD).

*(Fig.15): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 5th day of exposure, showing fatty degeneration (F).

*(Fig.16): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 7th day of exposure, showing necrosis (N) and inflammatory infiltration of WBCs (arrow).

*(Fig.17): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 9th day of exposure , showing severe necrosis (N) and infiltration of WBCs (arrow)

*(Fig.18): Section of Oreochromis niloticus liver treated with concentration (106_ 107 CFU / ml) of ETEC in the 9th day of exposure, showing large necrotic area (NA) occupied by WBCs and hemolysed blood.

*(Fig.19): Section of Oreochromis niloticus liver treated with concentration (109_ 1010 CFU / ml) of ETEC in the 1st day of exposure, showing pyknotic nuclei (P) of hepatocytes (arrow)

*(Fig.20): Section of Oreochromis niloticus liver treated with concentration (109_ 1010 CFU / ml) of ETEC in the 3rd day of exposure , showing fatty degeneration of hepatocytes (F) and hemorrhage (He)

*(Fig.21): Section of Oreochromis niloticus liver treated with concentration (109_ 1010 CFU / ml) of ETEC in the 3rd day of exposure, showing degeneration of pancreatic acini (D)

*(Fig.22): Section of Oreochromis niloticus liver treated with concentration (109_ 1010 CFU / ml) of ETEC in the 5th day of exposure, showing degeneration and necrosis (N) of pancreatic acini.

*(Fig.23): Section of Oreochromis niloticus liver treated with concentration (10^9 – 10^{10} CFU / ml) of ETEC in the 5th day of exposure, showing fatty degeneration of hepatocytes (F).

*(Fig.24):Section of Oreochromis niloticus liver treated with concentration (10^9 – 10^{10} CFU / ml) of ETEC in the 5th day of exposure, showing severe necrosis (N) and complete rupture of hepatocytes. The photograph demonstrated some blood cells and debris of hepatocyte.

DISCUSSION:

Among the biochemical profiles, monitoring of liver enzymes leakage into the blood has proved to be a very useful tool in liver toxicological studies (Osman et al., 2010). The transaminase, GPT and GOT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases (Ibrahim and Mahmoud, 2005).

In the present study, GPT and GOT increased in the 5th day of exposure in all concentrations. These results might be attributed to tissue damage, particularly liver (Palanivelu et al., 2005). In different fish species, including *Clarias gariepinus* caught from El Rahawy drain, polluted with sewage, agricultural and industrial wastes, GPT, and GOT were found to

increase (Authman et al., 2013) and in response to heavy metals (Mekkawy et al., 2011).

An increase in plasma GPT and GOT activities due to metals (Zn, Cu and Cd) was also found in experimental conditions (Zikic et al., 2001) as well as in fish chronically exposed to metals (Levesque et al., 2002). Tietz (1987) and Campel (1984) reported that, these enzymes liberate to the blood stream when the hepatic parenchymal cells are damaged. Shakoori (1990) reported that, the enzymatic increase is due to increased synthesis and induction of these enzymes.

De smet and Blust (1990) reported that, there is an increase in the activities of GOT and GPT in *Cyprinus carpio* exposed to cadmium. Alkaline phosphatase concentrations were higher than control in all period of exposure. The maximum concentration of alkaline phosphatase was determined in concentration (10^6 – 10^7 /ml E. coli) in the 7th day of exposure. Thus, the measurement of alkaline phosphatase and transaminase activities in the circulating fluid is frequently used as a diagnostic tool in water pollution studies (Adham et al., 1997).

The liver of *Oreochromis niloticus* treated with *Escherichia coli* demonstrated fatty degeneration, ballooning degeneration,

pyknotic nuclei of hepatocytes, necrosis of hepatocytes and pancreatic acini, hemorrhage, inflammatory infiltration of WBCs and accumulation of hemosiderin pigment.

Liver is a vital organ that is most affected by the contaminants in the water due to its role in detoxification and biotransformation processes. Olojo et al. (2005) observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead which agreed well with the present findings.

Necrosis in livers is not necessarily due to specific pollutants since little evidence linked damage to specific organic or inorganic compounds (Rabbitto et al., 2005). Necrosis is strongly associated with oxidative stress where lipid peroxidation is a clear source of membrane bilayer susceptibility (Avci et al., 2005). The oxidative forms may increase programmed cell death or disturbed cell homeostasis and cellular necrosis. Of all the liver pathologies, vacuolization of the hepatocytes, resulting from lipid dystrophies, occurred most frequently.

Hepatic lipidosis is believed to be a preneoplastic stage and has been observed in fish exposed to metals (Giari et al., 2007) and in feral fish from sites contaminated by mixtures of xenobiotics (Greenfield et al., 2008 and Triebkorn et al., 2008). Other studies revealed that preneoplastic lesions, such as hepatic foci of cellular alterations, characterized by basophilic, eosinophilic, vacuolated and clear cells, were early stages in the formation of hepatic neoplasia and so these could be used as histological biomarkers of different exposures (Stentiford et al., 2003 and Au, 2004).

In conclusion, the results of the experiment revealed that the liver of *O. niloticus* was highly susceptible to *E. coli* infection inducing elevated liver enzymes and reversible and irreversible liver damage and finally caused mortality in the highest concentration. These

bacterial toxins can be transmitted to human through water in several ways, such as infected fish. It is recommended to stop using treated sewage water in rearing fish in fish farms and check the validity of fish polluted by sewage in seas, rivers or lake.

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