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Molecular, Biochemical and Histological Effects of Tea Seed Cake on Different Organs of *Oreochromis niloticus*

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Abstract: Plant insecticides are usually used in our aquaculture for their benefits, but extensive use of them may impose a serious health threat to some aquatic populations like*Oreochromis niloticus* especially beside shrimp farms, so this work aimed to evaluate the toxic effects of tea seed on *Oreochromis niloticus.* Ninety-six hours (96hrs) LC_{50} of tea seed cake on *O. niloticus* were estimated by 14.85mg/l. Fish exposed to 4.95mg/l (1/3 LC_{50}) of tea seed cake showed abnormal swimming movement with congestion of internal organs.The serum, hepatic, renal, gills, spleen and intestinal tissues were taken for biochemical, molecular and histological investigation.The results revealed normocytic normochromic anemia with a significant increase in the levels of SOD and CAT activity and gene expression that reflects oxidative stress. General tissue damage in various organs was detected with high doses of tea seed cake. This was ameliorated by lowering dose of tea seed cake to 1/10 and 1/20 LC_{50}, indicating that; tea seed cake is a dose dependent plant insecticide and must be used with special care in regions beside aquaculture and not used in haphazard concentrations.

Key words: Tea Seed Cake · Oxidative Stress · LC_{50} · *Oreochromis niloticus*

INTRODUCTION

Many plants are considered a major source of structurally diverse biologically active substances [1]. Some plants contain compounds of various classes that have insecticidal, pesticide and molluscicidal properties, which are preferred unlike those synthetic chemical pesticides, which leave harmful residues in the aquatic environment [2]. Plant insecticides are believed to be friendlier to the environment as they are easily biodegraded, easily available, less expensive, lower toxicity and did not leave any residues in the environment [3]. A large number of plant products are commonly used for controlling these unwanted fish populations, such as the powdered seed of *Croton tiglium* and *Barringtoniaacutangula* [4], tea seed cake and Mahua oil cake [5].

Plant materials such as tea seed cake or Derris powder, are commonly used in Japan in shrimp culture ponds to kill selectively fish. The toxic plant products of tea seed cake and Derris root powder degrade within 7-12 days, but Derris root powder is not readily available throughout the world and is also expensive [6]. Tea seed cake produced from *Camellia*sp. Seeds after oil extraction it contains many active principles, especially saponin which represent 5.2-7.2%. Saponin is a steroid or triterpenoid glycosides, which are a large, diverse group of mainly plant-derived compounds [7].

The mechanism of action of tea seed cake is well known now through destroying red blood corpuscles leading to the blood heamolysis, reducing oxygen available for body and this is the reason for altering hemoglobin concentrations [8]. The toxic effect of tea seed cake is derived from damage caused to the respiratory epithelium of the gills by the detergent toxic action of the saponins [9]. Tea seed cake *Camellia sinensis* is commonly added to Water at a dose of 100 ppm resulted in the death of tilapia within 5 to 6 h [10]. Besides that, it has a destructive effect on gills of aquatic organisms [3].

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Tea seed cake was used by 10 and 25 mg/L to remove fishes (Mozambique tilapia and flathead goby) from shrimp ponds prior to stocking the ponds (7).

Very little information exists on the effect of tea seed cake on molecular level of antioxidant enzymes and pathological alterations in fish organs. Considering the molecular changes and the reliability of histopathological lesions as biomarkers of oxidative stress in fish under toxic conditions.Therefore, this research work was carried out to determine the toxicity of tea seed cake as well as establishing the incidence of molecular and pathological changes in different organs as a result of acute and chronic exposure of fish to tea seed cake.

MATERIALS AND METHODS

Experimental Fish: Six hundred twenty (620) fingerlings of *O.niloticus*with an average body weight of 20±1.0g were used. Fish were collected from the Abbassah Fish Farm, SharkiaProvince. They were apparently healthy and free from any external lesions. The fishes were held in glass aquaria containing 100L of dechlorinated tap water for acclimatization to laboratory conditions for up to seven days. The water in the aquaria was changed every 24 hour and maintained at atmospheric temperature, 30.0 ± 1.0°C; water temperature, 27.0 ± 1.5°C; pH, 7.0-7.2; dissolved oxygen, 7.5-8.0 mgL^{-1}; free carbon dioxide, 4.7-5.8 mgL^{-1}; bicarbonate alkalinity, 105.0-106.0 mgL^{-1}. The fish were fed on basal diet contain crude protein 32%. The amount of feed (On dry matter basis) given daily to fish was 8-10% of body weight and the fish were fed 3 times daily.

Tea Seed Cake: Tea seed cake with commercial name Yeling from Anhui, Chinawas used (Brown color cake in 50 kg plastic bags)

Experimental Design:

Determination of 96 Hrs LC_{50} of Tea Seed Cake (Acute Toxicity) in *O. niloticus* Fingerlings: Two hundred *O. niloticus*fingerlings were divided into 20 equal groups. All groups were exposed to different concentrations of Tea seed cake for Preliminary trials for zero and hundred % mortality of *O. niloticus* (Table 1). The 96 hrs LC_{50} was estimated using another 120 fingerlings [11]. Evaluation of general health condition of fish, clinical signs, post mortem lesions and mortality were recorded [12-14].

The Effect of 1/3, $^1/_{10}$ and $^1/_{20}$ 96 hrs LC_{50} of Tea Seed Cake on Health and Growth of *O. niloticus* fingerlings: Three hundred O. *niloticus* fingerlings were divided into 4 triplicate groups, each group with 25 fish density in each aquarium. The first group was kept as control, second, third and fourth groups were exposed to4.95, 1.48 and 0.74 mg/l which equal to 1/3, 1/10 and 1/20 of 96 hrs.LC_{50}, respectively. Tea seed cake (Soaked in water for 24 hours before use) concentrations were readjusted

Table 1: Preliminary trials for zero and hundred % mortality of *O. niloticus*exposed to different concentrations of tea seed cake

Group n=10`	Concentration of Tea seed cake (mg/L)	Mortality during 96 hrs 1st day	2nd day	3rd day	4th day	Total mortality	Total mortality %
1	0 (control)	0	0	0	0	0	0
2	1	0	0	0	0	0	0
3	2	0	0	0	0	0	0
4	3	0	0	0	0	0	0
5	4	0	0	0	0	0	0
6	5	0	0	0	0	0	0
7	6	0	0	0	0	0	0
8	7	0	0	1	0	1	10
9	8	0	0	2	0	2	20
10	9	0	2	0	1	2	20
11	10	0	0	2	0	2	20
12	12	1	1	0	1	3	30
13	13	1	1	0	1	3	30
14	14	1	0	1	1	3	30
15	15	2	1	2	2	7	70
16	16	4	3	3	0	10	100
17	17	5	4	1	0	10	100
18	18	9	1	0	0	10	100
19	19	10	0	0	0	10	100
20	20	10	0	0	0	10	100

every two days. Experimentlast for 4 days in acute exposure and for 8 weeks in chronic one. Clinical signs and postmortem lesions were detected and recorded during all duration of experiment for evaluation of general health conditions of fish and at the end of experiment; In addition, the mortality was recorded.

Sampling: Heparinized blood samples were collected from the caudal blood vessels for evaluation of RBCsusing heparin (1% Sol.)as anticoagulant (0.1 ml sol for 5 ml blood), Hb concentration, PCV, MCV and MCHC(15). Serum was separated for determination of alanine aminotransferase (ALT) by the method of Reitman and Frankel [16] creatinine, Husdan and Rapoport[17] and urea [18]. Tissue samples were collected from liver, kidney, intestine, gills and pancreasfor determination of superoxide oxide dismutase (SOD) activity, according to Packer and Glazer [19], serum catalase (CAT) activity according toAbei [20]. Tissue homogenates were prepared as follows (0.5 g of the tissues were homogenized in 5ml cold 20mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mMmannitol and 70 mM sucrose per gram tissue. Homogenates were centrifuged at 10,000 x g for 15min at 4°C and the supernatant was removed and stored at -80 °C until further biochemical analysis).

Histopathological Investigations: Specimens from the liver, Kidney, intestine, gills and pancreas, were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin. Five-micron thick paraffin sections were prepared and stained with hematoxylin and eosin(HE) dyes besides Prussian blue stain for hemosiderosis and Von Kossa stain for calcification [21] and examined microscopically.

Molecular Determination: Samples from liver, kidney and gills were collected rapidly, kept in liquid nitrogen and stored at -80 until molecular investigation of SOD and CAT gene expressions using a semi-quantitative RT-PCR [22]. Briefly, Total RNA was prepared using Qiagen RNA extraction kits, (Cat. No. 74104). The amount of extracted RNA was quantified and qualified using NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware USA. The purity of RNA was checked and ranged between 1.8 and 2.1, demonstrating the high quality of the RNA. The mRNA was stored at -20°C before RT-PCR. RNA was reversing transcribed using Super Script II RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in the presence of Random Primers (Promega, Charbonnièresles- Bains, France). Polymerase chain reaction (PCR) was performed using a 2720 thermocycler (Applied Biosystems, USA). Using PCR master mix (Qiagen USA) and using the specific primers for SOD "forward 5'-GGTGCCCTGGAGCCCTA-3' and reverse 5'-ATGCGAAGTCTTCCACTGTC-3 and for CAT "forward 5'- TCCTGAATGAGGAGGAGCGA-3' and reverse 5'-ATCTTAGATGAGGCGGTGATG -3', primer were designed using primer 3 programmer (http://biotools.umassmed.edu/bioapps/primer3_www.cgi), based on the published nucleotide sequence information of the *O.niloticus* SOD and CAT genes (GenBank accession no. JF801727.1 and JF801726.1), PCR conditions were a denaturation at 95 °C for 2 min followed by 28 cycles of 95°C, 1 min; 55°C, 1 min; 72°C, 1 min. PCR products were analyzed on a 2% agarose gel in 90 mMTrisborate, 2 mM EDTA buffer (TBE), pH 8 and visualized by staining with ethidium bromide and UV transillumination and analyzed by gel documentation system (Bio Doc Analyze, Biometra, Germany). The values for the specific targets were normalized according to those of â-actin to express arbitrary units of relative abundance of the specific messages (i.e., relative expression).

Statistical Analysis: The data was statistically analyzed using SPSS version 20. Statistical packages (IBM 1 New Orchard Road Armonk, New York 10504-1722 United States) and presented as a mean ± SD, n = 10. Statistical differences between groups performed using One-way analysis of variance (ANOVA). Duncan's test was used for testing the inter-grouping homogeneity. Statistical significance was set $p = 0.05$.

RESULTS

Acute Toxicity of Tea Seed Cake in *O. niloticus* fingerlings: Fish exposed to 1\3 LC_{50}96 h tea seed cake showed abnormal swimming movement, sluggish and did not respond to escape reflex. Postmortem findingsrevealedcongestion of internal organs and enlargement of liver (Fig.1).

The actual estimation of 96 hrs.$LC_{50}$of Tea seed cake in *O. niloticus* fingerlings was 14.85 mg/L (Table 2).

Fig. 1: *Oreochromis niloticus* fingerlings exposed to acute doses of tea seed cake, showed congestion of the internal organs with mucous secretion on gills.

Table 2: Estimation of 96 hrs LC_{50} of Tea seed cake in *O. niloticus*fingerlings

Group	Concentration of Tea seed cake (mg/L)	Total number of dead fish at 96 hrs	A	B	a x b	∑ a x b
I	6	0	1.5			
II	7.5	2	1.5	1	1.5	
III	9	2	1.5	2	3	
IV	10.5	3	1.5	2.5	3.75	
V	12	4	1.5	3.5	5.25	
VI	13.5	4	1.5	4	6	
VII	15	6	1.5	5	7.5	
VIII	16.5	6	1.5	6	9	
IX	18	7	1.5	6.5	9.75	
X	19.5	8	1.5	75	7.5	
XI	21	9	1.5	8.5	9	
XII	22.5	10	1.5	9.5	14.25	
		0	1.25			76.5

96 hrs LC_{50} = Highest dose - $\frac{\Sigma \text{ a x b}}{n}$

= 22.5 – $\frac{76.5}{10}$ = 22.5- 7.65 = 14.85 mg/L tea seed cake

Where:

a. Constant factor between two successive dosesb. The mean of dead fish in each group.

n. The number of fish in each group.$\sum$ (ax b) = sum of a x b

Table 3. Effect of tea seed cake on RBCs and some serum biochemical parameters

Parameters	Control	1/3 LC_{50} (4.95mg/l)	1/10 LC_{50}(1.48mg/l)	1/20 LC_{50} (0.74mg/l)
Erythrocytes count (x 10^6/µl)	1.30±.004^a	1.09±.011^C	1.23±.004^b	1.30±.009^a
Hemoglobin conc. (gm/dl)	4.37±.110^a	3.13±.042^c	3.77±.058^b	4.35±.408^a
To PCV %	21.0±.40^a	15.0±.478^c	18.0±.408^b	21.0±.408^a
M Mean corpuscular volume(Fl)	161.50±4.66^a	137.60±5.00^a	146.34±4.0^a	161.50±4.20^a
M Mean corpuscular hemoglobin conc.%	20.81±.52^a	20.86±.78^a	20.94±.64^a	20.72±.88^a
ALT (U/L)	50.27±0.64^c	77.02±0.63^a	68.14±1.8^b	50.13±1.9^c
Creatinine (mg/dl)	0.320±0.008^c	0.841±0.12^a	0.725±0.018^b	0.329±.01^c
Urea (mg/dl)	6.05±0.29^d	11.24±0.286^a	9.82±0.25^b	7.30±0.53^c

Means within the same raw carrying different subscript letters are significant at $p<0.05$.

Table 4: Effect of tea seed cake on tissue antioxidant enzymes activities

Parameters	Control	1/3 LC_{50} (4.95mg/l)	1/10 LC_{50}(1.48mg/l)	1/20 LC_{50} (0.74mg/l)
Hepatic SOD (ug/g tissue)	1.05±0.05^d	1.9±.011^a	1.41±.04^b	1.30±.07^c
Renal SOD (ug/g tissue)	0.86±0.01^d	1.13±.04^a	0.97±.03^b	0.91±0.08^c
Intestinal SOD (ug/g tissue)	0.48±0.008^d	0.68± 0.012^a	0.61±0.01^b	0.51± 0.009^c
Gills SOD (µg/g tissue)	1.02±0.19^d	1.41±0.31^a	1.275±0.29^b	1.065±0.2^c
Spleen SOD (µg/g tissue)	0.34±0.002^d	0.47±0.003^a	0.43±0.003^b	0.38±0.002^c
Hepatic CAT (µM H_2O_2 decomposed/g tissue)	1.34±0.02^c	2.07±0.03^a	1.63±0.03^b	1.38±0.002^c
Renal CAT (µM H_2O_2 decomposed/g tissue)	0.94±0.05^d	1.37±0.04^a	1.23±0.043^b	1.08±0.012^c
Intestinal CAT (µM H_2O_2 decomposed/g tissue)	0.62± 0.003^d	0.99± 0.004^a	0.85±0.009^b	0.55±0.001^c
Gills CAT (µM H_2O_2 decomposed/g tissue)	1.29±0.2^d	2.07±0.1^a	1.77±0.17^b	1.37±0.18^c
Spleen CAT (µM H_2O_2 decomposed/g tissue)	0.43±0.06^d	0.69±0.01^a	0.59±0.08^b	0.46±0.03^c

Means within the same raw carrying different subscript letters are significant at $p<0.05$.

Biochemical and Molecular Findings: Our results indicated that all used concentrationswere toxic and theywere dose dependant. The erythrogram revealed a significant decrease in the RBCs count, Hb concentration and PCV% with development of normocytic normochromic anemia.Thetoxicity was manifested by a significant increase in the serumlevelsof ALT, creatinine and urea (Table 3), as well the induction of the activities of the antioxidant enzymes; SOD and CAT in various tissues (Hepatic, renal, intestinal, Gills and pancreatic tissue) (Table 4)

Control Group (1): No microscopic abnormalities were seen (Figs 1-5).

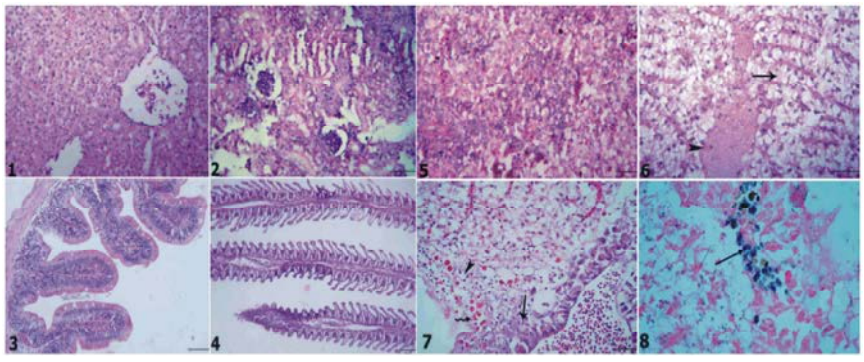
Group (2): Acute exposure to 1/3 $LC_{50}$of Tea Seed Cake.

The liver tissue showed severe vacuolation with pyknotic nuclei and intravascular hemolysis (Fig. 6). Hepatopancreatic necrosis with lymphocytes and EGCs infiltrations were seen besides hemosiderosis of brown pigments (Fig. 7). The hemosiderosis was stained blue with Prussianblue stain (Fig. 8). The portal areas revealed lymphocytes infiltrations and edema. The kidney shows severe hydropic degeneration and vacuolation in the renal tubular epithelia besides few interstitial round cells infiltrations (Fig. 9). Focal areas of coagulative necrosis and basophilic calcification were detected (Fig. 10). The calcification was stained black by Von Kossa stain (Fig. 11). The intestine showed clear enteritis represented by mucinous degeneration in the mucosa and leukocyte infiltrations in the submucosa besides sloughing and necrotic apical parts of the villi (Fig. 12). Sometimes, the intestine showed focal mucosal necrosis infiltrated with lymphocytes and inflammatory edema in the submucosa (Fig. 13). The gills showed epithelial proliferations with increased goblet cells and fusion at the base of gill filaments besides severe congestion of the lamellar blood capillaries, edema and leukocytic infiltration (Figs 14 and 15). Focal necrosis and desquamation of the lining epithelium were also visualized. The spleen showed mild depletion of the lymphoid cells with activation of melanomacrophages and hemosiderosis (Fig. 16). The latter was stained blue with Prussian blue stain (Fig. 17). Severe congestion in the splenic blood vessels and sinusoids was seen.

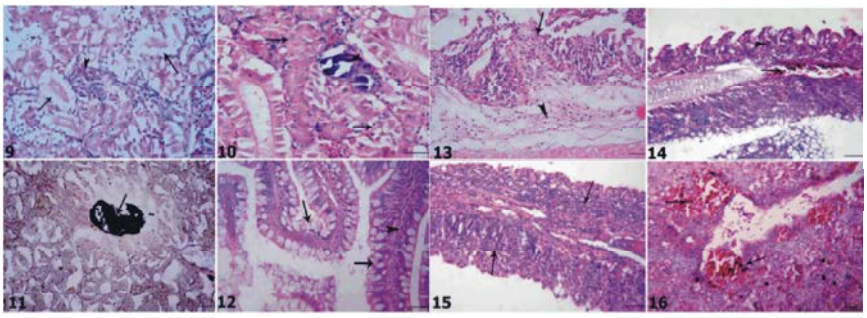
Group (3): Subacute exposure to 1/10 LC_{50} of Tea Seed Cake.

The liver revealed moderate vacuolation in the hepatocytes, hydropic degeneration and intravascular hemolysis (Fig. 18). Sometimes, the portal areas showed necrosis in the pancreatic acini and lymphocytes infiltrations. The kidneyshowed vacuolation in the renal epithelia and intravascular hemolysis (Fig. 19). Lymphocytes aggregations were noticed around necrotic renal tubules (Fig. 20). Hyaline and cellular casts were seen inside the lumina of some renal tubules. The intestine showed extensive desquamation and

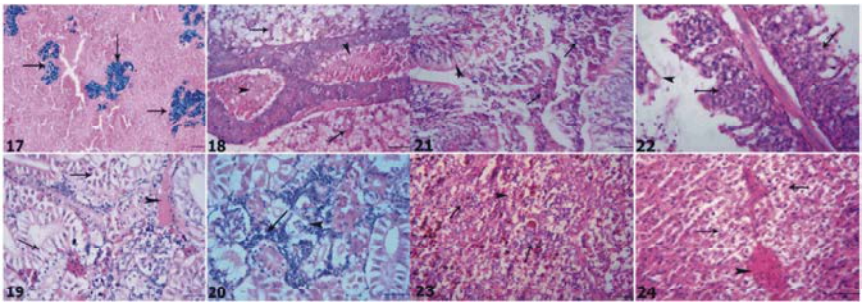
Histopathological Findings:



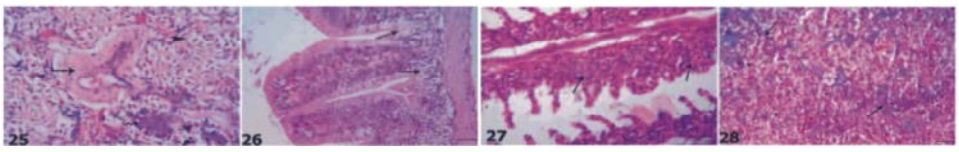
Figs 1-8: GPs (1 and 2).Normal control (GP 1) liver (1), kidney (2), intestine (3), gill (4) and spleen (5). Liver shows severe vacuolation with pyknotic nuclei (Arrow) and intravascular hemolysis (Arrowhead) (6). Liver (GP 2) shows hepatopancreatic necrosis (Arrow) with lymphocytes and EGCs infiltrations (Arrowhead) and hemosiderosis (Zigzag arrow) (7). Liver shows hemosiderosis stained blue (Arrow) by Prussian blue stain (8). HE (Bar = 100 μm) for 1-7.



Figs 9-16: Section in Kidney shows severe hydropic degeneration and vacuolation in the renal tubular epithelia (Arrows) and few round cells infiltration (Arrowhead) (9). Kidney shows focal coagulative necrosis (Arrows) and basophilic calcification (Arrowhead) (10), Kidney shows calcification stained black by Von Kossa stain (Arrow) (11). Intestine shows clear enteritis represented by mucinous degeneration in the mucosa (Arrows) and leukocyte infiltrations in the submucosa (Arrowhead) (12). Intestine shows focal mucosal necrosis infiltrated with lymphocytes (Arrow) and edema in the sub mucosa (Arrowhead) (13). Gill shows hyperplasia and increased goblet cells in the secondary lamellae with basal fusion (Arrowhead) and congestion (Arrow) (14). A higher magnification of previous fig. (14) to show the fusion and the leukocyte infiltration (Arrow) (15). Spleen shows mild depletion of the lymphoid cells with activation of melanomacrophages (Arrows) and hemosiderosis (16). HE (Bar = 100 µm).



Figs 17-24: Section in Spleen shows hemosiderosis stained blue with Prussian blue stain (Arrows) (17). Liver (gp 3) shows moderate vacuolation in the hepatocytes (Arrows) and intravascular hemolysis (Arrowheads) (18). Kidney shows vacuolation in the renal epithelia (Arrows) and intravascular hemolysis (Arrowhead) (19). Kidney shows lymphocytes aggregations (Arrow) around necrotic renal tubules (Arrowhead) (20). Intestine shows extensive desquamation and inflammatory cells in the lumen (Arrows) and mild mucinous degeneration in the lining epithelia (Arrowhead) (21). Gill shows focal hyperplasia and desquamation (Arrowhead) in the lining epithelium of secondary lamellae besides few leukocytes infiltration (Arrows) (22). Spleen shows mild increased in the lymphoid cells (Arrows) and hemosiderosis (Arrowhead) (23). Liver (gp 4) shows centrolobularhydropic degeneration with pyknotic nuclei (Arrows) and mild intravascular hemolysis (Arrowhead) (24). HE (Bar = 100 µm).



Figs 25-28: Section in Kidney shows vacuolation in the renal epithelia (Arrowhead), intravascular hemolysis with thickening and vacuolation of tunica media (Arrow) and few lymphocytes aggregation (Zigzag arrow) (25). Intestine shows mild mucinous degeneration in the lining epithelia (Arrows) (26). Gill shows basal fusion in the lining epithelium of secondary lamellae due to few leukocytes infiltration (Arrows) (27). Spleen shows normal or mildly hyperplastic lymphoid cells (Arrows) (28). HE (Bar = 100 µm).

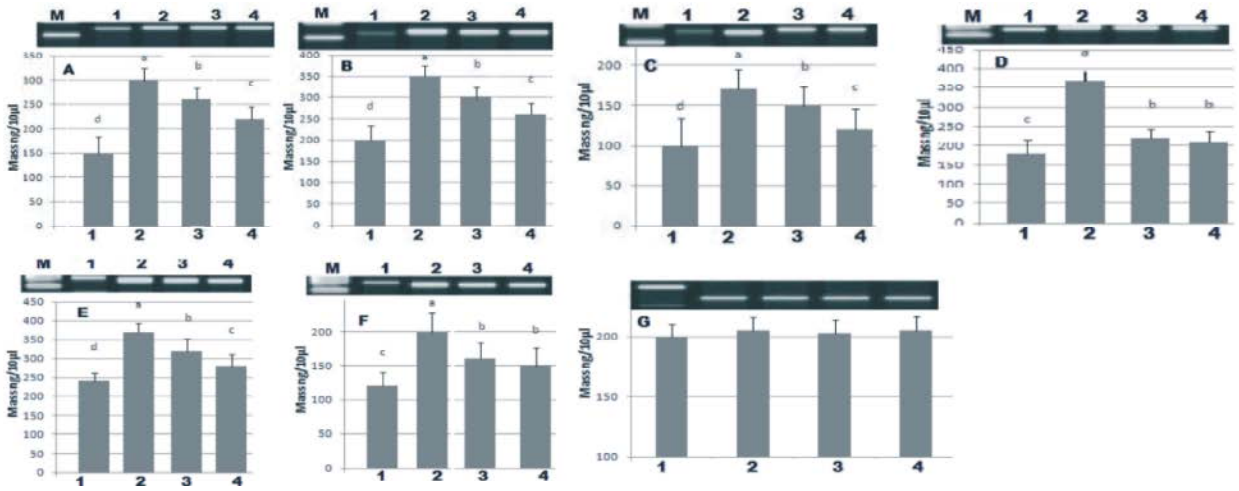


Fig. 29: Gene expression of Liver, Kidney and gills SOD and catalase gene expression A and B, C, D, E, F, G as a relative expression to ß actin gene. M,100bp DNA marker; 1, control group; 2, 1/3 LC_{50} group; 3, 1/10 LC_{50} group and 4, 1/20 LC_{50} group. On the molecular levels there was an induction of gene expression of antioxidant enzymes; SOD and CAT in liver, kidney and gills which refers to the toxicity implicated by different doses of tea seed cake.

inflammatory cells in its lumen and mild mucinous degeneration in the lining epithelia (Fig. 21). The muscular coat was focally hyalinized. The gills revealed focal hyperplasia and desquamation in the lining epithelium of secondary lamellae besides few leukocytes infiltration (Fig. 22). Numerous EGCs were seen at the base of the gill filaments. The spleen showed mild increased in the lymphoid cells and hemosiderosis (Fig. 23).

Group (4): Chronic exposure to 1/20 $LC_{50}$of Tea Seed Cake.

The liver showed centrolobularhydropic degeneration with pyknotic nuclei and mild intravascular hemolysis (Fig. 24). The kidney showed vacuolation in the renal epithelia, intravascular hemolysis with thickening and vacuolation of tunica media (Fig. 25). Few lymphocytes aggregations were also detected. The intestine shows mild mucinous degeneration in the lining epithelia with no evidence of leukocyte infiltrates (Fig. 26). The gills revealed a basal fusion in the lining epithelium of secondary lamellae due to few leukocytes infiltration (Fig. 27). The spleen showed normal or mildly hyperplastic lymphoid cells (Fig. 28).

DISCUSSION

The Tea seed cake is botanical pesticides that is widely used in aquaculture for elimination of predatory fishes in fish and prawn ponds. It can also be used in killing snails in pond or coastal cropland and underground pests in golf grassland.It can help shrimp exuviate and improve the quality of water. Nevertheless, it has several toxic effects on fish with behavior changes in O.*niloticus* especially when be used in large concentrations [23]. Therefore, this work has been established to clarify the deleterious effect of the residues of tea seed cake used in our aquatic media. (By estimating the 96hrs LC_{50}). By using different concentrations of tea seed cake varied from 1/3 to 1/20 of LC_{50}, our results indicate various levels of toxicity on O. *niloticus* fingerlings. The erythrogram revealed normocytic normochromic anemia in the second and third groups (Table 2), compared with control group. This may be due to hemolysis or decreased red cell production resulted from bone marrow depression or kidney lesions (15). The lesions recorded in the kidneys of these groups beside intravascular hemolysis (Figs. 6& 18) andhemosiderosis (Fig. 7) supported our findings. The biochemical markers for hepatic and renal toxicity showed a significant increase in the serum levels of ALT, creatinine and urea in fishes subjected to 1/3 LC_{50} (Table 4).This increase reflects the distraction occurred in hepatic and renal tissues (Figs.1-8). This destruction may be due to overproduction of free radicals that manifested by increase in the activity and gene expression of SOD and CAT presented in Table 4 and Figure 29. Tea seed cake in a concentration of 1/3 of LC_{50} was found to be

activator of SOD and CAT that are responsible for elimination of superoxide radicals and converting it into H_2O_2 then breakdown of it into H_2O and O_2 by catalase [24]. This refers to a state of overproduction of Reactive oxygen species (ROS) and production of hydroxyl radicals and indicates an oxidative stress in response to the toxin[25]. The increased H_2O_2 pool enhances the presence of redox active iron either from loosely bound Fe or by modulating the electron transport chain [26]. CAT gene expression and activities in liver and kidney tissues of fish exposed to tea seed cake were found to be higher than in control fish (Table 4 and figure 29). The stimulation of CAT gene expression and activities were clearly concentration dependent, as it increased in response to 1/10 and 1/20 LC_{50} of toxin but this increase was most pronounced in fish exposed to the highest concentration of toxin (1/3 LC_{50}). Fish have antioxidant defense mechanisms like other animals, which help to maintain health and prevent oxidation lesions. SOD and CAT are important antioxidant enzymes [27]. The enzymes are commonly used in toxicological tests as stress indicators [28]. SOD and CAT are scavengers of the reactive oxygen species, acting on superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) respectively [27].

The increase in hepatic and renal biochemical markers (ALT, creatinine and urea) ameliorated gradually thorough decreasing of tea seed cake concentration 1/10 and 1/20 LC_{50} (Table 3) but still significantly increased when compared with control samples.This improvement in serum hepatic and renal biomarkers concentration may be due to the moderate effect of tea seed cake on hepatocyte which manifested by moderate vacuolization and degeneration (Figs 18-28). The toxic effect of tea seed cake may be explained by its high level of saponin. Vinay *et al*. [27] reported that, the toxic effect of saponin which is manifested by increase of serum ALT and the histological observations showed hepatocyte injures, hypertrophy, cloudy swelling and vacuolization with architectural disarray, hepatic cell degeneration, kidney tubule necrosis with hemolytic erythrocytes in the hematopoietic tissue appeared. Changes observed in transaminases activities in liver and plasma of rats was speculated to be a reflection of liver damage occasioned by ingestion of saponin while the increase of plasma creatinine and urea might be due to kidney damage [28]. Ingestion of saponins results in hepatotoxic lesions manifested by small hemorrhage in hepatic lobules with destruction of liver and renal tubular cells [29].

The effect of tea seed cake on other fish organs was also very clear and runs in the same manner as liver and kidney, as it causes clear intestinal enteritis and focal necrosis with submucosal infiltration of lymphocyte with increase in the level of SOD and CAT activity in the group treated with 1/3 of LC_{50}. In addition, there were focal hyperplasia with leukocytes infiltration and increase in the lymphoid cells with hemosiderosis in gills and spleen respectively with an increase in antioxidant activities in these organs, which may reflect a general health effect and a general increase in the level of ROS in most organs. In the same aspect this increase and extensive destruction in tissue organs was also dose dependent as it decreased by reduction of the dose to 1/10 and 1/20 of LC_{50}.

CONCLUSION

The effect of extensive use of tea seed cake in the aquatic media is dose dependent. It affects the general health status and induces a state of oxidative stress manifested by an increase in the level of SOD and CAT activity and gene expression in different organs, with production of tissue destruction manifested by anemia, a significant increase in the serum biochemical markers (ALT, Urea and Creatinine) and histopathological examination.

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