1. INTRODUCTION

Plants are virtually inexhaustible source of structurally diverse biologically active substances [1]. Some plants contain compounds of various classes that have insecticidal, pesticidal and molluscidal properties. Unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment botanical insecticides are believed to be more environment friendly because they are easily biodegraded with no residues in the environment.

2. MATERIALS AND METHOD

2.1 Specimens collection

Healthy fingerlings of Nile tilapia (Oreochromis niloticus) provided by the Fish Hatchery of Central Laboratory for Aquaculture Research at Abbassa, Sharkia governorate, Egypt. Two hundred and eighty fingerlings with an average body weight 40 ± 0.1 g were randomly distributed. They were apparently healthy and free from any external lesions. Fish were kept in glass aquaria, each aquarium (60 x 50 x 30 cm) provided with an aerator and thermostatically controlled heater. The aquaria were filled with clean and dechlorinated water and containing tap water (temperature 26 ± 2 °C; pH 7.4 ± 0.18; dissolved oxygen (DO) 6.6 ± 0.78 mg/l; photoperiod 12:12 Light: Dark). Fish were fed on a commercial pellet diet containing 25% crude protein (3% of body weight per day) twice a day.

2.2 Experimental design

A total of 80 O. niloticus fingerlings were divided into 4 groups, each group with 20 fish density in each aquarium. Three replicates were used for each concentration, the first group was kept as control, second, third and fourth groups were exposed to 1/3, 1/10 and 1/20 of LC50 respectively (15.4 ± 0.8 mg L−1 of tea seed cake 1/3, 1/10 and 1/20 LC50). There was an increase of hepatic and renal biomarkers, alanin aminotransferase (ALT), creatinin and urea concentrations in serum this concomitant with different pathological changes in hepatic and kidney tissue mostly vacuolization, degeneration, necrosis, lymphocytic infiltration and haemolysis as well an induction of superoxid dismutas (SOD) and catalase (CAT) gene expression and increase of their activities in renal and hepatic tissues of the exposed fish.

2.3 Blood samples

Blood samples were collected from the caudal blood vein; blood samples for serum separation were collected without the addition of anticoagulants and then centrifuged at 3000 rpm for 20 min and stored at -20°C until further biochemical analysis. Serum was separated for determination of alanine aminotransferase (ALT) by the method of creatinine, urea Serum superoxide oxidase dismutase (SOD) activity was determined according to serum catalase (CAT) activity was determined according to the method of [10-14].

2.4 Histopathological investigations

The incidence of pathological changes in key organs like the liver and kidney, because of long term exposure of fish to tea seed cake.
Hepatic and renal SOD and CAT gene expressions were determined using a semi quantitative RT-PCR according to [15]. Total RNA was prepared from the frozen hepatic and renal powder using the E.Z.N.A. RNA Spin column RNA extraction kit (Omega Bio-Tech Int. Ltd, USA) following the manufacturer instructions. Concentrations of RNA were measured by spectrophotometry (OD 260 nm), and RNA integrity was electrophoretic ally verified using ethidium bromide. After DNase treatment (Ambion, Clincinesics, Montrouge, France), RNA was reverse transcribed using Super Script II RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in the presence of Random Primers (Promega, Charbonnières-Bains, France). Polymerase chain reaction (PCR) was performed using a 2720 thermocycler (Applied Biosystems, USA). Using PCR master mix (Qiagen USA) following the manufacturer instructions and the specific primers forward 5'-GGGGGCTGGCTGAGGCTCA-3' and reverse 5'- ATCCTGATGAGGAGGCA-GA and for CAT 'forward 5'- TCCTGAATGAGGAGGCA-3' and reverse 5'- ACCTTATGAGGGGCTTGATG-3', primer were designed using primer 3 programme based on the published nucleotide sequence information of 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 mM Trisborate, 2 mM EDTA buffer (TBE), pH 8, and visualizing by staining with ethidium bromide and UV transillumination, for quantitative evaluation, absolute optical densities (OD) of RT-PCR signals were obtained by densitometric scanning using an image analysis system (1- NC Manager; TDI Ltd.). The values for the specific targets were normalized according to those of GAPDH to express arbitrary units of relative abundance of the specific messages (i.e., relative expression).

3. RESULTS

This work was designed to investigate the toxic effects of three different concentrations (1/3, 1/10 and 1/20 of LC50) of tea seed cake on O. niloticus through monitoring their effects on functional and histological of both liver and kidney, our results revealed that all used concentrations were toxic and the degree of toxicity related to the concentrations. The toxicity was manifested significantly (P < 0.05; Ps 0.01) the concentrations of liver and kidney biochemical markers; serum ALT, creatinine and urea concentrations as well as the induction of the activities of the antioxidant enzymes; SOD and CAT. In both liver and kidney tissue (Table 1) on a molecular level there are induction of the gene expression of SOD and CAT genes (Figure 2 E, F). The induction of the gene expression of SOD and CAT genes increased significantly in the renal epithelia and intravascular hemolysis (Figure 2 B, C and D). The histopathological examination of both kidney and liver tissues confirmed the biochemical and molecular results and revealed that, the hepato pancreatic tissue showed a severe vacuolization with pyknotic nuclei and intravascular hemolysis, necrosis with lymphocytes and EGCs infiltrations and hemosiderosis that stained black by Von Kossa stain (Figure 2 B, C and D), but with 1/10 LC50; a moderate vacuolization in the hepatocytes and intravascular hemolysis were occurred (Figure 1 E) finally with 1/20 LC50; a centrolobular hydroptic degeneration with pyknotic nuclei and mild intravascular hemolysis were manifested (Figure 1 F). In relation to kidney tissue it had a severe hydroptic degeneration and vacuolization that subt the epithelia with few round cells infiltration, focal coagulative necrosis and basophilic calcification that stained black by Von Kossa stain (Figure 2 B, C and D), in 1/3 LC50, with 1/10 LC50 a vacuolization in the renal epithelia and intravascular hemolysis, lymphocytes aggregations around necrotic renal tubules were revealed (Figure 2 E and F), the same pathological manifestations were seen with 1/20 LC50 but in a low degree with thickening of tunica media (Figure 2 G).

4. DISCUSSION

Tea seed cake is botanical pesticides could be extensively used in aquaculture to eliminate predatory fishes in fish and prawn ponds. It also widely used in killing snails in pond or coastal cropland, earthworms in vegetable field and underground pests in golf grassland. It can help shrimp exuviate and improve the quality of water. It not left earthworms in vegetable field and underground pests in golf grassland. It also widely used in killing snails in pond or coastal cropland, earthworms in vegetable field and underground pests in golf grassland. It can help shrimp exuviate and improve the quality of water. It not left earthworms in vegetable field and underground pests in golf grassland. It also widely used in killing snails in pond or coastal cropland, earthworms in vegetable field and underground pests in golf grassland.

The stimulation of CAT gene expression and activities were clearly concentration dependant, it increased in response to 1/10 and 1/20 LC50 of toxin but the increase was most pronounced in fish exposed to the highest concentration of toxin (1/3LC50). CAT is an enzyme that scavenges hydrogen peroxide (H2O2) and hydroxyl peroxydase (H2O2) respectively [23].

REFERENCES


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