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ABSTRACT

Tea seed cake is a plant derivative with pesticide properties mainly used for eradication of unwanted fish, it easily degraded in water leaving an organic fertilizer so it is safe for the environment, this work aimed to study it's toxic effect on the widely farmed fish in Egypt; Oreochromis niloticus throughout this study its molecular, histological and biochemical effects on liver and kidney of O. niloticus fingerlings exposed to different concentration of tea seed cake (1/3, 1/10 and 1/20 LC50). There was an increase of hepatic and renal biomarkers, alanin aminotransferas (ALT), creatinin and urea concentrations in serum this concomitant with different pathological changes in hepatic and kidney tissue mainly vacuolization, degeneration, nicrosis, 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.

1. INTRODUCTION

Plants are virtually inexhaustible source of structurally diverse biologically active substances [1]. Some plants contain compounds of various classes that have insecticidal, pestcicidal and molluscicidal 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 the use of medicinal plants as effective alternatives of synthetic pesticides and fertilizers has gained more importance because they are highly toxic to the target pests [2,3]. Botanical pesticides are suggested as best alternatives to synthetic materials because of their easy biodegradability, easy availability, less expensive, lower toxicity against non-target organisms, greater effectiveness, and their comparative safety toward the environment and human beings [4]. The application of tea seed cake is very effective in eradicating predatory fish in prawn ponds [5]. An application rate of tea seed cake of 15 mg L−1 is considered suitable [6]. Since prawn farmers often apply excess amounts of tea seed cake in pond management, the concentration of saponin in the water and its effect on the Physiological and immune systems of cultured prawn are of primary concern the 24- and 96-h LC50 values (median lethal concentration) of saponin to shrimp, Marsupenaeus japonicas and juveniles have been reported to be 27.08 and 18.14 mg L−1, respectively [7]. Saponin has been reported to decrease the hemocyanin level and affect the acid-base balance of M. japonicus [8]. Very little information exists on the effect of plant pesticides on pathological alterations in fish organs. Considering the reliability of histopathological lesions as biomarkers of stress in fish under toxic conditions. This research work was carried out to establish the incidence of pathological changes in key organs like the liver and kidney, because of long term exposure of fish to tea seed cake.

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 ± 0.1g 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/1; 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 96 hrs LC50 respectively (15.4 ± 0.8 mg L-1 of tea seed cake (soaked in water for 24 hours before use) for 8 weeks [9].

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 oxide dismutase (SOD) activity was determined according to serum catalase (CAT) activity was determined according to the method of [10-14].

2.4 Histopathological investigations

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™.spin column RNA extraction kit (Omega Bio-Tech, Cat NO R6834-01, Canada) 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, Clinisciences, 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, Charbonnièresles- 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 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 programme 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 mM Trisborate, 2 mM EDTA buffer (TBE), pH 8, and visualized 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-D 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 by increasing significantly ($P \leq 0.05$; $P \leq 0.01$) 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 in liver and kidney tissues (Figure 3). The histological examination of both kidney and liver tissues confirmed the biochemical and molecular results and reveled that, the hepatopancreatic tissue showed a severe vacuolization with pyknotic nuclei and intravascular hemolysis, necrosis with lymphocytes and EGCs infiltrations and hemosiderosis that stained blue by Prussian blue stain at the 1/3 LC50 concentration (Figure1 B, C and D), but with 1/10 LC50; a moderate vacuolization in the hepatocytes and intravascular hemolysis were occured (Figure 1 E) finally with 1/20 LC50; a centrolobular hydropic degeneration with pyknotic nuclei and mild intravascular hemolysis were manifested (Figure 1 F). In relation to kidney tissue it had a severe hydropic degeneration and vacuolization in the tubular 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 reveled (Figure 2 E and F), the same pathological manifestation 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 contamination and poison through hydrolysing process in pond still crude protein and crude fibre, which can be assimilated thoroughly by aquatic creatures and algae bloom. Tea seed cake produced from Camellia sp. seeds after oil extraction it contains many active principles especially saponin which represent 5.2% - 7.2%. It has many toxicological effects on fish especially when be used in large concentrations. Our results indicated various levels of toxicity on O. niloticus fingerlings with respect to the different concentrations 1/3, 1/10 and 1/20 of LC50. On the serum level there was a high increase of liver and kidney toxicity biomarker ALT, creatinine and urea in fishes subjected to 1/3 LC50 (Table 1) , this may be due to the distraction that occurred in liver and kidney cells as seen in histopathological examinations which revealed a severe vacuolization and necrosis of hepatocyte (Figure 1B, C and D), Kidney shows severe hydropic degeneration and vacuolization in the renal tubular epithelia and few round cells infiltration, focal coagulative necrosis and basophilic calcification (Figure 2 B,C and D) the concentration of the biochemical markers (ALT, creatinine and urea) were decreased gradually thorough decreasing of tea seed cake concentration 1/10 and 1/20 LC50 (Table 2) but still significantly increased when compared with control samples, the improvement of serum hepatic and renal biomarkers concentration may be due to the moderate effect of tea seed cake on hepatocyt which manifested by moderate vacuolization and degeneration (Figure 1 E and F), Kidney shows moderate vacuolization and necrosis in the renal epithelia (Figure 2 E, F and G). The toxic effect of tea seed cake may be explained by its high level of saponin. The toxic effect of saponin of Quillaja saponaria that manifested by the 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 haemolytic erythrocytes in the hematopoietic tissue appeared [16]. In rat saponins mixture revealed mild cytolysis of the hepatocytes affecting predominantly the periportal region, nuclear vacuolation and prominent nuclei and sinusoidal congestion hepatocytes architectural disarray with extensive necrosis, kidney showed focal tubular atrophy and necrosis with epithelial cells vacuolation and tubular necrosis other investigators have reported untoward histopathological changes in rat /mice tissues following ingestion of saponin containing substances 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 [17-20]. Mice ingested saponins had a hepatotoxic lesion that manifested by small hemorrhage in many hepatic lobules, congestion of central veins and sinusoid, distraction of liver architecture and hepatic necrosis as well destruction of renal tubular cells and haemorrhage [21]. 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 1 and figure 3). 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 is known to detoxify reactive oxygen species in the cells. The increase of gene expression and activity of the CAT in fish in response to tea seed cake most probably occurs in response to an increase of reactive oxygen species indicating an oxidative stress in the animal in response to the toxin. The hepatic and renal SOD activities and gene expression were higher in fish exposed to tea seed cake than in control fish (Table 1 and figure 3) this increase is a concentration dependant. The increase in SOD activities and gene expression may be explained as a response to the toxic effect of tea seed cake on both liver and kidney tissues which may be induced the super oxide radical's generation. As with other animals, fish have antioxidant defense mechanisms, which help to maintain health and prevent oxidation lesions. SOD and CAT are important antioxidant enzymes [22-23]. The enzymes are commonly used in toxicological tests as stress indicators [24]. SOD and CAT are scavengers of the reactive oxygen species, acting on superoxide (O2-) and hydrogen peroxide (H2O2) respectively [23].

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