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Research Article
Glutathione-Enhancer™ Against Foodborne Aflatoxicosis of *Oreochromis niloticus* (Linnaeus, 1758)

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Abstract

Aflatoxicosis is the poisoning that caused by aflatoxins in both humans and animals including fish. The present study aimed to investigate the capability of dietary Glutathione-enhancer™ (GSH-En) to detoxify the chronic toxic effects of aflatoxin B_1 (AFB_1) on *Oreochromis niloticus* for 10 weeks. Fish with an average initial body weight of 28.75±0.14 g were distributed into five treatments (three replicates in each). The AFB_1 was added at a concentration of 150 μg kg^{-1} diet to all mash Basal Diet (BD) (T_2 C+,T_3, T_4 and T_5), except AFB_1-free mash BD was considered as a negative control (T_1 C-) group. AFB_1-BD was considered as a positive control (T_2 C+). However, graded levels (50, 100 and 200 mg GSH kg^{-1} diet) as GSH-En were added to AFB_1-BD T_3, T_4 and T_5, respectively. The results revealed the adverse impacts of AFB_1 (T_2 C+) on growth and feed efficiency, fish carcass composition, condition factor and hepato-somatic index, hematological and serum biochemical parameters, reduction in tissues content of glutathione reduced and total antioxidant capacity, hepatic histological alterations, besides AFB_1-bioaccumulation in the tissues among all toxic groups. Dietary GSH-En was more effective to alleviate these toxicological effects of AFB_1 increasing levels dependent. Hence, it could be concluded that efficient use of GSH-En as a food additive, especially into the high level (200 mg kg^{-1} diet) against the toxicity of AFB_1 on *O. niloticus*. Also, dietary addition of GSH-En at this level may lead to access to safe fish product for human consumption and create more friendly-environment conditions for both fish production and human health.

Key words: Fish, aflatoxins, glutathione, oxidative stress, liver, residues, *Oreochromis niloticus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins (AFs) are considered as unavoidable natural contaminants of foods and feedstuffs due to the lack of a single control measure and production by several strains of ubiquitously found toxigenic fungi, *Aspergillus flavus* and *A. parasiticus* (CAST., 2003). Prominently, the ingested AFs by fish are not totally eliminated from their system, instead they accumulate and cause greater threat not only to themselves but also to fish consumers (Madhusudhanan *et al*., 2006). Epidemiologically, it has been shown that 40% of hepatocellular carcinoma (HCC) in Africa is caused by AFs, when present in foodstuffs (Williams *et al*., 2004). Partially, Abdelhamid and Saleh (1996) confirmed the endemic outspread of AFs and ochratoxin in Egypt. Aflatoxin B_1 (AFB_1), is the major food contaminants of human and farm animals including fish in many areas of the world (IARC., 1993). The toxicity of AFB_1 occurs in the tissues such as the liver of the affected animal wherein it is converted to a reactive epoxide catalyzed by the mixed function mono-oxygenases belonging to the cytochrome P450 super family of enzymes (Guengerich *et al*., 1998). The highly reactive AFB_1 8, 9-epoxide is known to form derivatives with several cellular nucleic acids and proteins. Aflatoxicosis is a condition caused by aflatoxins in both humans and animals. It occurs in two general forms; 1st is acute primary aflatoxicosisis produced when moderate to high levels of aflatoxins are consumed. The 2nd is chronic primary aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (USAID., 2012). The susceptibility of individual animals including fish, to aflatoxicosis varies considerably depending on dose, duration of exposure, species, age, sex and nutrition (Murthy *et al*., 1975).

Aflatoxins are real public health problem and research should be continued to prevent their presence in food and to inhibit their harmful effects all over the world (Ayub and Sachan, 1997). In fish, consumption of low concentrations of AFs caused liver cells damage, impaired growth and feed utilization, reduced reproductively and suppressed the immunity responses (Agag, 2004). Since the discovery of the nature of AFs, aflatoxicosis has been investigated predominantly in freshwater aquatic species, especially in *Oncorhynchus mykiss* (Ellis *et al*., 2000; Alinezhad *et al*., 2011), *Ictarulus punctatus* (Gallagher and Eaton, 1995; Manning *et al*., 2005), *Oreochromis niloticus* (Deng *et al*., 2010; Selim *et al*., 2014), Gibel carp (*Carassius gibelio*) (Huang *et al*., 2011) and *Labeo rohita* (Madhusudhanan *et al*., 2006; Ruby *et al*., 2013).

Detoxification of AFs contaminated foods and feeds is a current problem, where the detoxification methods can be classified as physical, chemical or microbiological (Abdelhamid *et al*., 2002a; Agag, 2003). Many attempts were conducted for detoxification of AFs from the fish foods including; dietary addition of different adsorbents agents (Ellis *et al*., 2000; Abdelhamid *et al*., 2004b) microbial strains including yeast, molds and bacteria have been seemed for their ability to inactivate AFs (Volkl *et al*., 2004; Wu *et al*., 2009). Also, probiotic mixtures of Lactobacillus and *Propioni bacterium* may reduce bioavailability of dietary AFs (Ahokas *et al*., 1998). In addition, many chemicals have been tested for their ability to degrade AFs (Takahashi *et al*., 1995; Guarisco *et al*., 2008). However, the obtained results with these methods have not been optimal, because they may change the organoleptic characteristics and nutritional values of food (Madrigal-Santillan *et al*., 2010). Hence as a modern method for AFs detoxification, medicinal herbs or their extracts were effectively used (El-Barbary and Mehrim, 2009; Mehrim and Salem, 2013).

Chemoprevention has been examined as an attractive strategy to reduce losses due to AFB_1 contamination in feeds (Guarisco *et al*., 2008). Although, various chemicals have been evaluated in an almost isolated experimental form and have presented favorable results but not optimal; therefore, extensive studies on these agents should be carried out (Madrigal-Bujaidar *et al*., 2011). In this context, the conjugation of AFB_1 to glutathione (GSH) and its subsequent excretion is regarded as an important detoxification pathway in animals (Johnson and Guengerich, 1997; Smela *et al*., 2001). Glutathione, being a component of the mechanism of non-enzymatic antioxidative protection, plays important roles in antioxidant defense, in nutrient metabolism and in the regulation of cellular events. A deficiency of GSH sets off oxidative stress (Wu *et al*., 2004). Although GSH is synthesized mainly in the liver, orally ingested GSH is directly absorbed through the intestinal mucosa and acts as the functional component against oxidative stress (Hunjan and Evered, 1985). Therefore, the GSH content in various foods have become a matter of great interest. Consequently, more attempts are needed to determine the optimal levels of GSH for detoxification of AFs (El-Barbary, 2010) or to answer the main questions about the contribution of GSH conjugation system in removing AFs in different cellular systems (Ziglari and Allameh, 2013).

Hence, the present study was conducted to investigate the ability of dietary graded levels (0, 50, 100 and 200 mg GSH kg^{-1} diet) of GSH as a Glutathione-enhancer™ (GSH-En) capsules to detoxify the toxic effects of chronic oral administration of AFB_1 (150 μg kg^{-1} diet) on growth and feed efficiency, chemical composition of body, condition

factor (K_f) and hepatosomatic index (HSI), residues of AFB_1 in muscles and all viscera, hematological and serum biochemical parameters, glutathione reduced (GSH) in the liver and muscles and Total Antioxidant Capacity (TAC) in the liver, besides the liver histopathological alterations of Nile tilapia (*Oreochromis niloticus*) fingerlings for 10 weeks.

MATERIALS AND METHODS

Experimental procedures: This study was conducted in Fish Research Laboratory, Faculty of Agriculture, Al-Mansoura University, Al-Mansoura, Egypt. A total of 105 *O. niloticus* fingerlings, with an average initial body weight of 28.75±0.14 g were distributed into five treatments (three replicates in each) as shown in Table 1. Fish were stocked at seven fish per aquarium. Each glass aquarium (90×50×40 cm) was supplied with an air stone connected with electric compressor for water aeration. The replacement of the aquarium water was done partially every day to remove the wastes, then the chlorine-free tap water was added. Light was controlled by a timer to provide a 14 h light:10 h dark as a daily photoperiod.

Water quality parameters were measured including temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter), in all aquaria day by day. Mean values of water temperature ranged between 25.6 and 27.8°C, pH values 6.70-7.90 and dissolved oxygen 5.50-6.70 mg L^{-1}. All tested water quality criteria in the present study were suitable for rearing *O. niloticus* fing erlings as cited by Mehrim (2009).

Experimental diet preparation: The experimental Basal Diet (BD) used in this study contained 30% crude protein. The mashed ingredients of BD were bought from the local market and proximate chemical analysis was carried out according to AOAC (2004) as shown in Table 2. All ingredients were homogeneously mixed and the commercial anti-toxin agent GSH-En capsule was added at graded levels of 0, 50, 100 and 200 mg GSH kg^{-1} diet. Each hard gelatin capsule of GSH-En contains 50 mg GSH. It was manufactured by Marcyrl Pharmaceutical Industries, El-Obour City, Cairo, Egypt. The AFB_1 production was carried out according to Davis *et al*. (1966) using liquid yeast medium and *Aspergillus flavus* strain (NRRL 3145). The media which contain detectable amount of aflatoxin was mixed well with the basal diet to get AFB_1-contaminated diet. Then, AFB_1 was added at a concentration of 150 μg kg^{-1} diet to all mash diets (T_2 C+, T_3,T_4 and T_5), except the mash BD was considered as a negative control (T_1 C-) group, whereas the aflatoxic diet was

Table 1: Details of the experimental treatments

Treatments	Details
T_1 (C-)	Fish fed the Basal Diet (BD) free of AFB_1 (as a negative control)
T_2 (C+)	Fish fed the contaminated BD with 150 μg AFB_1 kg^{-1} diet (as a positive control)
T_3	Fish fed the contaminated BD with 150 μg AFB_1 kg^{-1} diet+50 mg glutathione kg^{-1} diet (as a Glutathione-enhancer™)
T_4	Fish fed the contaminated BD with 150 μg AFB_1 kg^{-1} diet+100 mg glutathione kg^{-1} diet (as a Glutathione-enhancer™)
T_5	Fish fed the contaminated BD with 150 μg AFB_1 kg^{-1} diet+200 mg glutathione kg^{-1} diet (as a Glutathione-enhancer™)

BD: Basal deit, AFB_1: Aftatoxin B_1

Table 2: Ingredients and proximate chemical analysis (percentage on dry matter basis) of the experimental basal diet

Ingredients	Percentage
Fish meal (70% CP)	8
Corn gluten (60% CP)	15
Soybean meal (44% CP)	28
Wheat bran	20
Yellow corn	20
Molasses	3
Corn oil	5
Vitamins and minerals premix[1]	1
Nutrient composition	
Dry matter (%)	93.53
Crude protein (%)	30.91
Ether extract (%)	7.23
Ash (%)	5.64
Total carbohydrates (%)	56.22
Gross energy (kcal 100 g^{-1} DM) (GE)[2]	473.64
Protein/energy (P/E) ratio (mg CP $kcal^{-1}$ GE)[3]	65.26

[1]Each 3 kg premix contains: Vit. A: 12000,000 IU, Vit. D_3: 3000,000 IU, Vit. E: 10,000 mg, Vit. K_3: 3000 mg, Vit. B_1: 200 mg, Vit. B_2: 5000 mg, Vit. B_6: 3000 mg, Vit. B_{12}: 15 mg, Biotin: 50 mg, Folic acid: 1000 mg, Nicotinic acid: 35000 mg, Pantothenic acid: 10,000 mg, Mn: 80 g, Cu: 8.8 g, Zn: 70 g, Fe: 35 g, I: 1 g, Co: 0.15 g, Se: 0.3 g, [2]GE (kcal 100 g^{-1} DM) = CP×5.64+EE×9.44+total carbohydrates×4.11 calculated according to NRC (1993), [3]P/E ratio (mg protein $kcal^{-1}$ gross energy) = CP/GE×1000

considered as a positive control (T_2 C+) as shown in Table 1. The experimental mashed diets were introduced manually twice daily at 9.00 am and 15.00 pm at 4% of the fish biomass in each aquarium. The fish were weighed every two weeks by a digital scale (accurate to ±0.01 g) to adjust their feed quantity according to the actual body weight changes, the fish biomass present in each aquarium. The fish were weighed every two weeks by a digital scale (accurate to ±0.01 g) to adjust their feed quantity according to the actual body weight changes, the fish biomass present in each aquarium.

Fish sampling and performance parameters: At the start (twenty fish as a pooled sample) and at the end of the experiment, fish samples (five fish in each treatment) were collected and kept frozen (-20°C) till the proximate analysis of the whole fish body according to AOAC (2004). Their energy content were calculated according to NRC (1993). Fish growth and feed efficiency parameters such as average Total Weight Gain (TWG, g), Average Daily Gain (ADG, gram per fish per day), Specific Growth Rate (SGR, percentage per day), Mortality Rate (MR, %), Feed Intake (FI, g), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Protein Productive Value (PPV, %) and Energy Utilization (EU, %) were calculated according to Lovell (2001).

Condition factor (K_f) and hepatosomatic index (HSI): Five fish in each treatment were randomly taken and anaesthetized to determine condition factor (K_f, %) and hepatosomatic index (HSI, %) parameters. Where, fish weight (W) and their Total Length (TL) were individually measured for the nearest 0.1 g and 0.01 cm, respectively to calculate K_f according to the Eq. 1:

$$K_f\ (\%) = \frac{W(g)}{TL^3(cm)} \times 100 \quad (1)$$

Then, fish were sacrificed to obtain the livers and they were individually weighed to determine the HSI according to the Eq. 2:

$$HSI\ (\%) = \frac{\text{Liver weight (g)}}{\text{Fish weight (g)}} \times 100 \quad (2)$$

Determination of AFB_1 residues: At the end of the experiment, three fish from each treatment were immediately taken for determination the residues of AFB_1. Fish were anaesthetized by transferring into a small plastic tank containing 10 L water supplemented with 3 mL pure clove oil (dissolved in 10 mL absolute ethanol). Then, fish were sacrificed, where muscles and all viscera (including liver) samples were taken to determine the residues of AFB_1 (μg kg^{-1}). Aflatoxin identification was performed by a modification of the HPLC-AFLATEST procedure Agillent 1200 Series, USA. The HPLC equipment with two pumps, column C18, Lichrospher 100 RP-18, (5 μm×25 cm) were used. The mobile phase consisted of water: methanol: acetonitrile (54: 29: 17, v/v/v) at flow rate of 1 mL min^{-1}. The excitation and emission wave length for AFB_1 was 362 nm (fluorescence detector). Total AFB_1 residues in tissues were quantitatively estimated according to the method described by Roos *et al*. (1997).

Hematological parameters: At the end of the experiment, blood samples were collected from the fish caudal peduncle of all treatments. Blood samples from three fish in each aquarium were randomly taken. Adequate amounts of whole blood (5 mL at each collection) were received in small plastic vials containing heparin which were used for the determination of hemoglobin (Hb) using commercial colorimetric kits (Diamond Diagnostic, Egypt). Total red blood cells (RBCs×10^6 mm^{-3}), platelets (×10^3 mm^{-3}), total white blood cells (WBCs×10^3 mm^{-3}) and their differential were counted according to (Dacie and Lewis, 1995) on an Ao Bright-Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany). However, the packed cell volume (PCV %) was measured according to Stoskopf (1993).

Serum biochemical measurements: Other blood samples (5 mL at each collection) were collected in dried plastic tubes and centrifuged for 20 min at 3500 rpm to obtain the blood serum. Serum samples were kept in deep freezer (-20°C) until the biochemical analysis was carried out. Serum biochemical parameters were measured as total protein according to Gornall *et al*. (1949), albumin according to Weichsebum (1946), globulin by difference according to Doumas and Biggs (1972) and total cholesterol according to the method described by Ellefson and Caraway (1976). Also, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were colorimetrically determined using commercial kits (Diamond, Diagnostic, Egypt) according to Reitman and Frankel (1957).

Glutathione reduced and total antioxidant capacity parameters: Three fish in each aquarium were randomly taken for determination the glutathione reduced (GSH, mg g^{-1}) in the liver and muscles and Total Antioxidant Capacity (TAC, mM g^{-1}) in the liver. Fish were anaesthetized and sacrificed to obtain the liver and muscle tissues. Then, tissues were homogenized in ice-cold buffer (5 mM potassium phosphate, pH 7.4 containing 0.9% sodium chloride and 0.1%

glucose) using a glass homogenizer immersed in an ice-water bath to yield a 10% homogenate. Homogenates were centrifuged at 4000 rpm for 15 min at 4°C. After centrifugation, the supernatant was collected and frozen at-20°C until analyzed. The TAC level was spectrophotometrically estimated at 532 nm following the method described by Galaktionova *et al.* (1998). While, GSH level was colorimetrically measured at 405 nm according to the method of Jollow *et al.* (1974).

Histopathological examination of the liver: At the end of the experiment, three fish in each aquarium were sacrificed and liver samples were taken for histopathological examination. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated using different grades of alcohol (70, 85, 96 and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six microns and stained with hematoxyline (H) and eosin (E) stains for preparing the histological slides for microscopic examination according to Roberts (2001).

Statistical analysis: All results are presented as mean values and standard errors of (Mean±SE) in three replicates (n = 3) of each treatment. All ratios and percentages were arcsine-transformed prior to statistical analyses. Data was subjected to one-way analysis of variance (ANOVA) using SAS (2004) procedure of the Statistical Analysis System (version 9.2) to detect the overall effects of treatments (T_1-T_5). The differences between mean of treatments were compared using Tukey's *post hoc* significant test and differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Growth and feed efficiency: Data of growth and feed efficiency of *O. niloticus* fed dietary AFB_1 and graded levels of GSH-En are shown in Table 3. Fish in T_1 (C-) group revealed the highest values in all growth and feed efficiency parameters and the best value in FCR among all treatments. Also, the administration of graded levels of GSH-En against the contaminated diets of AFB_1 to the experimental fish induced significant ($p \leq 0.05$) level-dependent increase in all growth and feed efficiency parameters, except FI and the best FCR value compare to the positive group (T_2 C+). Where, fish in the T_2 (C+) group revealed the absolutely lowest values in all growth and feed efficiency parameters but the highest values ($p \leq 0.05$) of MR among all treatments. No significant ($p \geq 0.05$) differences in FI were noticed among all treatments.

Chemical composition of fish body: Results of the whole body chemical composition of *O. niloticus* in T_2 (C+) group revealed significantly ($p \leq 0.05$) decreased DM and CP and increased EE and ash contents compared to the other groups (Table 4). Inversely trends were observed for T_1 (C-) group, where significantly ($p \leq 0.05$) increased DM and CP contents and decreased ($p \leq 0.05$) EE and ash were found compared to other groups without significantly differences with T_5 group. However, no significant ($p \geq 0.05$) differences in EC were detected among all treatments. In general, increasing the graded levels of GSH-En addition to AFB_1-contaminated diets alleviated the drastic affects of AFB_1 on fish body chemical composition parameters compared to the T_2 (C+) group.

Condition factor (K_f) and hepatosomatic index (HSI): Fish fed BD contaminated with AFB_1 only T_2 (C+) led to significantly ($p \leq 0.05$) decrease of K_f (Fig. 1a) and HSI (Fig. 1b) among all treatments. However, increasing levels of GSH-En addition to AFB_1-contaminated diets ameliorated the impaired affects of AFB_1 on K_f and HSI compared to T_2 (C+) group. These improvements were increased with increasing the addition levels of GSH-En. While, fish in T_1 (C-) and T_5 groups revealed significantly ($p \leq 0.05$) increased both of K_f (Fig. 1a) and HSI (Fig. 1b) among all treatments.

Table 3: Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on growth and feed efficiency parameters of *Oreochromis niloticus*

	Treatments					
Traits	T_1 (C-)	T_2 (C+)	T_3	T_4	T_5	p-value
Final weight (g)	83.57±1.88^{a}	59.83±2.42^{c}	64.10±2.96^{cb}	67.90±1.99^{b}	78.20±0.96^{a}	0.0001
TWG (g)	55.67±1.94^{a}	31.87±2.4^{c}	35.97±2.92^{cb}	39.83±1.81^{b}	50.17±1.05^{a}	0.0001
ADG (g per fish per day)	0.80±0.03^{a}	0.46±0.03^{c}	0.51±0.04^{cb}	0.57±0.03^{b}	0.72±0.02^{a}	0.0001
SGR (% per day)	1.57±0.04^{a}	1.08±0.06^{c}	1.17±0.07^{cb}	1.26±0.03^{b}	1.47±0.02^{a}	0.0001
MR (%)	0.00±0.00^{e}	42.76±2.45^{a}	28.48±0.09^{b}	14.38±0.07^{c}	9.53±0.04^{d}	0.0001
FI (g)	107.30±0.35	93.27±1.37	97.87±7.21	94.33±1.39	102.40±4.29	0.129
FCR	1.93±0.06^{c}	2.96±0.21^{a}	2.75±0.24^{ab}	2.38±0.14^{bc}	2.05±0.13^{c}	0.006
PER	1.68±0.05^{a}	1.10±0.07^{c}	1.20±0.11^{c}	1.37±0.08^{bc}	1.59±0.10^{ab}	0.003
PPV (%)	27.71±0.79^{a}	10.11±0.82^{c}	13.93±1.31^{b}	16.95±1.03^{b}	27.02±1.69^{a}	0.0001
EU (%)	15.84±0.44^{a}	8.79±0.54^{b}	9.81±0.85^{b}	11.14±0.64^{b}	16.16±0.99^{a}	0.0001

Mean in the same row having different small letters are significantly different ($p \leq 0.05$), TWG: Total weight gain, ADG: Average daily gain, SGR: Specific growth rate, MR: Mortality rate, FI: Feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein productive value, EU: Energy utilization

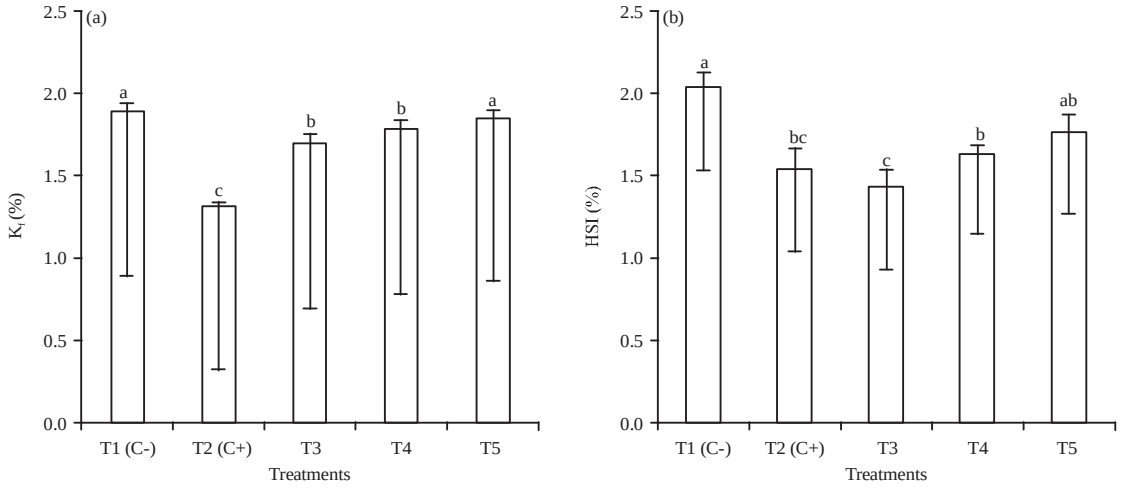

Fig. 1(a-b): Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on (a) Condition factor (K_f) and (b) Hepatosomatic index (HSI) of *Oreochromis niloticus*

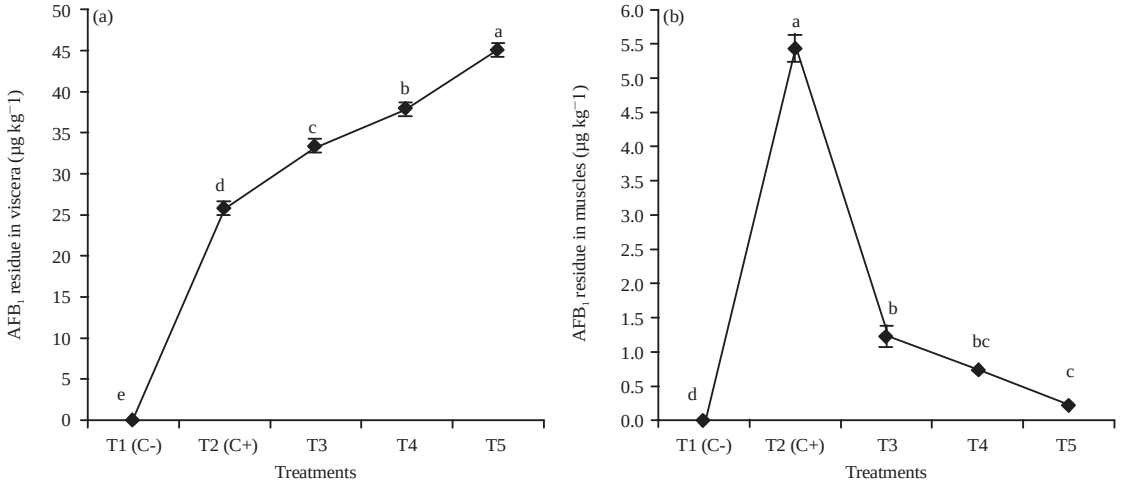

Fig. 2(a-b): Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on residues of AFB_1 in (a) All viscera and (b) Muscles of *Oreochromis niloticus*

Table 4: Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on whole body chemical composition of *Oreochromis niloticus*

		On dry matter basis			
Treatments	DM (%)	CP (%)	EE (%)	Ash (%)	EC (kcal 100 g^{-1})
At the start of the experiment	17.88	71.68	16.43	11.89	559.41
At the end of the experiment					
T_1 (C-)	23.37±0.56^{ab}	65.35±0.64^{a}	19.74±0.29^{d}	14.91±0.36^{b}	554.9±0.93
T_2 (C+)	20.15±2.09^{b}	53.94±0.90^{c}	26.51±0.62^{a}	19.55±1.51^{a}	554.5±10.81
T_3	20.40±0.48^{b}	59.60±1.14^{b}	23.81±0.54^{b}	16.60±0.62^{ab}	560.8±1.70
T_4	20.71±0.43^{b}	60.67±0.80^{b}	22.35±0.55^{cb}	16.98±1.29^{ab}	553.2±9.31
T_5	24.25±0.15^{a}	63.82±0.24^{a}	21.09±0.72^{c}	15.09±0.76^{b}	559.0±6.98
p-value	0.030	0.0001	0.0001	0.040	0.927

Mean in the same column having different small letters are significantly different ($p \leq 0.05$), DM: Dry matter, CP: Crude protein, EE: Ether extract, EC: Energy content

Residues of aflatoxin B_1 (AFB_1): Fish fed BD contaminated with AFB_1 only T_2 (C+) led to significantly ($p \leq 0.05$) increase of AFB_1 residues (µg kg^{-1}) in muscles (Fig. 2b) among all treatments. On the other hand, increasing levels of GSH-En addition to AFB_1-contaminated diets significantly reduced these bioaccumulations of AFB_1 in edible muscles but

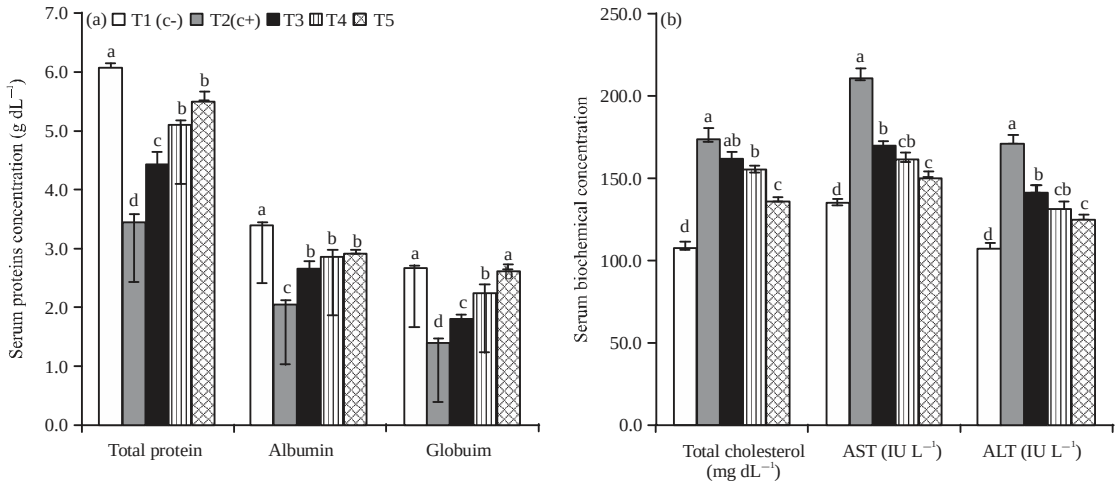

Fig. 3(a-b): Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on serum biochemical parameters of *Oreochromis niloticus*

Table 5: Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on some hematological parameters of *Oreochromis niloticus*

Treatments	Hb (g dL^{-1})	RBCs ($\times 10^6$ mm^{-3})	PCV (%)	Platelets ($\times 10^3$ mm^{-3})	WBCs ($\times 10^3$ mm^{-3})	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
T_1 (C-)	7.83±0.23^a	1.93±0.04^a	25.63±0.61^a	127.2±8.64^a	117.1±3.32^a	62.33±1.45^a	2.00±0.00	1.67±0.33
T_2 (C+)	3.83±0.15^d	0.93±0.09^d	15.37±0.52^d	66.87±1.96^c	61.53±1.95^e	39.00±1.15^c	1.00±0.00	1.00±0.00
T_3	5.73±0.22^c	1.13±0.09^{cd}	19.07±0.12^c	74.67±2.19^c	69.93±2.23^d	51.00±2.08^b	1.33±0.33	1.33±0.33
T_4	5.73±0.18^c	1.30±0.06^c	21.13±0.42^b	80.80±2.28^c	80.00±1.84^c	51.67±1.45^b	1.67±0.33	1.33±0.33
T_5	6.80±0.06^b	1.67±0.03^b	22.27±0.20^b	95.27±2.77^b	92.43±0.49^b	55.33±1.45^b	1.67±0.33	1.67±0.33
p-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.146	0.512

Mean in the same column having different small letters are significantly different ($p \leq 0.05$), HB: Hemoglobin, RBC_S: Red blood cells, PCV: Packed cell volume, WBC_S: White blood cells

increased in viscera compared to T_2 (C+) group. These enhancements were increased with increasing the addition levels of GSH-En (T_5). While, AFB_1 residues were not detected in both of viscera or muscles of fish fed BD free of AFB_1 T_1 (C-) compared to all treatments (Fig. 2a, b).

Hematological measurements: Hematological parameters of *O. niloticus* fed dietary AFB_1 and graded levels of GSH-En are shown in Table 5. Fish fed BD free of AFB_1 T_1 (C-) group had significantly ($p \leq 0.05$) increased all hematological parameters among all groups. Increased levels of dietary GSH-En in AFB_1-contaminated diets significantly ($p \leq 0.05$) alleviated the toxic effects on hematological parameters compared to fish fed AFB_1-contaminated BD only T_2 (C+). No significant ($p \geq 0.05$) differences in monocyte and eosinophil percentages were detected among all treatments.

Serum biochemical parameters: *Oreochromis niloticus* fed AFB_1-contaminated BD only T_2 (C+) had significantly ($p \leq 0.05$) decreased serum total protein, albumin and globulin (Fig. 3a) while significantly increased total cholesterol concentration, AST and ALT activities among all treatments (Fig. 3b). While, inversely trends were detected in these parameters of T_1 (C-) group among all groups. On the other side, increasing addition levels of dietary GSH-En in AFB_1-contaminated diets significantly ($p \leq 0.05$) increased serum total protein, albumin and globulin levels and ($p \leq 0.05$) decreased total cholesterol, AST and ALT levels compared to T_2 (C+) group. Hence, it could be noted that addition of GSH-En improved the liver (as a target organ for AFB_1) responses against the aflatoxicosis of the experimental fish.

Glutathione reduced and total antioxidant capacity parameters: Significant ($p \leq 0.05$) severe reduction was recorded in both of TAC (mM g^{-1}) concentration in the liver (Fig. 4a) and GSH (mg g^{-1}) in both liver and muscles (Fig. 4b) of *O. niloticus* fed AFB_1-contaminated BD only T_2 (C+) among all groups. However, the concentrations of TAC in the liver and GSH in the liver and muscles of the experimental fish were significantly ($p \leq 0.05$) increased by increasing levels of dietary GSH-En in AFB_1-contaminated diets compared to the T_2 (C+) group. On the other hand, these parameters were significantly ($p \leq 0.05$) increased in case of T_1 (C-) group among all groups.

Liver histopathology: Liver histological alterations of *O. niloticus* fed BD-contaminated with or without AFB_1 and dietary addition levels of GSH-En were illustrated in Fig. 5a-f. Fish fed BD free of AFB_1 T_1 (C-) showed normal structure of the

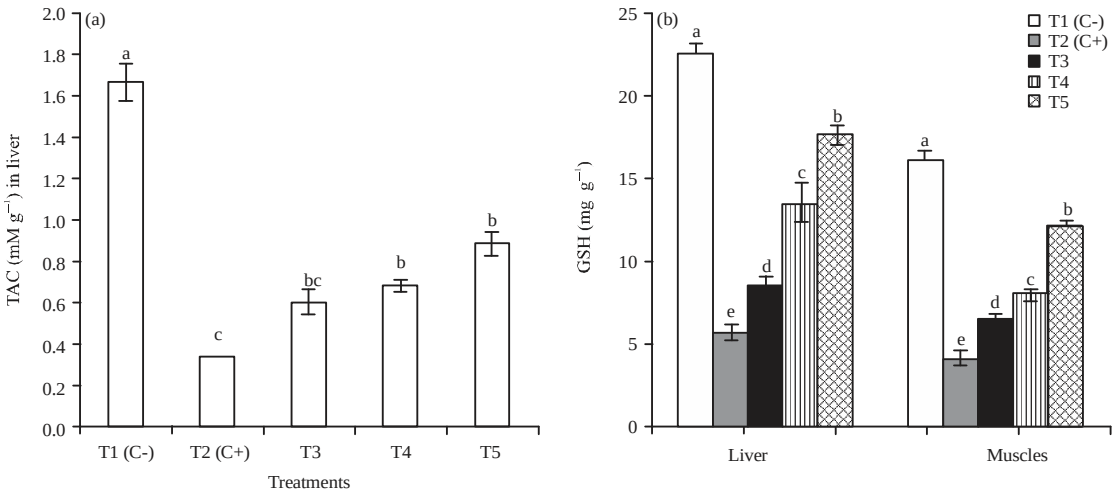

Fig. 4(a-b): Effect of dietary aflatoxin B_1 and Glutathione-enhancer™ on (a) TAC in liver and (b) GSH in liver and muscle of *Oreochromis niloticus*

hepatocytes around the Central Vein (CV) (Fig. 5a). While, there are severe alterations in the liver of fish fed BD contaminated with AFB_1 only T_2 (C+) such as dilatation, thickening and severe congestion of the Portal Vessels (PVs) adjacent with severe degeneration of the hepatocytes (Fig. 5b), large areas of severe hemolysis between the hepatocytes in the hepatic lobule, severe necrosis of the hepatocytes with areas of hemorrhage and fatty degeneration in the liver cells (Fig. 5c). These adversely affects of AFB_1 on histological structure of the liver were alleviated by increasing levels of dietary GSH-En in AFB_1-contaminated diets compared to the T_2 (C+) group (Fig. 5d-f).

DISCUSSION

Aflatoxins cause growth and feed efficiency suppression in fish. Similarly in the current findings, dietary AFB_1 reduced the survivability, total weight gain, average daily gain, specific growth rate and impaired FCR in many fish species, such as *O. niloticus* (Al-Faragi, 2014; Ayyat *et al*., 2014), *Clarius lazera* (Zaki and Fawzi, 2012) and *Labeo rohita* (Ruby *et al*., 2013). More recently, Mahfouz (2015) and Mahfouz and Sherif (2015) reported the adversely affects of AFB_1 on growth and feed efficiency parameters of *O. niloticus*. These reduction in growth and feed efficiency parameters in the experimental fish could be due to disturbance in metabolic process of lipids, carbohydrates and proteins by AFB_1 which leads to inhibition of carbohydrate and lipid metabolism and protein synthesis (Puschner, 2002). In addition, Russo and Yanong (2002) reported that aflatoxin causes loss of appetite. Hence, a general reduction in feed intake of *O. niloticus* fed AFB_1-contaminated diets have been documented by Jantrarotai and Lovell (1990) and Deng *et al*. (2010) in a dose-dependent fashion. Inversely, Huang *et al*. (2011) reported that no decreased feeding rate was observed in gibel carp fed AFB_1 less than 1000 μg kg^{-1}, indicating that gibel carp is not sensitive to dietary AFB_1. Consequently, the severity of AFs toxicity depend on many factors such as dose, age, sex, mode of application, duration of feeding and other factors such as stresses affecting an animal (Vainio *et al*., 1994). Thus, *O. niloticus* fed diets containing 2.25 mg AFB_1 kg^{-1} had changes in growth rate, whereas 10 mg AFB kg^{-1} produced hepatic lesions and 100 mg AFB_1 kg^{-1} caused severe hepatic necrosis and 60% MR (Tuan *et al*., 2002).

Similarly with the obtained results herein, *O. niloticus* fed a 200 μg AFB_1 kg^{-1} diet for 10 or 16 weeks, showed 34.34 and ~30.0% MR, respectively (Selim *et al*., 2014; Mahfouz, 2015), exposure of *O. niloticus* to 53.02-115.34 μg kg^{-1} for 120 days induced 67% MR (Cagauan *et al*., 2004). Also, Santacroce *et al*. (2008) suggested that dosages of 0.5-1 mg AFB_1 kg^{-1} diet resulted in 50% MR of *O. niloticus* as well as AFB_1 injected groups of *O. niloticus* revealed a significant increase in MR compared to AFB_1-not injected group (El-Barbary and Mehrim, 2009). Inversely with the previous findings and the obtained results herein, lower doses of AFB_1 (30 μg kg^{-1} or less) did not induce mortalities in the same species (Tuan *et al*., 2002), in hybrid tilapia (*O. niloticus*×*O. aureus*) (Deng *et al*., 2010) or in red tilapia (*O. niloticus*×*O. mossambicus*) (Usanno *et al*., 2005). These differences may be attributed to fish species, experimental conditions or duration of AFB_1 exposure (Ngethe *et al*., 1993) and generally cold water species are more sensitive than warm water species (Tuan *et al*., 2002).

Biochemically, AFs can affect metabolism of energy, carbohydrate, lipid, nucleic acid and protein (Ellis *et al*., 1991). In the present study, dietary AFB_1 led to harmful effects on body chemical composition of *O. niloticus*. Similar findings

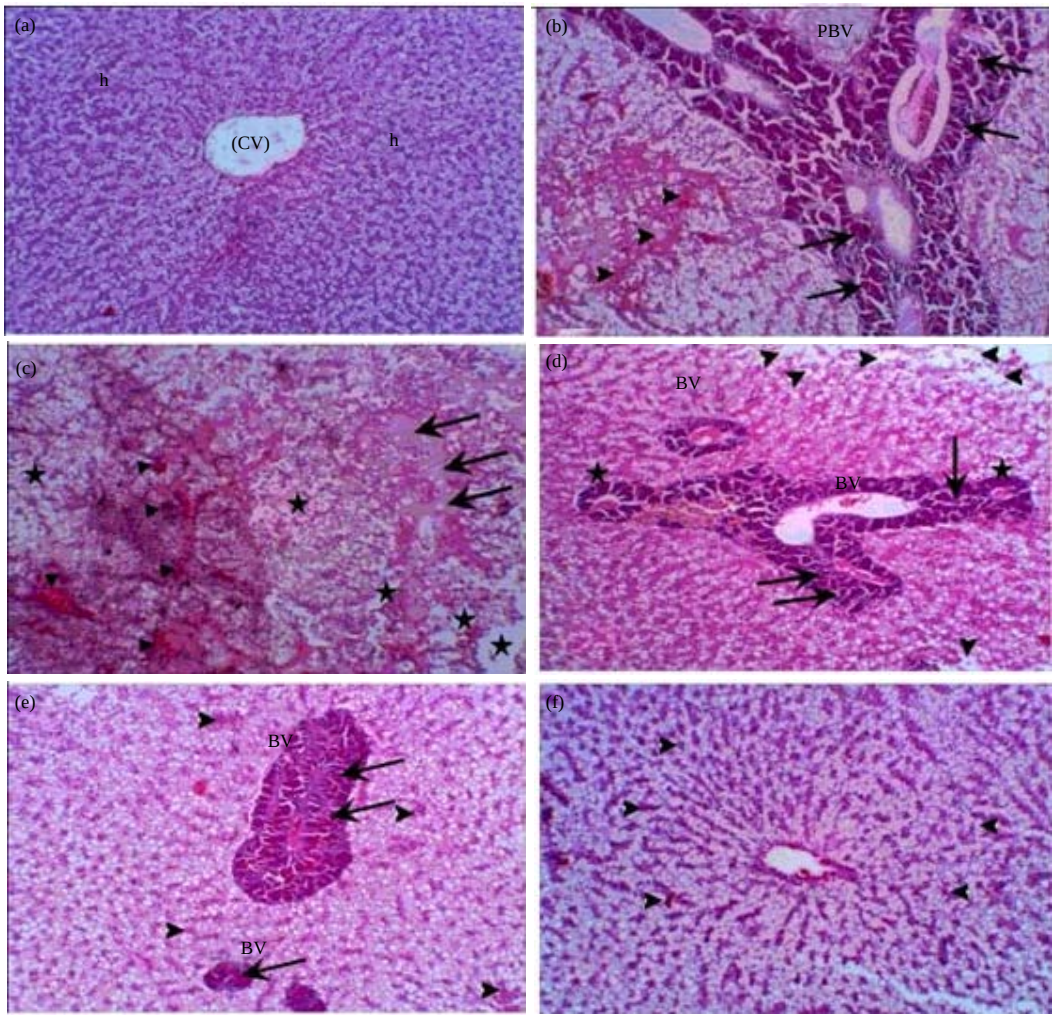

Fig. 5(a-f): Light micrograph of liver transverse sections of *O. niloticus* fed Glutathione-enhancer™ and aflatoxin B_1 (a) T_1 (C-) (BD free of AFB_1) showing normal structure of the hepatocytes (h) around the central vein (CV), (b) T_2 (C+) (Contaminated BD with 150 μg AFB_1 kg^{-1} diet) showing dilatation, thickening and severe congestion of the portal blood vessels (PBVs, arrows) adjacent with severe degeneration of the hepatocytes (arrows heads), (c) T_2 (C+): showing large areas of severe hemolysis between the hepatocytes in the hepatic lobule (arrows), severe necrosis of the hepatocytes with areas of hemorrhage (arrows heads) and fatty degeneration in the liver cells (stars), (d) T_3: (Contaminated BD with 150 μg AFB_1 kg^{-1} diet+50 mg glutathione kg^{-1} diet) showing diffuse fatty droplets in the liver lobule (arrows heads), enlargement and severe congestion of the blood vessels (BVs, arrows) and severe congestion of the bile ducts (stars), (e) T_4: (Contaminated BD with 150 μg AFB_1 kg^{-1} diet+100 mg glutathione kg^{-1} diet) showing moderate necrosis of the hepatocytes (arrows heads) and monocytes and fibroblast infiltration of BVs (arrows) and (f) T_5: (Contaminated BD with 150 μg AFB_1 kg^{-1} diet +200 mg glutathione kg^{-1} diet) showing moderate necrotic hepatocytes around CV (arrows heads) (×100, H and E stains)

herein were reported by Abdelhamid *et al.* (2004b, 2007).The reduction in CP% of fish body treated by AFB_1 may be due to significant reduction in the utilization of dietary CP and retention of DM and CP by AFB_1 (Johri *et al.*, 1986). In addition, AFB_1 hepatotoxic effects in fish generate a reduced amount of total protein owing to the inhibition of protein synthesis induced by the binding of aflatoxin adducts to cellular macromolecules (Sahoo *et al.*, 2001). Where, aflatoxin metabolites appear to have an effect on protein and amino acid metabolism; hence, researchers have examined the

possibility of increasing dietary protein levels as a means of alleviating aflatoxicosis (Waldroup, 1997). In this regard, Abdelhamid *et al*. (1994) found that raising dietary protein, amino acids and/or energy levels alleviated the toxic effects of AFB_1 on body chemical composition of broiler chicks.

Condition factor (K_f) is a measure used to describe fish health, particularly in relation to the degree of nourishment (Nash *et al*., 2006). In the present study, aflatoxicated diet T_2 (C+) caused significant ($p \leq 0.05$) reduction in K_f and HSI of *O. niloticus* among other groups. These reductions in both of K_f and HSI are potently related with the adversely affected of AFB_1 on the growth and feed efficiency parameters obtained herein or in the previous studies (Zaki *et al*., 2008; Salem *et al*., 2010). Similarly (Deng *et al*., 2010) reported an AFB_1-induced hepatic disorder in hybrid tilapia characterized by decreased lipid content, HSI and abnormal hepatic morphology. More recently, Mahfouz and Sherif (2015) reported that 100 μg AFB_1 kg^{-1} diet decreased HSI of *O. niloticus*. In addition, AFs, specifically AFB_1 is eventually secreted in the liver where it has been shown to be toxic to cells (Moreau, 1979). The AFs in the liver are degraded in two phases by biotransformation to a more toxic product and detoxification to a less toxic and easily excretable product (Hodgson and Levi, 1987).

Mycotoxins, including AFs, are of increasing concern in aquaculture because their contamination is persistent in fish flesh and residues have been found in marketed fish products at further than acceptable levels (Santos *et al*., 2010). In liver cells, AFB_1 is converted to several active metabolites that may be transmitted to edible animal products, giving rise to potential public health risks (Puschner, 2002). More recently, Al-Hizab *et al*. (2015) revealed that aflatoxin residues may cause massive histopathological changes to the liver tissue and caution should be exhibited in its use for human and animal consumption. The current findings revealed that fish fed AFB_1-contaminated diet T_2 (C+) led to highest bioaccumulation of AFB_1 in muscles among all treatments. However, this harmful bioaccumulation of AFB_1 was improved by increasing addition level of dietary GSH-En. Thus, it could be noted that AFB_1 had potent effects to accumulate in the fish tissues particularly in the edible tissue (muscles). On the other hand, addition of GSH-En to AFB_1-contaminated diets caused releasing of AFB_1 in fish viscera, hence reduced AFB_1 bioaccumulation in the edible muscles which potentially related with increasing levels of dietary GSH-En.

Similarly with the obtained results herein, the higher residues of AFB_1 were detected in whole body of *O. niloticus* (El-Banna *et al*., 1992; El-Barbary and Mehrim, 2009) than that reported in other species. Also, AFB_1 residue was detected in muscles of *O. mossambicus* (Varior, 2003), in the flesh of American channel catfish (Lovell, 1984) and in the edible muscles of sea bass (El-Sayed and Khalil, 2009). Which, suggesting a significant risk for transmission of AFB_1 to the human consumer, since the US Food and Drug Administration has situate a 5 μg kg^{-1} limit for AFB_1 in human foods. Nevertheless, AFB_1 residues were not detected in whole body of *O. niloticus*, when it analysis directly at the end of the experiment (Abdelhamid *et al*., 2002b) and after different freezing periods (1st, 2nd, 4th, 8th and 12th week, Abdelhamid *et al*., 2004a) or either shrimp (Bautista *et al*., 1994) or red tilapia (Usanno *et al*., 2005) after 60 days of exposure even at the highest exposure level. This disagreement may be due to the different pathways of metabolism of AFB_1 in these species, level of AFB_1, duration of exposure to AFB_1 or the differences in the experimental conditions.

Generally, the promising positive effects of dietary GSH-En against aflatoxicosis of *O. niloticus*, to eliminate the toxic effects of AFB_1 on mentioned parameters herein may be due to GSH conjugation is the major pathway for detoxification of AFs metabolites and is important in determining the resistance of different species to the hepatotoxic effects of AFB_1 (Bailey *et al*., 1996). Hence, it could be noted that these protective effects of dietary GSH-En against the toxic effects of AFB_1 on the experimental fish were potently doses related of GSH-En. Where, GSH is involved in the protection of the cells against the effect of Reactive Oxygen Species (ROS) which is produced in excessive rate in case of oxidative stress (Meister and Anderson, 1983). Like in the hepatic detoxification of AFs and other chemicals, GSH acts as antioxidant and has many functions in membrane maintenance and stability as well as in reducing oxidative stress factors and the high ROS produced from the process of lipid peroxidation (Stewart *et al*., 1996; Halliwell, 2007).

Changes of hematological parameters are important for the evaluation of fish physiological responses against environmental stresses (Hrubec *et al*., 2000). All hematological parameters in the present study, were negatively affected by dietary AFB_1. Similarly, these harmful changes in hematological parameters were reported by aflatoxicosis in *O. niloticus* (El-Barbary and Mehrim, 2009; Selim *et al*., 2014), *O. aureus* (Rizkalla *et al*., 1997), *Clarious lazara* (Zaki *et al*., 2011) and *Labeo rohita* (Mohapatra *et al*., 2011). More recently, Mahfouz and Sherif (2015) reported that 100 μg AFB_1 kg^{-1} diet negatively impacted *O. niloticus* hematological profiles which potentially related with adversely affects on HSI or histological structure of the liver. In addition, the decrease in Hb, RBCs, PCV indicated anemia, possibly due to

erythropoiesis, hemosynthesis and osmoregulatory dysfunction that occur owing to inhibiting the activities of several enzymes involved in heme biosynthesis (ATSDR., 2005) or due to an increase in the rate of erythrocyte destruction in hematopoietic organs (Jenkins *et al*., 2003).

Fish serum may reflect status of many biochemical processes in the metabolism. Hence, chronic aflatoxicosis may be diagnosed by determining serum biochemical and hematological alterations (Oguz *et al*., 2000). In the present study, the lowest significant ($p \leq 0.05$) serum total protein, albumin and globulin levels but the highest significant total cholesterol, AST and ALT levels were recorded in *O. niloticus* fed AFB_1 contaminated-BD T_2 (C+) among all treatments. These findings clearly indicated that AFB_1 had stressful effects on the hepatic tissue, consistent with those previously reported by Abdelhamid *et al*. (2007) and El-Sayed and Khalil (2009). In this respect, the reduced level of total protein is indicative of the toxic effects of AFB_1 to the liver due to the failure in synthesis of the proteins (Mohamed and Metwally, 2009), whereas the reduced globulin levels in AFB_1-treated fish might have been the result of lymphocytolysis (Sahoo *et al*., 2001). The increased activities of ALT and AST reported herein have been attributed to the damaged structural integrity of the liver (Mehrim *et al*., 2006), because liver is the major target organ of AFB_1 as well as liver is rich in AST and ALT and changes in plasma levels of these enzymes may be indicative of liver dysfunction (Kapila, 1999). Additionally, the release of these cellular enzymes into the blood stream corresponds the stressful conditions imposed upon AFB_1-treated fish (El-Sayed and Khalil, 2009). Similarly, with the current observations of biochemical parameters, AFB_1 led to reduction of serum total protein and globulin concentrations and increase AST and ALT activity of *O. niloticus* (El-Barbary and Mehrim, 2009). The increase in ALT and AST activities reported by Rasmussen *et al*. (1986) may be due to the liver affection in case of aflatoxicosis. Significant ($p<0.05$) increases in serum ALT, AST and creatinine levels and significant decreases in serum total protein and albumin concentrations were reported of *O. niloticus* fed contaminated diet with 200 μg AFB_1 kg^{-1} diet (Selim *et al*., 2014) or 250 μg AFB_1 kg^{-1} diet (Ayyat *et al*., 2014). More recently, Mahfouz and Sherif (2015) reported that the mean serum AST, ALT and ALP activities were significantly ($p<0.05$) increased following exposure of *O. niloticus* to 100 μg AFB_1 kg^{-1} diet for 6 or 12 weeks.

The mechanism of action of mycotoxins on the cell is mediated through the production of free radicals and ROS. Hence, oxidative stress can also occur when there is a decrease in the antioxidant capacity of a cell (Trush and Kenslar, 1991). The hepatic antioxidants represent the major defense against toxic liver lesions. The current findings showed that *O. niloticus* fed AFB_1-contaminated BD T_2 (C+) suffered from oxidative stress as indicated by the significant decrease of TAC in the liver or GSH in the liver and muscles of fish. These results are in agreement with those reported by Rizzardini *et al*. (2003) and Choi *et al*. (2010) suggested that oxidative stress may be due to direct effect of AFs or by the metabolites formed and the free radicals generated during the formation of these metabolites. This increased depletion of GSH herein or in the previous studies, leads to abnormally high levels of ROS found in cells affected by AFB_1 due to uncoupling of metabolic processes resulting from the lack of GSH for GSH-peroxidase catalysis of O_2 to H_2O_2 leading to lipid peroxidation and compromised cell membranes (Stewart *et al*., 1996). It is well known that the protection system against oxidative stress depends mainly on GSH concentration by the activities of GSH related enzymes, such as glutathione reductase and peroxidase (GSH-PX) (Abdel-Wahhab *et al*., 2006). Hence, results in the present study demonstrated that dietary GSH-En improved the serum antioxidant status in *O. niloticus* during chronic aflatoxicosis. In the same concern, several studies have demonstrated that GSH plays an important role in the detoxification of the reactive and toxic metabolites of AFs and the liver necrosis begins when the GSH stores are almost exhausted (Abdel-Wahhab *et al*., 2006, 2010) which may explain the decrease in TAC. Where, the increase of GSH concentration may be due to the decreased lipid peroxidation caused by GSH-En supplementation. The results presented herein show similarity with the findings of previous studies on the antioxidant effects of L-carnitine (Rani and Panneerselvam, 2001; Guzman-Guillen *et al*., 2013).

The organ most severely affected by AFB_1 is the liver and the primary lesions include hemorrhagic necrosis, fatty acid infiltration and bile duct proliferation (Coulombe, 1993). The liver histological alterations of *O. niloticus* fed AFB_1-contaminated BD herein, indicated that AFB_1 has induced severely histological changes in the hepatic tissues. These results confirmed the biochemical findings and they are in agreement with those previously reported (El-Barbary and Mehrim, 2009; Mehrim and Salem, 2013). In a previous work, Amany *et al*. (2009) reported that *O. niloticus* fed AFB_1-contaminated diet showed severe histopathological changes included severe hyperplasia changes in the liver tissue. In addition, the liver of *O. mossambicus* fed contaminated diet with AFB_1 showed necrosis and biliary epithelial cell proliferation with damage being more severely with increasing concentrations of AFB_1 in the diet (Varior, 2003).

More recently, several histopathological alterations were observed in *O. niloticus* liver fed aflatoxicated diets with 20 and 100 μg AFB_1 kg^{-1} diet for 12 weeks (Mahfouz and

Sherif, 2015). Thus, severely histological alterations in the liver tissue by AFs have reported not only in *Oreochromis* sp. but also in other fish species included *Channa punctatus* (Verma and Pandey, 1987), *Labeo rohita* (Sahoo *et al*., 2001) and *Cyprinus carpio* (Al-Faragi, 2014).

In the present study, *O. niloticus* fed AFB_1-contaminated BD have showed adversely affects on all measured parameters, typical to those reported in the literature of aflatoxicosis. Conversely, these negative alterations due to AFB_1 were significantly improved by increasing levels of dietary GSH-En of *O. niloticus* as an antitoxin agent in the current study. Hence, GSH conjugation is the major pathway for detoxification of AFs metabolites and is important in determining the resistance of different species to the hepatotoxic effects of AFB_1 (Bailey *et al*., 1996). Additionally, the capacity of dietary GSH-En to promote GSH concentration was also shown and this antioxidant action may be due to the decrease of lipid peroxidation during the chronic aflatoxicosis in *O. niloticus*. In this respect, antioxidant action of dietary GSH-En may be related to the transport of fatty acids into mitochondria for β-oxidation and thus to the decrease of lipid usage and protection of the cell membrane against toxic Reactive Oxygen Species (ROS) and other free radicals (Kalaiselvi and Panneerselvam, 1998).

CONCLUSION

It could be concluded the destructively effects of dietary AFB_1 on the growth and feed efficiency, hematological, biochemical parameters and hepatic histological alterations, besides the AFB_1-bioaccumulation in the tissues of *O. niloticus*. While, dietary glutathione-enhancer™ (GSH-En) was effective to eliminate these toxicological effects of AFB_1, especially with the high level (200 mg kg^{-1} diet). Where, it is evident that in conclusion, GSH-En has a potential antioxidant activity and a protective effect against AFB_1 toxicity and this protection was dose dependent. In addition, further attempts would provide more specific information concerning the response of antioxidant defenses in different fish species under various conditions as well as on the regulatory mechanisms of this response. Such potential studies will, no doubt, benefit aspects related to aquaculture production, human health and friendly environmentally conditions.

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REFERENCES

AOAC., 2004. Association of Official Analytical Chemists of Official Methods of Analysis. AOAC International, Arlington, Virginia, USA.

ATSDR., 2005. Draft toxicological profile for lead. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, Georgia, USA., pp: 102-225.

Abdel-Wahhab, M.A., H.H. Ahmed and M.M. Hagazi, 2006. Prevention of aflatoxin B_1-initiated hepatotoxicity in rat by marine algae extracts. J. Applied Toxicol., 26: 229-238.

Abdel-Wahhab, M.A., N.S. Hassan, A.A. El-Kady, Y.A. Khadrawy and A.A. El-Nekeety *et al*., 2010. Red ginseng extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats. Food Chem. Toxicol., 48: 733-742.

Abdelhamid, A.M., T.M. Dorra, S.E. Mansy and A.E. Sallam, 1994. Effect of raising dietary protein, amino acids and/or energy levels as an attempt to alleviate severity of the chronic aflatoxicosis by broiler chicks. Arch. Anim. Nutr., 46: 339-345.

Abdelhamid, A.M. and M.R. Saleh, 1996. Are aflatoxin and ochratoxin endemic mycotoxins in Egypt? Proceedings of the Conference Foodborne Contamination and Egyptian's Health, November 26-27, 1996, University of Mansoura, Mansoura, Egypt, pp: 51-59.

Abdelhamid, A.M., A.M. Ahmed and K.M. El-Meleigy, 2002a. Detoxification of aflatoxins-contaminated diet by some physical and chemical means. J. Agric. Sci. Mansoura Univ., 27: 8213-8224.

Abdelhamid, A.M., F.F. Khalil, M.I. El-Barbary, V.H. Zaki and H.S. Hussein, 2002b. Feeding Nile tilapia on Biogene to detoxify aflatoxic diets. Proceedings of the 1st Conference on Animal and Fish Production, Sept. 24-25, Mansoura, pp: 207-230.

Abdelhamid, A.M., A.E. Abdel-Khalek, A.I. Mehrim and F.F. Khalil, 2004a. An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementations with chicken-hatchery by-products (egg shells) and shrimp processing wastes (shrimp shells). 2-On clinical, blood and histological parameters. J. Agric. Sci. Mansoura Univ., 29: 6175-6196.

Abdelhamid, A.M., A.I. Mehrim and F.F. Khalil, 2004b. Detoxification of aflatoxin-contaminated diet of tilapia fish using dietary supplementation with egg shell, Betafin, clay or silica. J. Agric. Sci. Mansoura Univ., 29: 3163-3174.

Abdelhamid, A.M., M.F.I. Salem, A.I. Mehrim and M.A.M. El-Sharawy, 2007. Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 1-Fish performance, feed and nutrients utilization, organs indices, residues and blood parameters. Egypt. J. Nutr. Feeds, 10: 205-223.

Agag, B.I., 2003. Prevention and control of mycotoxins in feeds. Assiut Univ. Bull. Environ. Res., 6: 149-166.

Agag, B.I., 2004. Mycotoxins in foods and feeds: 1-aflatoxins. Ass. Univ. Bull. Environ. Res., 7: 173-206.

Ahokas, J., H. El-Nezami, P. Kankaanp, H. Mykknen and S. Salminen, 1998. A pilot clinical examining the ability of a mixture of *Lactobacillus* and *Propion bacterium* to remove aflatoxins from the gastrointestinal tract of healthy. Egypt. Volunteers Revue Med. Vet., 149: 568-572.

Al-Faragi, J.K., 2014. The efficacy of prebiotic (β-glucan) as a feed additive against toxicity of aflatoxin B_1 in common carp, *Cyprinus carpio* L. J. Aquacult. Res. Dev., Vol. 5. 10.4172/2155-9546.1000240

Al-Hizab, F.A., N.A.M. Al-Gabri and S.E.M. Barakat, 2015. Effect of Aflatoxin B1 (AFB1) residues on the pathology of camel liver. Asian J. Anim. Vet. Adv., 10: 173-178.

Alinezhad, S., M. Tolouee, A. Kamalzadeh, A.A. Motalebi and M. Nazeri *et al*., 2011. Mycobiota and aflatoxin B_1 contamination of rainbow trout (*Oncorhinchus mykiss*) feed with emphasis to *Aspergillus* section *Flavi*. Iran. J. Fish. Sci., 10: 363-374.

Amany, M.K., M.E.G. Hala, M.N.A. Mohammad and M.A. Abdel-Wahab, 2009. Pathological studies on effects of aflatoxin on *Oreochromis niloticus* with application of different trials of control. Egypt. J. Comp. Pathol. Clin. Pathol., 22: 175-193.

Ayub, M.Y. and D.S. Sachan, 1997. Dietary factors affecting aflatoxin Bi carcinogenicity. Mal. J. Nutr., 3: 161-179.

Ayyat, M.S., G.A. Abd Rahman, H.I. El-Marakby, N.A.B. El-Hakem and A.A.A. Hessan, 2014. Toxicity and biochemical hazards induced by exposure of *Nile tilapia* to aflatoxin and their amelioration. Global J. Environ. Sci. Toxicol., 1: 1-19.

Bailey, G.S., D.E. Williams and J.D. Hendricks, 1996. Fish models for environmental carcinogenesis: The rainbow trout. Environ. Health Perspect., 104: 5-21.

Bautista, M.N., C.R. Lavilla-Pitogo, P.F. Subosa and E.T. Begino, 1994. Aflatoxin B_1 contamination of shrimp feeds and its effect on growth and hepatopancreas of pre-adult *Penaeus monodon*. J. Sci. Food Agric., 65: 5-11.

CAST., 2003. Mycotoxins: Risks in plant, animal and human systems. Task Force Report No. 139, January, 2003. Council for Agricultural Science and Technology, Ames, Iowa, USA., Pages: 191.

Cagauan, A.G., R.H. Tayaban, J.R. Somga and R.M. Bartolome, 2004. Effect of aflatoxin-contaminated feeds in *Nile tilapia* (*Oreochromis niloticus* L.). Proceeding of the 6th International Symposium on Tilapia in Aquaculture Manila, September 12-16, 2004, Philippines, pp: 172-178.

Choi, K.C., W.T. Chung, J.K. Kwon, J.Y. Yu and Y.S. Jang *et al*., 2010. Inhibitory effects of quercetin on aflatoxin B_1-induced hepatic damage in mice. Food. Chem. Toxicol., 48: 2747-2753.

Coulombe, Jr. R.A., 1993. Biological action of mycotoxins. J. Dairy Sci., 76: 880 -891.

Dacie, J.V. and S.M. Lewis, 1995. Practical Haematology. 8th Edn., Churchill Livingstone, Edinburgh, Scotland.

Davis, N.D., U.L. Dioner and D.W. Dridge, 1966. Production of aflatoxins B_1 and G_1 by *Aspergillus flavus* in a semisynthetic medium. Applied Microbiol., 14: 378-380.

Deng, S.X., L.X. Tian, F.J. Liu, S.J. Jin and G.Y. Liang *et al*., 2010. Toxic effects and residue of aflatoxin B_1 in tilapia (*Oreochromis niloticus* × *O. aureus*) during long-term dietary exposure. Aquaculture, 307: 233-240.

Doumas, B.T. and H.G. Biggs, 1972. Determination of Serum Albumin in Standard Method of Clinical Chemistry. In: Standard Methods of Clinical Chemistry, Cooper, G.R. (Ed.). Vol. 7, Academic Press, New York, USA.

El-Banna, R., H.M. Teleb, M.M. Hadi and F.M. Fakhry, 1992. Performance and tissue residue of tilapias fed dietary aflatoxin. J. Vet. Med. Giza, 40: 17-23.

El-Barbary, M.I. and A.I. Mehrim, 2009. Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus*. J. Fish. Aquatic Sci., 4: 178-190.

El-Barbary, M.I., 2010. Antiaflatoxigenic activity of chemical antioxidants (glutathione and glutathione enhancer) on *Oreochromis niloticus*. Egypt. J. Aquat. Res., 36: 203-215.

El-Sayed, Y.S. and R.H. Khalil, 2009. Toxicity, biochemical effects and residue of aflatoxin B_1 in marine water-reared sea bass (*Dicentrarchus labrax* L.). Food Chem. Toxicol., 47: 1606-1609.

Ellefson, R.D. and W.T. Caraway, 1976. Fundamentals of Clinical Chemistry. In: Lipids and Lipoproteins, Tietz, N.W. (Ed.). W.B. Saunders Co., Philadelphia, PA., pp: 506-510.

Ellis, R.W., M. Clements, A. Tibbetts and R. Winfree, 2000. Reduction of the bioavailability of 20 μg kg^{-1} aflatoxin in trout feed containing clay. Aquaculture, 183: 179-188.

Ellis, W.O., J.P. Smith, B.K. Simpson, J.H. Oldham and P.M. Scott, 1991. Aflatoxins in food: Occurrence, biosynthesis, effects on organisms, detection and methods of control. Crit. Rev. Food Sci. Nutr., 30: 403-439.

Galaktionova, L.P., A.V. Molchanov, S.A. El'chaninova and B. Varshavskii, 1998. Lipid peroxidation in patients with gastric and duodenal peptic ulcers. Klinicheskaia Laboratornaia Diagnostika, 6: 10-14.

Gallagher, E.P. and D.L. Eaton, 1995. *In vitro* biotransformation of aflatoxin B_1 (AFB_1) in channel catfish liver. Toxicol. Applied Pharmacol., 132: 82-90.

Gornall, A.G., C.J. Bardawill and M.M. Parid, 1949. Determination of serum proteins by means of the biuret reaction J. Biol. Chem., 177: 751-766.

Guarisco, J.A., J.O. Hall and R.A. Coulombe Jr., 2008. Mechanisms of butylated hydroxytoluene chemoprevention of aflatoxicosis-inhibition of aflatoxin B_1 metabolism. Toxicol. Applied Pharmacol., 227: 339-346.

Guengerich, F.P., W.W. Johnson, T. Shimada, Y.F. Ueng, H. Yamazaki and S. Langouet, 1998. Activation and detoxication of aflatoxin B1. Mutat. Res./Fund. Mol. Mechan. Mutagen., 402: 121-128.

Guzman-Guillen, R., A.I. Prieto, C.M. Vazquez, V. Vasconcelos and A.M. Camean, 2013. The protective role of L-carnitine against cylindrospermopsin-induced oxidative stress in tilapia (*Oreochromis niloticus*). Aquat. Toxicol., 132-133: 141-150.

Halliwell, B., 2007. Oxidative stress and cancer: Have we moved forward? Biochem. J., 401: 1-11.

Hodgson, E. and P.E. Levi, 1987. A Textbook of Modern Toxicology. Appleton and Lange, New York, USA., ISBN-13: 9780838589151, Pages: 386.

Hrubec, T.C., J.L. Cardinale and S.A. Smith, 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* Hybrid). Vet. Clin. Pathol., 29: 7-12.

Huang, Y., D. Han, X. Zhu, Y. Yang, J. Jin, Y. Chen and S. Xie, 2011. Response and recovery of gibel carp from subchronic oral administration of aflatoxin B_1. Aquaculture, 319: 89-97.

Hunjan, M.K. and D.F. Evered, 1985. Absorption of glutathione from the gastro-intestinal tract. Biochimica Biophysica Acta (BBA)-Biomembr., 815: 184-188.

IARC., 1993. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 56, International Agency for Research on Cancer, Lyon, France, pp: 245-395.

Jantrarotai, W. and R.T. Lovell, 1990. Subchronic toxicity of dietary aflatoxin B_1 to channel catfish. J. Aquat. Anim. Health, 2: 248-254.

Jenkins, F., J. Smith, B. Rajanna, U. Shameem, K. Umadevi, V. Sandhya and R. Madhavi, 2003. Effect of sub-lethal concentrations of endosulfan on hematological and serum biochemical parameters in the carp *Cyprinus carpio*. Bull. Environ. Contam. Toxicol., 70: 993-997.

Johnson, W.W. and F.P. Guengerich, 1997. Reaction of aflatoxin B_1 *exo*-8,9-epoxide with DNA: Kinetic analysis of covalent binding and DNA-induced?hydrolysis. Proc. Natl. Acad. Sci. USA., 94: 6121-6125.

Johri, T.S., R. Agarwal and V.R. Sadfagopan, 1986. Surveillance of aflatoxin B_1 content of poultry feedstuffs in and around Bareilly district of Uttar Pradesh. Indian J. Poult. Sci., 21: 227-230.

Jollow, D.J., J.R. Mitchell, N. Zampaglione and J.R. Gillette, 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology, 11: 151-169.

Kalaiselvi, T. and C. Panneerselvam, 1998. Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. J. Nutr. Biochem., 9: 575-581.

Kapila, R., 1999. Biochemical status of cold water fishes. Bulletin No. 3, National Research Center on Cold Water Fisheries, U.P., India.

Lovell, R.T., 1984. Microbial toxins in fish feeds. Aquacult. Mag., 10: 34-36.

Lovell, R.T., 2001. Nutrition and Feeding of Fish. 2nd Edn., Haworth Press, New York, ISBN: 9780412077012, pp: 24-28.

Madhusudhanan, N., S.N. Kavithalakshmi, E.R.B. Shanmugasundaram and K.R. Shanmugasundaram, 2006. Aflatoxin B_1-induced DNA damage in *Labeo rohita*: Protective effect of an antioxidant supplement, Amrita Bindu. Basic Clin. Pharmacol. Toxicol., 98: 473-479.

Madrigal-Bujaidar, E., O. Madrigal-Santillan, I. Alvarez-Gonzalez and J.A. Morales-Gonzalez, 2011. Aflatoxin B1-Prevention of its Genetic Damage by Means of Chemical Agents. In: Aflatoxins-Detection, Measurement and Control, Torres-Pacheco, I. (Ed.). InTech Publication, Rijeka, Croatia, ISBN: 978-953-307-711-6, pp: 251-282.

Madrigal-Santillan, E., J. Morales-Gonzalez, N. Vargas-Mendoza, P. Reyes-Ramirez and S. Cruz-Jaime *et al*., 2010. Antigenotoxic studies of different substances to reduce the DNA damage induced by aflatoxin B_1 and ochratoxin A. Toxins, 2: 738-757.

Mahfouz, M.E. and A.H. Sherif, 2015. A multiparameter investigation into adverse effects of aflatoxin on *Oreochromis niloticus* health status. J. Basic Applied Zool., 71: 48-59.

Mahfouz, M.E., 2015. Ameliorative effect of curcumin on aflatoxin B_1-induced changes in liver gene expression of *Oreochromis niloticus*. Mol. Biol., 49: 275-286.

Manning, B.B., M.H. Li and E.H. Robinson, 2005. Aflatoxins from moldy corn cause no reductions in channel catfish *Ictalurus punctatus* performance. J. World Aquacult. Soc., 36: 59-67.

Mehrim, A.I., A.M. Abdelhamid, A.A.M. Abo Shousha, M.F.I. Salem and M.A.M.M. El-Sharawy, 2006. Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 2-Clinical, biochemical and histological parameters. J. Arab. Aquacult. Soc., 1: 69-90.

Mehrim, A.I., 2009. Effect of dietary supplementation of Biogen® (Commercial probiotic) on mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. J. Fish. Aquat. Sci., 4: 261-273.

Mehrim, A.I. and M.F. Salem, 2013. Medicinal herbs against aflatoxicosis in Nile tilapia (*Oreochromis niloticus*): Clinical signs, postmortem lesions and liver histopathological changes. Egypt. J. Aquat. Res., 3: 13-15.

Meister, A. and M.E. Anderson, 1983. Glutathione. Annu. Rev. Biochem., 52: 711-760.

Mohamed, A.M. and N.S. Metwally, 2009. Antiaflatoxigenic activities of some plant aqueous extracts against aflatoxin-b1 induced renal and cardiac damage. J. Pharmacol. Toxicol., 4: 1-16.

Mohapatra, S., N.P. Sahu, A.K. Pal, A.K. Prusty, V. Kumar and S. Kumar, 2011. Haemato-immunology and histo-architectural changes in *Labeo rohita* fingerlings: Effect of dietary aflatoxin and mould inhibitor. Fish Physiol. Biochem., 37: 177-186.

Moreau, C., 1979. Moulds, Toxins and Food. 2nd Edn., John Whey and Sons, Chichester, UK., Pages: 477.

Murthy, T.R.K., M. Jemmali, Y. Henry and C. Frayssinet, 1975. Aflatoxin residues in tissues of growing swine: effect of separate and mixed feeding of protein and protein-free portions of the diet. J. Anim. Sci., 41: 1339-1347.

NRC., 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC., USA., ISBN-13: 9780309048910, Pages: 114.

Nash, R.D.M., A.H. Valencia and A.J. Geffen, 2006. The origin of Fulton's condition factor-setting the record straight. Fisheries, 31: 236-238.

Ngethe, S., T.E. Horsberg, E. Mitema and K. Ingebrigtsen, 1993. Species differences in hepatic concentration of orally administered ^{3}H-AFB$_1$ between rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis niloticus*). Aquaculture, 114: 355-358.

Oguz, H., T. Kececi, Y.O. Birdane, F. Onder and V. Kurtoglu, 2000. Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Res. Vet. Sci., 69: 89-93.

Puschner, B., 2002. Mycotoxins. Vet. Clin. North Am.: Small Anim. Pract., 32: 409-419.

Rani, P.J.A. and C. Panneerselvam, 2001. Carnitine as a free radical scavenger in aging. Exp. Gerontol., 36: 1713-1726.

Rasmussen, H.B., K. Larsen, B. Hald, B. Moeller and F. Elling, 1986. Outbreak of liver-cell carcinoma among saltwater-reared rainbow trout *Salmo gairdneri* in Denmark. Dis. Aquat. Organisms, 1: 191-196.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 53-56.

Rizkalla, E.H., M.H. Zahra, A. Deab and M.F. Farag, 1997. Haematological responsiveness of *Oreochromis aurous* to dietary aflatoxins contamination and the role of selenium and vitamin E. Alexandria J. Vet. Sci., 13: 423-438.

Rizzardini, M., M. Lupi, S. Bernasconi, A. Mangolini and L. Cantoni, 2003. Mitochondrial dysfunction and death in motor neurons exposed to the glutathione-depleting agent ethacrynic acid. J. Neurol. Sci., 207: 51-58.

Roberts, R.J., 2001. Fish Pathology. 3rd Edn., Churchill Livingstone, London, ISBN-13: 978-0702025631, Pages: 492.

Roos, A.H., H.J. van der Kamp and E.C. Marley, 1997. Comparison of immunoaffinity columns with Florisil/C18 columns for the determination of aflatoxins in animal feed and maize. Mycotoxin Res., 13: 2-10.

Ruby, D.S., A. Masood and A. Fatmi, 2013. Effect of aflatoxin contaminated feed on growth and survival of fish *Labeo rohita* (Hamilton). Curr. World Environ., 8: 479-482.

Russo, J.A.R. and R.P.E. Yanong, 2002. Molds in fish feed and aflatoxicosis. UF/IFAS Fact Sheet FA-95, Institute of Agricultural Sciences, University of Florida, USA. http://fisheries.tamu.edu/files/2013/09/Molds-in-Fish-Feeds-and-Aflatoxicosis.pdf.

SAS., 2004. SAS Statistical Guide for Personal Computer. SAS Institute Inc., Cary, NC., USA.

Sahoo, P.K., S.C. Mukherjee, S.K. Nayak and S. Dey, 2001. Acute and subchronic toxicity of aflatoxin B$_1$ to rohu, *Labeo rohita* (Hamilton). Indian J. Exp. Biol., 39: 453-458.

Salem, M.F.I., M.T. Shehab El-Din, M.M.M.A. Khalafallah, S.H. Sayed and S.H. Amal, 2010. Nutritional attempts to detoxify aflatoxic effects in diets of tilapia fish (*Oreochromis niloticus*). J. Arabian Aquacult. Soc., 5: 195-206.

Santacroce, M.P., M.C. Conversano, E. Casalino, O. Lai, C. Zizzadoro, G. Centoducati and G. Crescenzo, 2008. Aflatoxins in aquatic species: Metabolism, toxicity and perspectives. Rev. Fish Biol. Fish., 18: 99-130.

Santos, G.A., I. Rodrigues, K. Naehrer and P. Encarnacao, 2010. Mycotoxins in aquaculture: Occurrence in feed components and impact on animal performance. Aquacult. Eur., 35: 503-513.

Selim, K.M., H. El-Hofy and R.H. Khalil, 2014. The efficacy of three mycotoxin adsorbents to alleviate aflatoxin B$_1$-induced toxicity in *Oreochromis niloticus*. Aquacult. Int., 22: 523-540.

Smela, M.E., S.S. Currier, E.A. Bailey and J.M. Essigmann, 2001. The chemistry and biology of aflatoxin B$_1$: from mutational spectrometry to carcinogenesis. Carcinogenesis, 22: 535-545.

Stewart, R.K., C.J. Serabjit-Singh and T.E. Massey, 1996. Glutathione *S*-transferase-catalyzed conjugation of bioactivated aflatoxin B$_1$ in rabbit lung and liver. Toxicol. Applied Pharmacol., 140: 499-507.

Stoskopf, M.K., 1993. Fish Medicine. 1st Edn., W.B. Saunders Co., Philadelphia, ISBN-13: 9780721626291, Pages: 882.

Takahashi, N., D.M. Stresser, D.E. Williams and G.S. Bailey, 1995. Induction of hepatic CYPIA by indole-3-carbinol in protection against aflatoxin B$_1$ hepatocarcinogenesis in rainbow trout. Food Chem. Toxicol., 33: 841-850.

Trush, M.A. and T.W. Kensler, 1991. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic. Biol. Med., 10: 201-209.

Tuan, N.A., J.M. Grizzle, R.T. Lovell, B.B. Manning and G.E. Rottinghaus, 2002. Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B$_1$. Aquaculture, 212: 311-319.

USAID., 2012. Aflatoxin: A synthesis of the research in health, agriculture and trade. Feed the Future: The Office of Regional Economic Integration, USAID East Africa Regional Mission, Nairobi, Kenya.

Usanno, O., S. Chaisilapasung, N. Sukrakanchana and K. Supamattaya, 2005. Effects of aflatoxin B$_1$ on sex reversed red tilapia (*Oreochromis niloticus* Linn. x *O. mossambicus* Peters). Songklanakarin J. Sci. Technol., 27: 187-197.

Vainio, H., E. Heseltine and J. Wilbourn, 1994. Priorities for future IARC monographs on the evaluation of carcinogenic risks to humans. Environ. Health Perspect., 102: 590-591.

Varior, S., 2003. Biochemical and histopathological effects of aflatoxin on *Oreochromis mossambicus* (Peters). Ph.D. Thesis, Faculty of Marine Sciences, University of Science and Technology, Kerala, India.

Verma, S.K. and S. Pandey, 1987. Effect of aflatoxin B_1 on the Haematopoietic tissue of air breathing teleost *Channa punctatus*. J. Aquac., 5: 13-21.

Volkl, A., B. Vogler, M. Schollenberger and P. Karlovsky, 2004. Microbial detoxification of mycotoxin deoxynivalenol. J. Basic Microbiol., 44: 147-156.

Waldroup, P.W., 1997. Managing molds and mycotoxins in poultry feeds. ASA Technical Bulletin Vol. PO33-1997, American Soybean Association, Singapore, pp: 1-17. http://biotechconsultancy.net/files/news/technical/Manageing-Molds-And-Mycotoxins-In-Poultry-Feeds.pdf

Weichsebum, T.E., 1946. Method for determination of albumin in serum blood. Am. J. Clin. Pathol., 16: 40-40.

Williams, J.H., T.D. Phillips, P.E. Jolly, J.K. Stiles, C.M. Jolly and D. Aggarwal, 2004. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences and interventions. Am. J. Clin. Nutr., 80: 1106-1122.

Wu, G., Y.Z. Fang, S. Yang, J.R. Lupton and N.D. Turner, 2004. Glutathione metabolism and its implications for health. J. Nutr., 134: 489-492.

Wu, Q., A. Jezkova, Z. Yuan, L. Pavlikova, V. Dohnal and K. Kuca, 2009. Biological degradation of aflatoxins. Drug Metab. Rev., 41: 1-7.

Zaki, M.S., N.E. Sharaf, H. Rashad, S.O. Mostafa and O.M. Fawzi, 2008. Diminution of aflatoxicosis in *Tilapia nilotica* fish by dietary supplementation with fix in toxin and *Nigella sativa* oil. Am. Eurasian J. Agric. Environ. Sci., 3: 211-215.

Zaki, M.S., O.M. Fawzi and I.M. Zytuun, 2011. Reduction of alfatoxin in *Clarious lazara* catfish by ginseng extract and *Nigella sativa* oil. J. Am. Sci., 7: 591-596.

Zaki, M.S. and O. Fawzy, 2012. Effect of aflatoxins B_1 on endocrine status in cat fish (*Clarious lazera*). Life Sci. J., 9: 419-422.

Ziglari, T. and A. Allameh, 2013. The Significance of Glutathione Conjugation in Aflatoxin Metabolism. In: Aflatoxins-Recent Advances and Future Prospects, Razzaghi-Abyaneh, M. (Ed.). Chapter 13, InTech Publsher, Rijeka, Croatia, ISBN: 978-953-51-0904-4, pp: 267-286.