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Chemoprevention and Therapeutic Efficacy of Glutathione Against Aflatoxicosis in Nile Tilapia (*Oreochromis niloticus)*

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Abstract: This study evaluated the efficacy of reduced glutathione (GSH) in ameliorating aflatoxicosis in *Oreochromis niloticus*. Three and 6 mg/kg b.w. of GSH were injected either pre- or post-treatment with 6 mg/kg b.w. of aflatoxin B_1 (AFB_1) administrated as a single, intraperitoneal injection. AFB_1 was dissolved in Dimethylsulphoxide (DMSO 25%) while GSH was dissolved in saline water just before use directly injected to the fish groups. In total, 240 adult *O. niloticus* were divided into 8 treatments (T_1-T_8) with 3 replicates. T_1 was a negative control treatment (25% DMSO). T_2 was the positive control group (AFB_1). Groups T_3 and T_4 were injected with 3 and 6 mg/kg b.w. of GSH, respectively. Groups T_5 and T_6 were injected with GSH at the start of the experiment and with AFB_1 after 7 days (pre-treatment). Groups T_7 and T_8 were injected with GSH after injection with AFB_1 (post-treatment). At the end of the experiment (14 and 21 days), samples were collected for analysis. The results show that AFB_1 has significant potency for increasing aspartate transaminase (AST), alanine transaminase (ALT) in blood and reducing the total protein (TP), albumin (AL) and globulin (GL) in blood of *O. niloticus*. In addition, the negative effects of AFB_1 on hepatosomatic index (HSI), lipid peroxidation and total antioxidant capacity in liver of *O. niloticus* aflatoxicosis were recorded in this study. Microscopically, there were hepatic lesions in AFB_1- injected fish. Moreover, these results showed that the ability of GSH when use after aflatoxicosis to counteract the toxic effects of AFB_1 on most parameter exanimate of *O. niloticus* could be better than its usage before aflatoxicosis (as a pre- treatment). In addition, the low level 3 mg/kg b.w. of GSH could be better than the high level. Finally, the optimal levels of GSH for detoxification of aflatoxin effects need more studies.

Key words: Aflatoxin B_1 · Detoxification · Glutathione · Antioxidants · Histopathology

INTRODUCTION

Aflatoxins are small molecular weight toxic compounds produced by certain toxigenic strains of the molds *Aspergillus flavus* and *Aspergillus parasiticus*, under suitable physical, chemical and biological factors [1].

AFB_1 is a potent hepatocarcinogen and hepatotoxin in some fish species, such as rainbow trout and it has been recently classified as a group 1 carcinogen [2]. Jantrarotai *et al*. [3] reported that aflatoxin is among the most common contaminants causing great economic losses in aquaculture, where aflatoxin can invade the aqua-feed chain either directly by using feed ingredients contaminated with AFB, or indirectly by the growth of the toxigenic fungi on the feed.

Fish that exposed to chronic or acutic toxicity of aflatoxin develop various health problems including reduction of growth performance and feed utilization, increased mortality, immunosuppression with consequent enhanced susceptibility to infectious diseases and dangerous histopathological changes in internal organs in addition to the mutagenic and carcinogenic effects [4-11]. The mechanism of action of aflatoxin on the cell is mediated through the production of free radicals and reactive oxygen species (ROS) [12, 13]. Where in

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aflatoxicosis, Aflatoxin B_1 is metabolically biotransformed in liver to form the reactive intermediates AFB1-8,9-epoxide (AFBO) [14], that may, in part, be responsible for the carcinogenic activity of AFB_1 [15].

The detoxification of AFBO also occurs in liver through the conjugation or Phase II enzymes, where glutathione S-transferase-mediated conjugation with reduced glutathione (GSH) [16, 17]. AFB_1-glutathione (GSH) conjugation is the major detoxification pathway of aflatoxin metabolites in the liver [18]. The resulting conjugate is often less toxic than the parent compound and its increased hydrophilicity can make it more readily excretable from the body. Most fish have a second group of biotransformation enzymes referred to as conjugation or Phase II enzymes [19]. However, this process consumes a big amount of GSH, which is an intracellular antioxidant and thus indirectly accelerates the production of reactive oxygen species (ROS) in the cytosol. This AFB_1-induced elevated ROS level also contributes to the hepatic cytotoxic and carcinogenic effects [20]. Therefore, stimulation of the antioxidant defence system like reduced glutathione (GSH) may reduce the risk of AFB-mediated carcinogenesis. Reduced glutathione is normally present in tissue at relatively high concentrations [21] and is a key component of antioxidant defense mechanisms [22, 23]. In addition, it is one of the most important factor protecting from oxidative attacks by reactive oxygen species, because GSH acts as a reducing agent and free-radical trapper and is known to be a cofactor substrate and/or GSH-related enzymes [24].

El-barbary [25] reported that, both of glutathione and glutathione enhancer (GSH and GSH-EH)have the ability to conjugate with AFB_1 and to be excreted from the body when *O. niloticus* fish injected with 9mg AFB_1/kg b.w. before injection with both of 5 and 10 mg GSH /kg b.w. In addition, the same author reported that the positive effects of both of GSH and GSH-EH on overcoming the toxic effects of AFB_1 could be attributed to the anti-oxidative properties of these materials. Denzoin *et al.* [26] showed that both free GSH (200 mg/kg) and niosomal GSH (14 mg/kg) treatments were highly effective in reducing both hepatotoxicity and hematotoxicity in cats that were intoxicated with a dose of 150 mg/kg acetaminophen.

The major objective of the present study was to evaluate the chemoprevention (pre-treatment) and therapeutic (post-treatment) efficacy of glutathione against aflatoxicosis in Nile tilapia.

MATERIALS AND METHODS

Preparation of aflatoxin B_1: Aflatoxin B_1 was produced in liquid medium (potato dextrose) by *Aspergillus parasiticus* (NRRL. 2999) according to the procedure of Ready *et al*. [27]. Aflatoxin B_1 was dissolved in chloroform and quantitatively estimated by thin layer chromatography TLC [28]. The chloroform was evaporated to dryness on a rotary vacuum evaporator at 40°C and AFB_1 was redissolved in 25% DMSO (1:3 water) to the appropriate concentration. AFB_1 was freshly dissolved in DMSO before each injection.

Glutathione: Reduced GSH was obtained from Sigma Chemical Co. This antioxidant was dissolved in saline water as described in the experimental design.

Experimental Protocol: This experiment was designed to evaluate AFB_1 effects on some biochemical parameters and histopathological alteration of liver in addition to evaluate the efficacy of glutathione (GSH) in ameliorating aflatoxicosis in *O. niloticus*. Two hundred and forty of *O. niloticus* fingerlings, with a mean weight of 40g, were obtained from El-Serw Fish Research Station, were randomly divided into 8 groups (T_1-T_8) with 30 fish in each group and were maintained in three glass aquaria (70X40X30 cm). The fish were acclimated to the aquarium conditions for a week before the experiment was initiated and the experimental period was 14 day. The aquaria were provided with air stones and all fish were fed twice daily at a feeding rate of 3% of the actual body weight. AFB_1 was used at 6 mg/kg b.w. (1/6 of the LC_{50}, according to El-Barbary [29]). The experiment was designed as follows:

- Group T_1 was injected intraperitoneal (I.P.) with 0.5 ml DMSO per fish as a control group fish at the start of the experiment.
- Group T_2 was injected (I.P.) with AFB_1 (6 mg/kg b.w.) at the start of the experiment.
- Group T_3 was injected (I.P.) with GSH (3 mg/ kg b.w.) at the start of the experiment.
- Group T_4 was injected (I.P.) with GSH (6 mg/ kg b.w.) at the start of the experiment.
- Group T_5 was injected (I.P.) with GSH (3 mg/ kg b.w.) at the start of the experiment and with AFB_1 (6 mg/kg b.w.) after 7 days (pre-treatment).

- Group T_6 was injected (I.P.) with GSH (6 mg/ kg b.w.) at the start of the experiment and with AFB_1 (6 mg/kg b.w.) after 7 days (pre-treatment).
- Group T_7 was injected (I.P.) with AFB_1 (6 mg/kg b.w.) at the start of the experiment and with GSH (3 mg/ kg b.w.) after 7 days (as a post-treatment).
- Group T_8 was injected (I.P.) with AFB_1 (6 mg/kg b.w.) at the start of the experiment and with GSH (6 mg/ kg b.w.) after 7 days (as a post-treatment).

At the end of the experiment (14 and 21 days), samples were collected for analysis. El-barbary [25] reported that fish injected with DMSO alone showed approximately absence of toxicity and any side effects due to DMSO administration.

Collection of Samples: The fish were sacrificed at the end of experiment (at 14 or 21 day) and blood was collected by cardiac puncture, then plasma separated and stored at -20°C until further use. Hepatic tissues of six individuals per group were quickly dissected and weighted them to assay the HSI, then fixed in Bouin's fluid for histological examination. Moreover, in each treatment, hepatic tissues of six individual fish were used for determination of lipid peroxidation and total antioxidant.

Assessment of Liver Function: Blood samples were collected and the plasma was separated by centrifugation with 3000×g for 15 min at 4°C. The activities of hepatic enzymes (AST and ALT) and the concentrations of plasma total protein, globulin and albumin, were determined using commercial calorimetric kits (Stanbio Laboratory Diagnostic, USA).

Hepatosomatic Index (HSI): At the end of the test, three fish were randomly selected from each group and body weight and liver weight recorded. The hepatosomatic index for each fish was calculated according to White and Fletcher [30] HSI = liver weight (g)/body weight (g) x 100

Preparation of Liver Homogenate: Liver samples (each fish treatment were performed in triplicate) were homogenized using a Teflon Pestle connected to a Braun homogenizer motor (25 strokes per minute at 1000 rev/min), in ice cold homogenate buffer (0.34M sucrose and 1mM potassium dihydrogen phosphate, pH 7.0), the liver homogenate was diluted to yield a 5% (w/v) liver homogenate. The homogenate was then centrifuged at 5000 rpm for 30 minutes at 4°C to precipitate insoluble materials. The supernatant was used to assay for lipid peroxidation and total antioxidant.

Determination of Lipid Peroxidation and Total Antioxidant Capacity: Lipid peroxidation products, measured as malondialdehyde (MDA) concentration were quantified by the thiobarbituric acid (TBARS) method and the MDA concentration was expressed as nanomoles per mg protein by the method of Stocks and Donnandy [31]. While total antioxidant were measured by using commercial calorimetric kits (Biodiagnostic, Egypt).

Histological Examination: The liver samples were fixed in Bouin's fluid for 12 h and dehydrated in a graded ethanol series. The hepatic were then embedded in paraffin, thin sectioned to 5ìm, stained with hematoxylin and eosin (H&E), observed and photographed using ICC50 HD camera and Leica LASEZ microscope.

Analytical Methods: At the end of the experiment (either at the 14^{th} or 21^{st} day), the obtained data were statistically analyzed by one-way analysis of variance using a software SAS [32]. When the F-test was significant, least significant difference was calculated according to Duncan [33] for the comparison among means.

RESULTS

Biochemical Blood Parameters: Biochemical blood plasma profiles of the control and experimental treatments fish are given in Table 1. Hepatic enzyme activities (ALT and AST), total protein and albumin were tested in the present study. As shown in Table 2 the activity of both enzymes which are usually used to evaluate the hepatic function, were dramatically and significantly ($p \leq 0.05$) increased approximately in all aflatoxicosis fish whether injected with or without GSH (except T_6) comparing to the control group (T_1). These results indicate that T_2 (injected with AFB_1 alone) showed higher value of hepatic enzymes than the all control groups (T_1, T_3, T_4).

The negative alteration due to AFB_1 was significantly improved by using GSH, particularly at its low level and at the post- treatment (T_7). However, AFB_1 treated decreased the concentrations of total protein and globulin (Table 1) comparing to the control group (T_1). Total protein (TP) was similar in all aflatoxicosis treatments, except T_6 reflect lowest level among all treatments.

Table 1: Hepatic enzymes activities, plasma protein, albumin and globulin concentrations in fish injected with AFB_1 alone or with glutathione

Treatments		AST U/L	ALT U/L	TP(g/dL)	AL(g/dL)	GL(g/dL)
Control	T_1	38.80^{f}±0.5	18.3^{e}±0.04	3.10^{a}±0.03	1.13^{b}±0.04	1.97^{a}±0.05
	T_2	165.7^{b}±1.8	24.9^{a}±0.20	2.50^{d}±0.02	1.17^{b}±0.08	1.33^{c}±0.10
	T_3	55.40^{e}±0.8	14.36^{d}±0.1	2.80^{b}±0.02	1.33^{a}±0.02	1.47^{c}±0.21
	T_4	32.5^{g}±0.52	17.5^{c}±0.04	2.62^{c}±0.02	1.23^{ab}±0.02	1.39^{c}±0.00
Pre- treatment	T_5	64.3^{d}±1.00	17.3^{c}±0.04	2.50^{d}±0.03	1.28^{ab}±0.05	1.22^{d}±0.03
	T_6	210.7^{a}±2.8	21.3^{b}±0.04	1.80^{e}±0.05	0.83^{c}±0.09	0.97^{e}±0.05
Post-treatment	T_7	59.4^{d}±1.00	11.7^{d}±0.10	2.70^{c}±0.20	1.35^{a}±0.02	1.35^{c}±0.02
	T_8	83.0^{e}±0.70	12.6^{d}±0.10	2.90^{b}±0.02	1.27^{ab}±0.02	1.63^{b}±0.02

Within each column, means superscript with different letters are significantly different at ($P \leq 0.05$)

Table 2: Effects of AFB_1 on hepaticsomatic index of fish injected with AFB_1 alone or with glutathione.

Treatments		Body weight	Liver weight	HSI
Control	T_1	39.8^{a}±0.60	0.47^{b}±0.01	1.19^{ab}±0.01
	T_2	38.5^{a}±0.50	0.34^{c}±0.00	0.88^{b}±0.03
	T_3	38.6^{a}±1.05	0.33^{c}±0.01	0.85^{b}±0.05
	T_4	41.5^{a}±0.70	0.51^{b}±0.05	1.23^{ab}±0.11
Pre- treatment	T_5	41.6^{a}±1.10	0.40^{c}±0.05	0.96^{b} ±0.10
	T_6	42.6^{a}±2.20	0.66^{a}±0.01	1.55^{a}±0.20
Post-treatment	T_7	39.7^{a}±0.80	0.58^{a}±0.02	1.46^{a}±0.02
	T_8	38.7^{a}±0.01	0.44^{a}±0.02	1.4^{ab}±0.03

Within each column, means superscript with different letters are significantly different at $P \leq 0.05$.

Hepatosomatic Index (HSI): The results of relative liver weight (HSI) are given in Table 2. The data reveal that the *O. niloticus* injected with aflatoxin alone (T_2), showed a higher decrease in the HSI values compared with other groups. However, the results showed that GSH has significant potency for increasing the HSI levels in *O. niloticus* injected with AFB_1 either in pre-treatments or post-treatments compared with the positive control (T_2). While the low level of GSH in control group fish (T_3) showed lower value of HSI than the all GSH treatments.

Effects of GSH and AFB_1 on Lipid Peroxidation and Total Antioxidant Capacity: The malondialdehyde (MDA) values, as an index of lipid peroxidation, after 14 days were showed in Fig. 1. Hepatic MDA level was significantly increased in *O. niloticus* injected with AFB_1 either alone (T_2) or with glutathione (T_5-T_8), when compared to the control group (T_1). Also, this results showed that glutathione has significant potency for reducing the MDA values in *O. niloticus* liver either was injected alone (T_3, T_4) or with AFB_1 as a pre-treatments (T_6) or post-treatment (T_7, T_8) comparing with T_2 except T_5 which reflect no significant difference between its value and T_2 values. Fig. 2 showed that significant differences observed in total antioxidant capacity (TAC) values among some fish treatments. AFB_1 has significant potency for reducing the TAC values in *O. niloticus* liver, either it was injected alone (T_2) or with both of the two concentration of glutathione (T_5-T_8) comparing with negative control (T_1). However no significant difference was observed among AFB_1-treatments (T_5-T_8) comparing with AFB_1-control (T_2). The two levels of glutathione led to significant increase in TAC values in the same treatments (T_5-T_8) compared to T_2. Nevertheless, no significant difference between pre-treatment and post-treatment in TAC values in *O. niloticus* liver at the different doses of GSH. In addition, the low dose of GSH treatment (T_3) showed a higher significant increase in the TAC value compared with all other treatments.

Histopathological Alterations of *O. Niloticus* Liver: Effect of aflatoxin B_1 and GSH on histological liver of control and experimental fish were showed in Figs. 3 & 4. The liver is associated with detoxification and biotransformation processes; it is one of the organs most affected by toxic. The histopathological lesions in liver of *O. niloticus* fish injected with AFB_1 with and without different doses of GSH were studied in the present work. In the control fish treatment, no histological changes were observed in liver (Figure 3a). Fish injected with 6mg AFB_1 / kg b.w. (T_2) showed severe lesions in the liver, in form of focal areas of necrosis between the hepatocytes invaded with inflammatory cells (b, x300) and very severe hemolytic diffused between clearly necrotic hepatocytes (c, x300) besides increased vacuolation in hepatocytes and necrosis in hepatocytes (d, x600). Normal structure of hepatocyte were observed in T_3 which fish injected with 3 mg /kg b.w. of GSH alone (Figure 3e), while slight hemorrhage between hepatocytes with dilation in sinusoids in addition to hemosiderin accumulation around blood vessels were observed in T_4 fish, which injected with the high dose of GSH, 6 mg/kg b.w, (Figure 3f). Vacuolar degeneration in the hepatocytes and severe haemolysis and hemosiderin accumulation between the hepatocytes were observed in both of pre-treatments T_5 and T_6 (Figure 4a & 4b, respectively) besides hepatocytes necrosis in T_6.

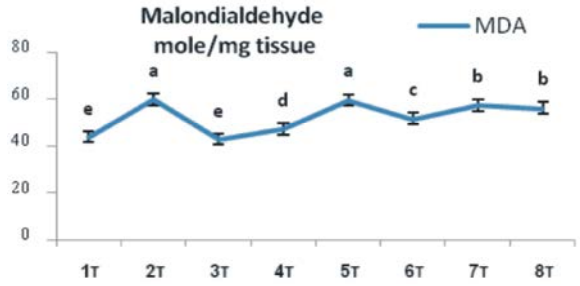

Fig. 1: MDA level in the liver of *O. niloticus* after 14 day of injected with 3 and 6 mg GSH /kg b.w. with and without AFB1 either pre-or post-treatment of GSH. a-c: Different letters indicate significantly different mean values among treatments at ($P \leq 0.05$).

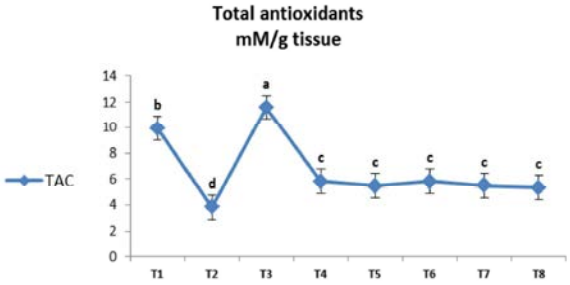

Fig. 2: ATC level in the liver of *O. niloticus* after 14 day of injected with 3 and 6 mg GSH /kg b.w. with and without AFB1 either pre-or post-treatment of GSH. a-c: Different letters indicate significantly different mean values among treatmentsat ($P \leq 0.05$).

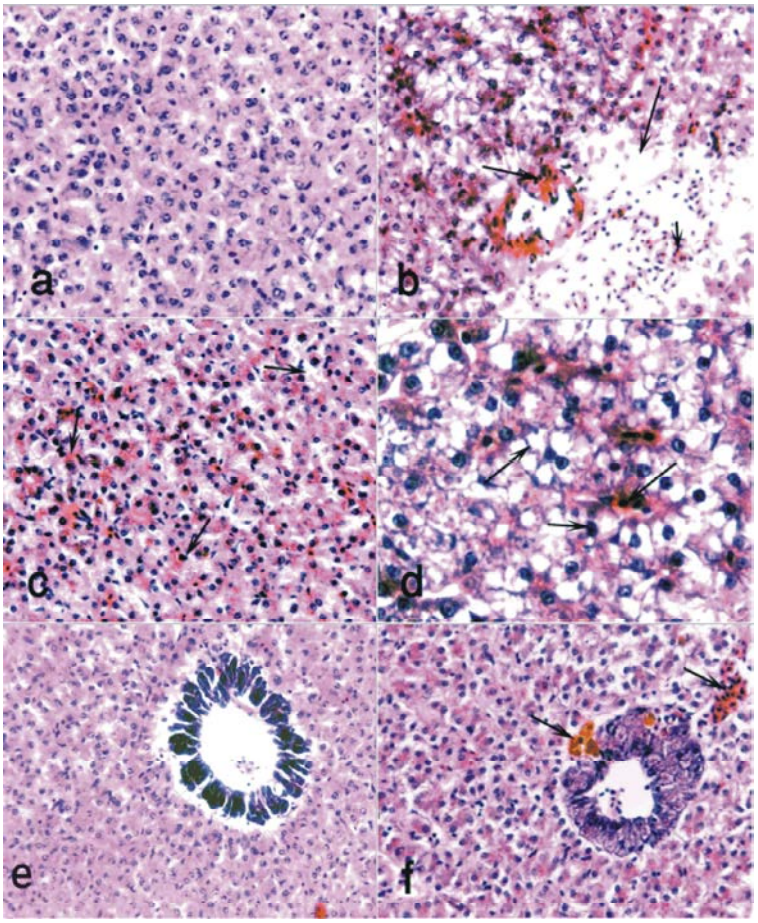

Fig. 3: Histopathological changes in liver of *O niloticus* controls treatments (T_1-T_4) stained with H&E. (a): control fish group showing normal structure (T_1, x300). (b -d): fish injected with AFB_1 (6mg/kg B.W., T_2) showing focal areas of necrosis between the hepatocytes invaded with inflammatory cells (b, x300) and very severe small hemolytic between clearly necrotic hepatocytes (c, x300) besides increased vacuolation in hepatocytes and necrosis in hepatocytes (d, x600). Whereas fish injected with GSH alone at 3 and 6 mg/kg B.W. (e &f respectively) showing normal structure (e, x200) (f): showing slight hemosiderin accumulation around blood vessels with slight hemorrhage between hepatocytes with dilation in sinusoids (f, x200)

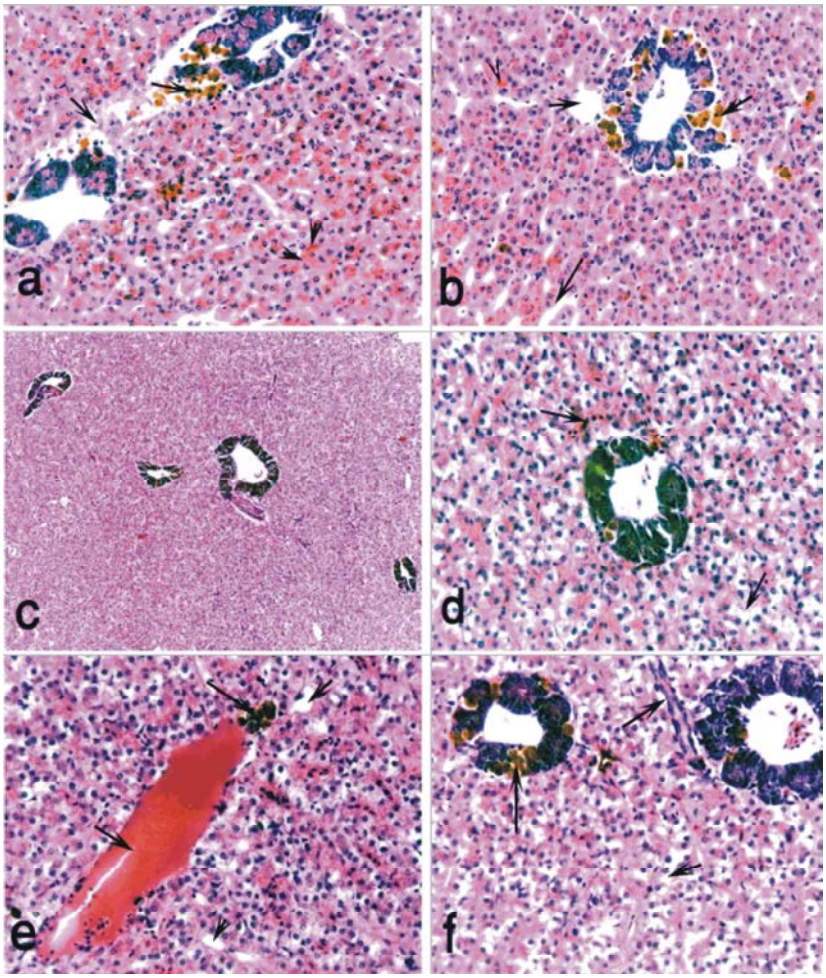

Fig. 4: Histopathological changes in liver of *O niloticus* injected with glutathione either before or after aflatoxisosis (T_5, T_6 and T_7, T_8) at the both of low and high doses of GSH (T_5, T_7 and T_6, T_8, a &b, respectively). T_5, T_6 showing congestion and dilation in blood sinusoids with pycnotic nuclei in the hepatocytes and hemosiderin accumulation around blood vessels (a x400), in addition vacuolar degeneration in the hepatocytes with disappearance of hepatocyte wall and hepatocyte necrosis (bx400). (c, d); T_7 showing normal structure of the liver (c x100) with slight congestion and dilation in blood sinusoids beside hemosiderin accumulation around blood vessels (d x200). (e, f); T_8 showing severe haemolysis besides hemorrhage in blood vessels and degeneration vacuoles in hepatocytes (ex250). (f); showing degeneration vacuoles in hepatocytes with necrosis nuclei in the hepatocytes and fibrosis (x250).

The lesions in fish of post- treatments (T_7, T_8) were different between them. Where, T_7 showing normal structure of the liver (Figure 4c) with slight congestion and dilation in blood sinusoids beside hemosiderin accumulation around blood vessels (Figure 4d). The high dose of glutathione (T_8) reflected severe haemolysis and hemorrhage in portal blood vessels and degeneration vacuoles in hepatocytes (Figure 4e). Also, T_8 showing degeneration vacuoles in hepatocytes and necrosis nuclei in the hepatocytes with fibrosis (Figure 4f).

DISCUSSION

Liver is known to be the principal target organ for aflatoxicosis in fish [34]. After the invasion of aflatoxins into the liver, aflatoxin metabolites react negatively with different cell proteins, lipid metabolism and protein synthesis, finally inducing liver necrosis [35]. The levels of serum ALT and AST were found to be increasing with aflatoxicosis in Nile tilapia. This alteration in blood parameters among fish treatments may be due to the

alterations in histological structure of livers of AFB_1-injected fish leading to inhibition of blood synthesis, where liver plays an important role in this process. The increase in the activities of these enzymes (ALT and AST) in plasma is indicative for liver damage and thus causes alteration in liver function and may be due to the partial necrosis of hepatocytes. Whereas Awad *et al.* [36], Youssef and Ashry [37] attributed the increase in activity of AST and ALT enzymes to the hepatotoxic effect of AFB_1 and consequently hepatic cell damage and liver dysfunction. Saber [38] reported the same observations in AFB-exposed *O. niloticus,* concluding the increase in transaminases activities in blood might be due to necrosis of liver.

In this study, plasma from the aflatoxin-injected fishes revealed a significant reduction in the total protein; globulin and albumin levels (Table 3). The decrease in the protein level could be correlated with severe damage of hepatocytes as indicated by histopathological studies in addition, this decrease might be contributed to the binding of aflatoxin to DNA, then decrease total serum proteins synthesizes by the liver [39]. Whereas the reduced globulin levels in AFB_1 treated fish may have been the result of lymphocytolysis [40]. Moreover, in this study, GSH showed clear trend in decreasing the negative effect of the AFB_1 on hepatic enzymes activity and total protein in aflatoxicosis Nile tilapia only when used after aflatoxicosis (in post-treatments). However, GSH has significant potency for increasing the HSI levels in *O. niloticus* injected with AFB_1 in both of pre- and post-treatments compared with the positive control (T_2). While the low level of GSH in control group fish (T_3) showed lower value of HSI than the all GSH treatments.

The biological parameters are sometimes indicative of toxicant effects [41]. The results of HSI of *O. niloticus* injected with aflatoxin alone (T_2), showed a higher decrease in the HSI values compared with other groups. In agreement, Zychowski *et al.* [42] found that HSI decreased significantly in groups exposed to AFB_1. Histopathological changes in the liver and lowered HSI suggest a progression towards AFB1-induced heptocarcinogenesis. In agreement, in the study of Deng *et al.* [43] AFB_1 induced a significant loss in liver mass, which was also observed in another aflatoxicosis study on tilapia [44]. One explanation of this AFB_1-induced depressed liver growth is with respect to the decreased hepatic lipid synthesis. In general, liver weight is positively related to the liver lipid content, however, in the animal exposed to high AFB_1, the incorporation of free fatty acids into liver triglycerides is dramatically decreased and the mobilization of lipid in adipose tissue to plasma is inhibited [45]. This is also in accordance to Deng *et al.* [43], who reported that the decreased liver lipid content in the liver lesion, which could be induced by high oxidative stress triggered by AFB_1 exposure, could also contribute to the abnormal liver growth. In contrast, Figueiredo-Fernandes *et al.* [46] suggested a positive relationship between the relative liver weight and the xenobiotic.

Antioxidant defenses of fish can be used as biological indicators of aquatic environmental health [47]. The antioxidant defense system playing complementary role in the prevention of oxidative damage resulting from ROS generated during AFB_1 metabolism. Lipid peroxidation (as MDA) is one of the main manifestations of oxidative damage and it has been found to play an important role in the toxicity and carcinogenicity. In this study, the positive relationship between the MDA and aflatoxicosis were investigated in the results in Fig. 1. Where the hepatic MDA levels were significantly increased in O. *niloticus* injected with AFB_1 in all aflatoxicosis treatments (T_2, T_5-T_8) compared to the control group (T_1), that is similar with several reports indicated that exposure to AFB increased LP in liver. Yin *et al.* and Abdel-Wahhab *et al.* [48, 49] reported that, AFB_1 injected enhanced LP as indicated by the significant increase in liver MDA level, which directly results of free radical-mediated toxicity. Moreover, this results showed that GSH has significant potency for reducing the MDA values in *O. niloticus* liver either was injected alone (T_3, T_4) or with AFB_1as a pre-treatments (T_6) or post-treatment (T_7, T_8) comparing with T_2, except T_5 group which reflect no significant difference between it and T_2 values. The protective effect of GSH was attributed to its ability to stabilize the pollutants by scavenging the free radicals and thereby blocking LPO development [50].

The protective and adaptive role of GSH against oxidative stress-induced toxicity is well established in aquatic animals [51, 52]. While the negative effect of AFB_1 on TAC was reported in Fig. 2, where AFB_1 has significant potency for reducing the TAC values in *O. niloticus* liver, either it was injected alone (T_2) or in all AFB_1-treatments (T_5-T_8) comparing with control (T_1). Koracevic *et al*. [53] described that the low total antioxidant capacity could be indicative of oxidative stress or increased susceptibility to oxidative damage.

Shyamal *et al.* [54] reported that the potent antioxidant properties would help to reduce cell damage caused by AFB_1. Zuo *et al*. [55] reported that T-AOC activity in liver was decreased and hepatic cells were damaged in chicks fed with AFB1. Also, GSH led to significant increase in TAC values at the two levels of GSH. While no significant difference between pre-treatment and post-treatment in TAC values in *O. niloticus* liver at the different doses of GSH. In addition, the low dose of GSH treatment (T_3) showed a higher significant increase in the TAC value compared with all other treatments. Furthermore, the association of glutathione peroxides (GPX) with AFB or its metabolites [56] may explain the reduced level of TAC. In the same concern, several studies on the mechanisms of AFs-induced liver injury have demonstrated that glutathione plays an important role in the detoxification of the reactive and toxic metabolites of AFs and the liver necrosis begins when the glutathione stores are almost exhausted [56-57], which may explain the decrease in TAC. Finally, the increased level of MDA and the decreased activity of TAC may be attributed to the free radical formation that initiated chain reactions of direct and indirect bond formation with cellular molecules due to AFB_1.

Histopathological changes observed in the hepatic of *O. niloticus* injected with AFB_1. T_2 showed severe lesions in the liver, in form of focal areas of necrosis between the hepatocytes invaded with inflammatory cells, very severe hemolytic diffused between clearly necrotic hepatocytes, besides increased vacuolation in hepatocytes that lost their normal polygonal structure and had prominent vacuolization. This may be attributed to the direct toxic effects of aflatoxin on hepatocytes. This lesions described by El-Barbary and Mehrim [58] and El-Barbary [25], who reported hepatic lesions manifested by severing congestion with large areas of hemorrhages, necrotic hepatocytes and dilatation in blood vessels in the liver of *O. niloticus* injected with 9 and 18 mg AFB_1/ kg b.w. In addition, Huang *et al.* [59] reported that vacuolar degeneration, a sign of hepatocyte apoptosis, was actually found in the liver of tilapia injected with AFB_1.

In this study, the effects of the two levels of GSH on liver histology were studied in T_3 & T_4. No significant changes were observed in the liver histology of fish injected with the low dose of GSH (3 mg/kg b.w. alone without AFB_1, Figure 3e). However, fish injected with the high dose of GSH, 6 mg/kg b.w., observed some histopathological alteration in liver, these results are partially supported by the results reported by El-Barbary [25], which reported that fish injected with 10 mg /kg b.w. GSH showed severe diffusion of hemosiderin and accumulation around blood vessels. Therefore, these results showed that the pathological changes in the liver of the fish treatments injected with the high level of glutathione appeared to be more than in the fish treatments injected with the low level of GSH. These pathological changes increased in the pre-treatments comparing to post-treatment. That may be attributed to the characteristics of GSH, which is easily oxidized and can be regenerated very rapidly [60-62] so, its efficiency to play an essential role in biochemical and pharmacological reactions to counteract the toxic effects of AFB_1 on the fish could be lower. Few previous studies on the role of GSH on ameliorating toxicity, Denzoin *et al*. [26] reported that both free GSH (200 mg/kg) and niosomal GSH (14 mg/kg) treatments were highly effective in reducing both hepatotoxicity and hematotoxicity in cats that were intoxicated with a dose of 150 mg/kg Acetaminophen (APAP). While, El-Barbary [25] reported that, the histopathological findings revealed that the GSH at the tested levels (5 and 10 mg/kg b.w) could not have potency to overcoming the side effects of AFB_1 (9 and 18 mg/kg b.w) on the liver histology.

CONCLUSION

It could be concluded that the negative effects of AFB_1 on liver function, HSI, lipid peroxidation, total antioxidant capacity and liver histology of *O. niloticus* aflatoxicosis were recorded in this study. Moreover, these results showed that the ability of GSH when use after aflatoxicosis to counteract the toxic effects of AFB_1 on *O. niloticus* could be better than its usage before aflatoxicosis (as a pre- treatment). In addition, the low-level 3mg/kg b.w. of GSH could be better than the high level. Finally, the optimal levels of GSH for detoxification of aflatoxin effects need more studies.

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