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Full Length Research Paper

Assessment of toxicity and clastogenicity of sterigmatocystin in Egyptian Nile tilapia

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The increasing presence of genotoxic pollutants in the aquatic environment has led to the development of quick monitoring methods. Sterigmatocystin (Stg) is closely related to mycotoxins and has the carcinogenic potency in the experimental animal models. The exposure to genotoxic agents will give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprints. Therefore, we have applied the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects of Stg and to determine if the Egyptian montmorillonite (EM) has a protective effect against Stg. The experiment was conducted *in vivo* to evaluate the ability of EM at a level 0.5 mg/kg body weight (bw) to prevent the toxicity and genotoxicity induced by Stg in the Nile tilapia fish. Fishes were orally administrated with EM in corn oil with or without Stg (1.6 µg/kg bw) twice a week for 4 weeks. Blood and tissue samples were collected at the end of the treatment. The results revealed that Stg had genotoxic and toxicopathological effects in *Oreochromis niloticus* fish. The genotoxic effects were indicated by appearance of some changes in polymorphism band patterns including lost of stable bands or occurrence of new bands. There also exists a distinct distance between the band patterns of exposed fish and protected or control fish samples. The effects on the tissues were manifested by different histopathological lesions in different organs including hyperplastic proliferation of branchial epithelium, necrobiotic changes in hepatic tissue and destruction of components of the spleen. These responses were virtually abolished or markedly decreased when fishes were exposed to EM combined with Stg. It could be conclude that addition of EM resulted in the inhibition of the toxicity and clastogenicity of Stg.

Key words: Fish, DNA fingerprinting, montmorillonite, sterigmatocystin.

INTRODUCTION

Mycotoxins are structurally diverse fungal metabolites that can contaminate the ingredients of animal feed and human food (Wang and Groopman, 1999). To date, mycotoxins with carcinogenic potency in experimental animal models include aflatoxins, sterigmatocystin, ochratoxin, zearalenone, and some *Penicillium* toxins, such as citrinin, luteoskyrin, patulin, and penicillic acid (Ueno and Kubota, 1976; CAST, 1989; Wang and Groopman, 1999; Tuan et al., 2002; Misumi, 2004). Sterigmatocystin (Stg) is produced from several species such as *Aspergillus versicolor, A. nidulans, Penicillium luteum*, and *Bipolaris* species (Wang and Groopman, 1999; Sivakumar et al., 2001). Stg is closely related to aflatoxin mycotoxins and is a precursor of aflatoxin biosynthesis (Barnes et al., 1994), however, the acute and chronic toxicities of Stg are considerably lower (Scudamore et al., 1997). Stg is carcinogenic in mice (pulmonary adenocarcinomas) and rats (hepatocellular carcinomas) following oral administration and is classified as an IARC class 2B carcinogen (i.e., as possibly carcinogenic to humans) (IARC, 1976; 1987). The toxicity of Stg is primarily confined to the liver and kidneys. However, lung tumors were also observed in newborn mice treated by a single subcutaneus injection (5 mg/kg) of Stg (Gujii et al., 1976). Stg is one of predominant

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contaminating mycotoxins in foods and grains of incidence areas of malignant tumors in China (Huang et al., 2002).

Most of the carcinogenic mycotoxins are genotoxic agents which produce chromosomal aberrations, micronuclei, sister chromatid exchanges and chromosomal strand breaks, as well as DNA adducts in rodent and human cells (Wang and Groopman, 1999). Numerous studies indicated that the activation of the mycotoxins aflatoxins B_1, G_1, B_2, G_2, aflatoxicol and Stg by S9 fraction and microsomal preparation of livers of several species significantly increases the chromosome breaking function, lethality and DNA damaging effect (Stich and Luishes, 1975; IARC, 1993). In addition, aflatoxins inhibit DNA synthesis, DNA-dependent RNA polymerase activity, messenger RNA synthesis, and protein synthesis (Busby and Wogan, 1985; McLean and Dutton, 1995). Pathologically, the effect of mycotoxins in fish was first reported by Ashley et al. (1964) and Halver (1965). The histopathology of the principle lesion was that of an invasive hepatocarcinoma. However, the available literatures on the effect of mycotoxins on tissues rather than liver were scanty in tilapias. Because Stg may significantly affect animal and human health, protection of food and feedstuffs from these poisons is of great demand. Several reports indicated that the phyllosilicate clay, hydrated sodium calcium aluminosilicate (HSCAS), which is currently available as an anticaking agent for animal feeds, may prevent disease associated with aflatoxicosis in animals, including chickens, turkey poults, pigs and minks (Phillips et al.,1988; Phillips, 1999). Recent studies have shown that the addition of HSCAS, bentonite, or montmorillonite to the aflatoxin-contaminated diets can greatly reduce the bioavailability of toxins in the gastrointestinal tract due to the high adsorptive properties of these clays (Mayura et al., 1998; Abdel-Wahhab et al., 2002, 2005). Up to 85% of performance losses due to aflatoxins have been recovered by the addition of 0.5 g clay/kg contamined diets, and alterations in serum clinical chemistry profiles indicative liver damage, teratogenic effects and chromosomal aberrations due to aflatoxin have been prevented (Lindemann et al., 1993; Abdel-Wahhab et al., 2002, 2005).

Due to these earlier findings, a variety of other common clay and zeolitic minerals are now being added to feeds as aflatoxin binders. Unfortunately, many of these binders due to their interaction with nutrients and other important feedborne chemicals may be nonselective in their action and may pose significant hidden risks (Abdel-Wahhab et al., 2005). Therefore, research to establish a sensitive animal model which can be used to compare the efficacy and safety of potential sorbents is warranted. The Nile tilapia (*Oreochromis niloticus*) may represent such a model, since it is highly susceptible to nutritional deficit and is extremely vulnerable to toxic insult from diverse chemicals including Stg (Abdel-Wahhab et al., 2005). The present study was aimed to evaluate the ability of the possible protective effects of EM against Stg-induced genotoxicity and histopathological alterations in the Nile tilapia as a sensitive model for mycotoxicosis.

MATERIALS AND METHODS

Chemicals

Sterigmatocystin was purchased from Sigma Chemicals Co. (St. Louis, OH). Egyptian montmorillonite (EM) was kindly provided by the Ceramic Department, National Research Center, Dokki, Giza, Egypt. All reagents were of the highest purity that commercially available.

Oreochromis niloticus

Two-month old of Nile tilapia (*Oreochromis niloticus*) fish weighing 90 ± 10 g were purchased from EL-Ibrahimia Fish Farm (Sharkia, Egypt) and transported in large plastic water containers supplied with battery aerators as a source of oxygen. Fishes were maintained on *ad libitum* standard fish died (free from Stg) at the Animal House, Faculty of Veterinary Medicine, Cairo University (Giza, Egypt). After an acclimation period of one week, fishes were divided into 5 experimental groups (8 fishes/group) and each group was individually placed into a fish aquarium containing dechlorinated tap water. The water was circulated 15 times a day, and the average water temperature was 14.5 ± 3.7°C and the pH was in the range of 7.2 – 8.2.

Experimental design

Fishes within treatment groups were treated intragastric for 4 weeks (2 doses/week) as follow: group 1, untreated control; group 2, treated with Stg (1.6 µg/ kg body weight dissolved in corn oil); group 3, treated with EM alone; group 4, treated with EM plus Stg; group 5, treated with corn oil alone. Fish treated with EM alone or in combination with Stg were given an amount of the sorbent equivalent to 5 g/kg (w/w) of the estimated maximum daily intake of feed dissolved in corn oil. Mortality rate was recorded daily, where blood samples were collected from the gills of fishes at the end of experimental period for DNA isolation. Tissue samples from different organs were collected for histopathological examination.

Molecular analysis

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook et al., 1989). The purity of the DNA preparation was judged by examining the ratio of absorbency at 260 to 280 nm (Aquardo et al., 1992).

RAPD-PCR analysis

To generate RAPD profiles from tilapia DNA, 21 oligodecamers (21-mer random primers: A01, A02, A03, A04, A05, A06, A07, A08, A09, A10, A12, A15, A20, C03, C05, C06, C07, C09, C12, C15 and C20) (Table 1) from the Operon Technologies were used. DNA amplification reactions were performed under conditions reported by Williams et al. (1990) and Plotsky et al. (1995). PCR amplification was conducted in 50 µl reaction volume containing

Table 1. Sequence of primers employed.

Primer	Sequence	Primer	Sequence
A01	5'-CAGGCCCTTC-3'	---	---
A02	5'-TGCCGAGCTG-3'	---	---
A03	5'-AGTCAGCCAC-3'	C03	5'-GGGGGTCTTT-3'
A04	5'-AATCGGGCTG-3'	---	---
A05	5'-AGGGGTCTTG-3'	C05	5'-GATGACCGCC-3'
A06	5'-GGTCCCTGAC-3'	C06	5'-GAACGGACTC-3'
A07	5'-GAAACGGGTG-3'	C07	5'-GTCCCGACGA-3'
A08	5'-GTGACGTAGG-3'	---	---
A09	5'-GGGTAACGCC-3'	C09	5'-CTCACCGTCC-3'
A10	5'-GTGATCGCAG-3'	---	---
A12	5'-TCGGCGATAG-3'	C12	5'-TGTCATCCCC-3'
A15	5'-TTCCGAACCC-3'	C15	5'-GACGGATCAG-3'
A20	5'-GTTGCGATCC-3'	C20	5'-ACTTCGCCAC-3'

100 ng genomic DNA, 100 µM dNTPs, 40 nM primer (Operon, Almeda, CA, USA), 2.5 units of Taq DNA polymerase and 5 µl promega 10X Taq DNA polymearse buffer. The reactions were carried out in Thermocycler (Perkin-Elmer 9700) programmed with a first denaturation of 5 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C and finally, one cycle at 72°C for 5 min. The PCR product was analyzed by electrophoresing 25 µl of the amplified mixture on agarose gel. The Gel-Pro Analyzer (Media Cybernetics) was used to document ethidium bromide DNA gels.

Histopathological examination

Tissue samples from gills, liver, spleen, kidneys and muscles were collected for histopathological examination. The samples were preserved in 10% buffered neutral formalin, washed in tap water, dehydrated in alcohol and cleared in xylene (Bancroft et al., 1996).

RESULTS

RAPD fingerprinting pattern

The genomic density of the fingerprints, generated by the arbitrary primers used, was evaluated among tilapia fish (blood cells DNA). From the twenty one primers used, only eight (A06, A09, A12, A20, C06, C07, C15, C20) gave positive and detectable bands. These eight primers amplified a total of 124 different bands, ranging from 83 to 1860 bp. Over all samples, the number of RAPD bands generated per primer varied between 16 and 24 bands, with a mean of 20 bands per primer.

Of the 124 scorable bands, 18 were similar "monomorphic" for control, EM and Stg plus EM samples (A06-1078, A06-929, A06-603, A06-456, A09-571, A09-428, A09-364, A09-327, A20-1293, A20-718, C6-1055, C6-446, C6-368, C7-1106, C7-347, C15-592, C15-333, C20-301) (Table 2). However, only one band (C6-578) was monomorphic for all control-, Stg-, EM- and Stg plus EM-samples (Table 2). The stable bands that appear in the control and protected tilapia are species-specific. They represent the common characteristics of the total normal tilapia. The DNA of the samples treated with Stg alone analyzed with all eight primers revealed the appearance of 29 new bands (A06-1106, A06-891, A09-840, A09-712, A09-513, A09-477, A09-378, A09-192, A12-436, A20-1323, C6-1193, C6-1078, C6-466, C6-428, C6-324, C7-1459, C7-696, C7-322, C15-1123, C15-953, C15-781, C15-571, C15-321, C20-1323, C20-1103, C20-929, C20-627, C20-491, C20-299) which did not appear in the samples of other groups (Table 2). These new bands could be considered as "genus diagnostic" markers which can attributed to Stg treatment. While, the genomic DNAs of the tilapia samples protected with EM against Stg did not appear any of these new bands. Furthermore, RAPD primers: C07 and C20 in the exposed samples displayed lost of some stable bands which occurred in the DNA of control and protected fish samples (Figure 1).

Clinical signs and histopathological findings

The main clinical signs were noticed in fish treated only with toxin. Where, after three days post administration the fishes were darker in color, unbalanced swimming together with death of 25% of the fish from this group. The other groups did not revealed any clinical abnormalities.

In comparison to fish treated with Stg, examined fish tissues from other groups were apparently normal except the intestine of the fish treated with toxin and clay. This intestine showed hyperplasia of the epithelial lining with marked activation of mucous secreting cells (Figure 2a). On the other hand, fishes treated only with the toxin had some lesions in several organs. The branchial tissue of

Table 2. Size in base pair of detected tilapia markers.

Marker	PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM
A06-1574	+					A09-987			+		+	**A12-1656**	+					A20-1623	+				
A06-1353	+					A09-840			+			A12-1574		+				A20-1323			+		
A06-1223		+				A09-766	+					A12-1423				+		A20-1293	+	+		+	+
A06-1106			+			A09-725				+		A12-990	+		+			A20-1154		+			
A06-1078	+			+	+	A09-712			+			A12-970		+				A20-1128	+		+		+
A06-1055		+				A09-699					+	A12-929				+		A20-1103					
A06-1012	+					A09-661	+	+				A12-891					+	A20-839				+	
A06-949					+	A09-571	+				+	A12-682	+					A20-718	+			+	
A06-929		+		+		A09-541		+				A12-655		+				A20-704			+		+
A06-910	+					A09-513			+			A12-603					+	A20-517		+			
A06-891			+			A09-495				+	+	A12-508	+					A20-380			+	+	+
A06-835				+		A09-486		+				A12-499		+				A20-298	+	+	+		+
A06-817	+					A09-477			+			A12-462					+	A20-296				+	
A06-603	+			+		A09-428	+			+		A12-453				+		A20-284	+	+			
A06-590		+	+			A09-421		+	+		+	A12-436			+			A20-254		+			
A06-530				+		A09-385					+	A12-405	+					**C6-1423**			+		
A06-476		+				A09-378			+			A12-328		+				C6-1319		+	+	+	+
A06-466			+		+	A09-364	+			+		A12-280		+				C6-1193			+		
A06-456	+			+		A09-358		+	+			A12-271	+		+			C6-1078			+		
A06-338		+	+		+	A09-352					+	**A20-1860**					+	C6-1055		+			+
A06-331				+		A09-327	+				+	A20-1818	+					C6-1012			+	+	
A06-324	+					A09-321		+	+	+		A20-1698		+	+			C6-733	+				
A09-1004		+				A09-192			+			A20-1660				+	+	C6-590	+				

Table 2: Continued

	PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM		PC	NC	Stg	Stg+EM	EM
C6-578		+	+	+	+	C7-641					+	C15-752					+	C20-301		+			+
C6-530		+	+			C7-628				+		C15-603		+				C20-299			+		
C6-466			+			C7-570					+	C15-592	+			+	+	C20-298	+				
C6-456	+					C7-405	+					C15-571			+			C20-279	+	+			
C6-446		+		+		C7-347	+			+	+	C15-495	+										
C6-428			+			C7-341		+				C15-339					+						
C6-368	+				+	C7-322			+			C15-333		+		+							
C6-360		+	+	+		C7-246				+		C15-327	+										
C6-324			+			C7-242					+	C15-321			+								
C7-1698					+	C7-238	+	+				C20-1323			+								
C7-1574	+	+				C7-83					+	C20-1103			+								
C7-1459			+			C15-1272	+	+				C20-929			+								
C7-1319					+	C15-1246				+	+	C20-910		+									
C7-1134				+		C15-1123			+			C20-891	+										
C7-1106	+	+			+	C15-1078	+					C20-664					+						
C7-929				+	+	C15-1059		+				C20-652				+							
C7-910		+				C15-953			+			C20-627			+								
C7-891	+					C15-936					+	C20-491			+								
C7-837	+					C15-919				+		C20-467		+									
C7-696			+			C15-795				+		C20-380	+										
C7-655	+	+				C15-781			+			C20-303				+							

+: Each marker was found in control and treated samples.
PC: fish treated with corn oil, NC: untreated fish, Stg: fish treated with Stg, Stg+EM: fish treated with EM plus Stg, EM: fish treated with EM alone.

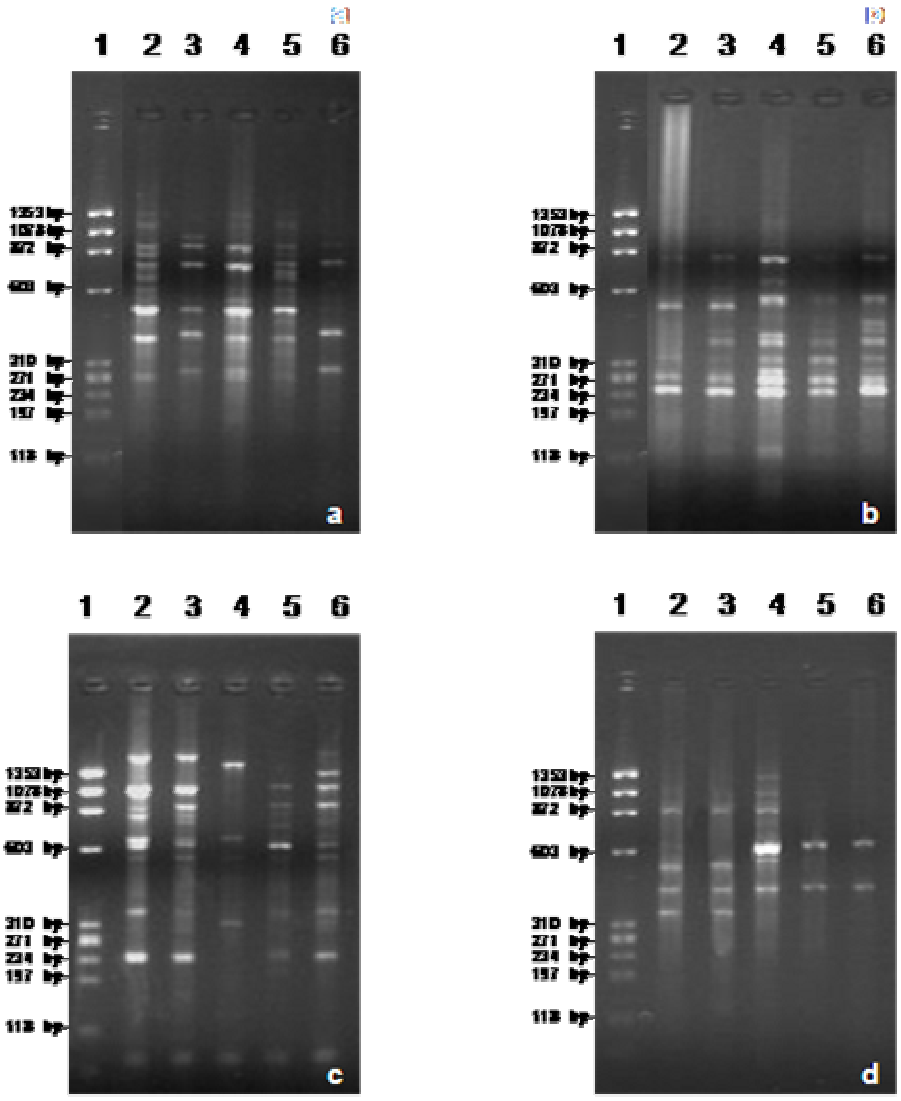

Figure 1. Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA. (a) Represents PCR products with primer A06, (b) represents PCR products with primer A09, (c) represents PCR products with primer C07 and (d) represents PCR products with primer C20. The DNA marker is in lane 1. Lane 2 represents fish treated with corn oil, lane 3 represents untreated fish, lane 4 represents fish exposed to Stg (1.6 µg/kg body weight dissolved in corn oil), lane 5 represents fish treated with EM plus Stg and lane 6 represents fish exposed to EM alone.

the gills in this group showed hyperplasia, lamellar oedema and hemorrhages. The hyperplastic lesion appeared either diffuse with fusion between secondary gill lamellae (Figure 2b) or focal in the form of three dimensional lamellar hyperplasia (Figure 2c). In other cases, oedema was more prominent where the oedematous fluid accumulated between the cells of secondary gill lamellae (Figure 2d). Free red blood cells were common between the branchial tissue epithelium indicating hemorrhages. The hepatic tissue in this group showed large number of eosinophilic granular cells and melanophores infiltrating the area of hepatopancrease (Figure 3a). The melanophores were also demonstrated diffusely in the hepatic tissue. The lesion in other cases was advanced and characterized by necrosis and lysis of the hepatic cells (Figure 3b). The spleenic tissue showed severe pathological lesions. In some cases, marked hemorrhages and eosinophilic granular cells aggregation were present in the area of melano-macrophage centers (Figure 3c). In other cases, the melanophores were aggregated around the blood capillary of the splenic ellipsoids. Areas of necrosis in spleen were also noticed in other cases where the necrotic tissue was surrounded and invaded by melanophores (Figure 3d).

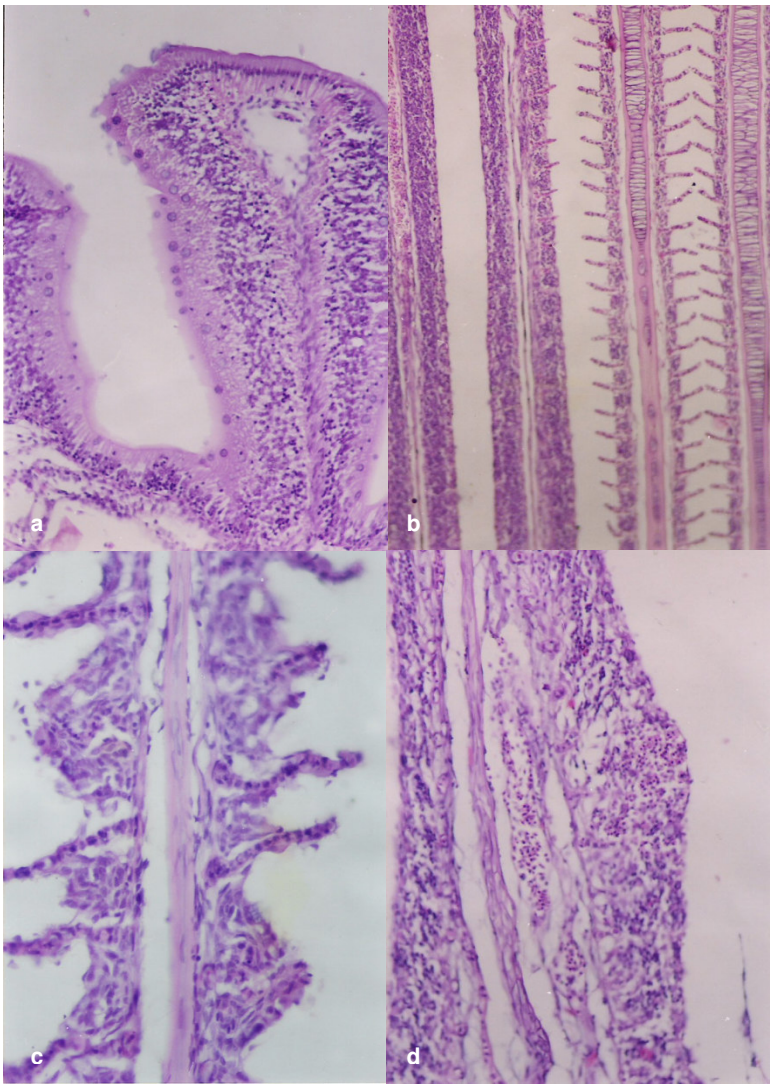

Figure 2. Photomicrographs of several organs of *o. niloticus* fish treated with Stg in combination with EM or with Stg alone: (a) Intestine of *o. niloticus* fish treated with both Stg and EM showing hyperplasia of the epithelial lining with marked activation of mucous secreting cells (H&E stain x200). (b) Gills of *o. niloticus* fish treated with Stg only showing diffuse lamellar hyperplasia (H&E stain x100). (c) Gills of *o. niloticus* fish treated with Stg only showing focal hyperplasia in the form of three dimensional lamellar hyperplasia (H&E stain x400). (d) Gills of *o. niloticus* fish treated with Stg only showing hemorrhages between the branchial tissue epithelium (H&E stain x200).

DISCUSSION

Stg is a mycotoxin and a precursor of aflatoxin which is produced by *Aspergillus versicolor* (Misumi, 2004). It is classified as one of predominant contaminating mycotoxins in the foodstuff of animal and human. Wang and Groopman (1999) and Misumi (2004) reported that mycotoxins with carcinogenic potency in experimental animal models include aflatoxins and sterigmatocystin, which are currently believed to act by disrupting the signal transduction pathways of the target cells. From this regard, we evaluated the ability of EM to bind Stg *in vivo* and, therefore, prevent its effect. In the present investigation, treatment of the fishes with Stg was comparable to previous doses reported in the literature (Matsushima and Sugimura, 1976; Hendricks et al., 1980; Abdel-Wahhab et al., 2005). The selected dose of EM was based on the *in vitro* results of Abdel-Wahhab et al. (2005). As a result of Stg-treatment, we have found that the mortality rate between fishes was relatively high

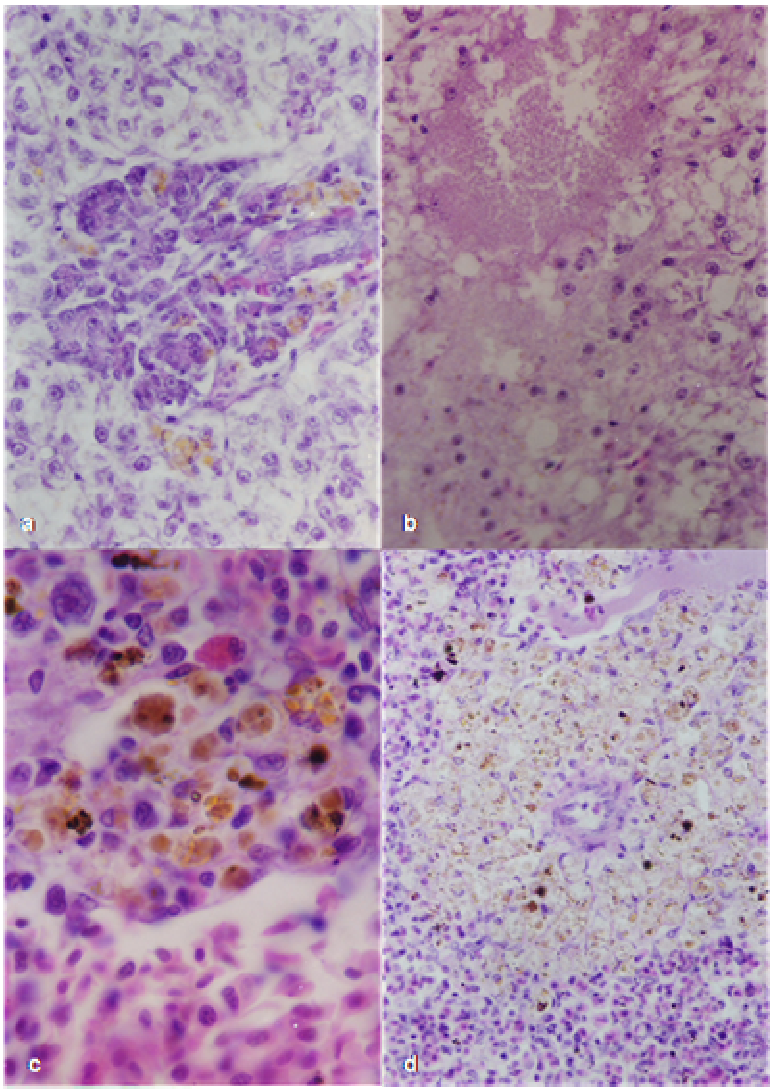

Figure 3. Photomicrographs of several organs of *Oreochromis niloticus* fish treated with Stg alone: (a) Liver of *O. niloticus* fish showing melanophores infiltrating the area of hepatopancrease (H&E stain x400). (b) Liver of *O. niloticus* fish showing necrosis and lysis of the cells (H&E stain x400). (c) Spleen of *O. niloticus* fish showing marked hemorrhages and eosinophilic granular cells aggregation in the area of melano-macrophage centers (H&E stain x1000). (d) Spleen of *O. niloticus* fish showing melanophores aggregated around the blood capillary of the splenic ellipsoids (H&E stain x400).

(25%). Also, treatment with Stg caused some histopathological lesions in many of the tilapia organs (liver, spleen and gills), where these parameters were comparable to the control fishes as well as fishes treated with EM alone. These histopathological lesions in tilapia liver were observed as marked necrosis and melanophores aggregation. Consistent with our observations, Bunger et al. (2004) reported that Stg was able to cause a higher toxicity in the A-549 lung cell line and Hep-G2 liver cells when mycotoxins were tested for cytotoxicity in four established cell lines. In addition, Majeed et al. (1984) described pathological lesions of mycotoxins in rainbow trout liver and detailed the neoplasm induced by such toxins in the hepatic tissue. In contrary, our results did not detected any neoplastic changes in the hepatic cells which may be explained on the bases of time factor required for induction of such lesion or some factors elaborating toxic effects on fish liver. Braunbeck et al. (1984) demonstrated considerable differences between fish species as well as between fish sex in the responsiveness to organic toxins. The influence of Stg on tilapia spleen was observed, where

hemorrhages, eosinophilic granular cells aggregation and necrotic changes were common. Ma et al. (2003) found that Stg was able to enhance the development of intestinal metaplasia and increase gastrin levels in *H. pylori*-infected Mongolian gerbils. The toxic effects of some mycotoxins on the immune system of the fishes were clearly described (Roberts et al., 1978; Ferguson, 1989). In the branchial tissue, lamellar hyperplasia and fusion together with oedema were common. The fusion and hyperplasia of gill lamellae may be induced by the effect of the toxin which alters glycoprotein in the mucus covering of the cells, thereby affecting the negative charge of the epithelium and favoring adhesion to adjacent lamellae (Ferguson, 1989). Furthermore, Sivakumar et al. (2001) suggsted that the histological lesions of liver collected from male albino rats treated with Stg were attributed to reduction in the levels of antioxidants such as glutathione, ascorbic acid, and alpha-tocopherol.

In the current study, Stg treatment resulted in an increase of new bands which did not appear in the fishes of other groups. Furthermore, some RAPD primers displayed some changes in polymorphism band patterns including lost of stable bands. Numerous studies on rat bone marrow and fish kidney showed that DNA damage such as chromatid gaps and chromatid breaks were the most frequent types of aberrations observed with aflatoxin and Stg (Ferguson et al., 1986; Basaran et al., 1993; Abdel-Wahhab et al., 2005). The clastogenicity of Stg arising from the toxin molecules might be related to forming covalent adducts and disturb the DNA (Wang and Groopman, 1999). Cao et al. (2000) found that polymorphism band patterns resulted by Stg treatment in Stg-treated human lung fibroblast cells were due to molecular alterations at exon 8 of p53 gene, a tumor suppressor gene. Furthermore, Xie et al. (2000) suggested that the molecular alterations in p53 gene caused due to Stg treatment, forms a feedback loop with a protein of MDM2 gene, a p53 target gene, and inhibits p53-mediated G1 arrest. Where, the induction of MDM2 may contribute to the failure of G1 arrest in Stg-treated cells.

Abdel-Wahhab et al. (2002) stated that hydrated sodium calcium aluminosilicate (HSCAS) and montmorillonite were found to protect the laboratory animals from the toxic and teratogenic effects of aflatoxins, where they are effective in the protection against aflatoxin B_1 and suppressing damage in the DNA and chromosomal aberrations as well as alterations in serum biochemical parameters. HSCAS clay acts as an aflatoxin enterosorbent that tightly and selectively binds these poisons in the gastrointestinal tract of animals, thereby decreasing the toxin bioavailability and subsequent toxicities (Mayura et al., 1998; Wang et al., 2005). The results of Abdel-Wahhab et al. (2005) indicated that the binding capacity of EM to Stg reached 98% and the adduction was able under different conditions of pH and temperature. The binding capacity of EM may be due to the large molecular structure of montmorillonite which increases the adsorption of organic compounds in each of its layers (Fushiwaki and Urano, 2001). Montmorillonite, which is commonly the main constituent of the clay know as bentonite, has the property of adsorbing organic substances either on its external surfaces or within its inter laminar spaces by the interaction with or substitution for the exchange cations present in their spaces (Latif and Quisenberry, 1968; Abdel-Wahhab et al., 2002). Our results indicated that EM did not have any negative action on the overall fish health as indicated by histopathological findings. Moreover, it had protected the fishes from the mutagenic effects of Stg. Addition of EM to Stg resulted in a preventing of occurrence the new bands or loss of the stable bands which appeared with Stg treatment. These findings support the earlier reports that the basic mechanism appears to involve sequestration of Stg in the gastrointestinal tract and chemisorption (i.e. tight binding) to EM which results in reduction in toxin bioavailability (Abdel-Wahhab et al., 2002; Wang et al., 2005). The *in vivo* adsorption of Stg in the gastrointestinal tract of the fishes by EM was confirmed by the determination of Stg residual in fish tissues (Abdel-Wahhab et al., 2005). The concentration of Stg in the edible tissues was significantly reduced in fishes treated with EM plus Stg. These findings suggest a strong binding capacity of EM to Stg resulting in a decrease in the clastogenicity and bioavailability of Stg in the gastrointestinal tract and subsequent reduction in the distribution to different organs. The mechanism of inhibitory effect of EM on Stg binding to DNA resulted in low genotoxicity may be appear to be related to induction of detoxification enzymes. Wang and Groopman (1999) elucidated that the risk for aflatoxins hepatocarcinogenesis can be modified in animals when they bounded by a number of chemoprotective such as antioxidants or ethoxyquin (EQ) produced a dramatic reduction in the binding of aflatoxin B to hepatic DNA. The inhibitory effect of EQ on aflatoxin B binding to DNA appeared to be related to induction of detoxification enzymes, since rats fed 0.4% EQ for 7 days showed a 5-fold increase in hepatic cytosolic glutathione *S*-transferase specific activities.

In conclusion, the results from the current study have indicated that EM has the ability to reduce the histopathology lesions of Stg and prevent its clastogenicity in Nile tilapia fishes. Also, tilapia fishes can be used as a sensitive model to examine effectiveness and safety of other protectors against mycotoxins.

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