Prevention of cytogenetic, histochemical and biochemical alterations in *Oreochromis niloticus* by dietary supplement of sorbent materials

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**SUMMARY**

The objectives of the current study were to evaluate the ability of Egyptian bentonite (EB) and montmorillonite (EM) to protect against biochemical, histochemical and genotoxicity induced by aflatoxin B₁ using the micronucleus (MN) assay, chromosomal aberrations and random amplified polymorphism DNA (RAPD-PCR) analysis in Tilapia fish. Six groups of fish were treated for 3 weeks and included the control group, AFB₁-treated group and the groups treated with EB or EM alone or in combination with AFB₁. At the end of experiment period, blood samples were collected for MN, testosterone and biochemical assays. Chromosomal aberrations were determined in kidney tissues, DNA fragmentation test was determined in liver and testes whereas; histochemical study was carried out on liver, testes and gills. The results indicated that a significant decrease in total protein, albumin, globulin, testosterone and DNA content in liver, gills and testes accompanied with a significant increase in number of MnRBCs, total chromosomal aberrations in kidney and DNA fragmentation in testis and liver of fish received AFB₁ alone. Fish treated with EB or EM alone were comparable to the control regarding the biochemical parameters except testosterone in EB-treated group which was significantly decreased. Both clays induced a significant increase in number of MnRBCs, chromosomal aberrations in the kidney, DNA fragmentation in testes but not in liver of EB-treated group. The combined treatment with AFB₁ and EB or EM succeeded to improve all the tested parameters towards the control values although it did not normalize them. Moreover, the improvement was pronounced in the group received EM plus AFB₁. It could be concluded that EB and EM have the ability to tightly bind AFB₁ in the gastrointestinal tract of fish resulting in decreasing its bioavailability. Moreover, the two tested clays were safe and can be use as potential aflatoxins binders in animal feed.

**Key words:** Aflatoxins; bentonite; montmorillonite; Clays; sorbent materials; Fish; Cytogenetic
INTRODUCTION

Aflatoxins are the most potent natural toxic metabolites produced by molds contaminating foods, feed ingredients and products of animal origin (Schoental, 1967). Many feed ingredients used in aquaculture, such as cottonseed, peanuts, corn, soybean, maize, rice, dried fish, shrimp and fish-meals, have been found to be frequently contaminated with aflatoxins (Ellis et al., 2000; Cagauan et al., 2004 and Spring 2005). At the present time, increased use of ingredients of plant origin in aquafeed formulations for fish breeding has intensified the potential onset for aflatoxicosis in farming systems due to the carryover of high loads of aflatoxin contamination by vegetable sources. As a result, the problem of mycotoxins contamination in aquaculture has amplified (Tacon et al., 1995 and Spring 2005). For the most part, aflatoxin contamination of aquafeed is greatly widespread, especially in countries with humid tropical climates owing to many factors, among which are permissive climatic conditions to mold growth and inappropriate methods of feed processing and storage (Murjani, 2003).

Aflatoxins exert a substantial impact on the fish farming production, causing disease with high mortality and a gradual decline of reared fish stock quality, thus representing a significant problem in aquaculture systems (Santacroce et al., 2008). Aflatoxins and their animal biotransformation products, known as foodborne carcinogens, have been associated with serious harmful effects on the health of humans and animals (Hussein and Brasel, 2001; Puschner, 2002 and Williams et al., 2004). A wide variety of biological effects such as toxicity, carcinogenicity, teratogenicity, genotoxicity, impairment of immune and reproductive system have been recorded in most animal species (Steyn 1995, Abdel-Wahhab and Kholif, 2008).

Several reports have indicated that the phyllosilicate clay which is currently available as an anticaking agent for animal feeds, may prevent disease associated with aflatoxicosis in farm animals (Phillips, 1999). Recent studies have shown that the addition of different clay materials to the aflatoxin-contaminated diets can greatly reduce the bioavailability of toxins in the gastrointestinal tract (Phillips et al., 1988; May-
ura et al., 1998; Abdel-Wahhab et al., 1998, 1999, 2002) due to the high adsorptive properties of these clays. The toxic effects due to aflatoxins were reversed by the addition of clay at 5% (w/w) to the contaminated diet, and alterations in serum clinical chemistry profiles indicative of liver damage, teratogenic effects and chromosomal aberrations due to aflatoxin have been prevented (Lindemann et al., 1993 and Abdel-Wahhab et al., 1998, 2002). On the basis of these earlier findings a variety of other common clay and zeolitic minerals are now being added to feeds as ‘aflatoxin binders’ without appropriate in vitro and in vivo testing. Many of these may be non-selective in their action and may pose significant hidden risks due to interaction with nutrients and other important food-borne chemicals. The major objectives of this study were to evaluate the protective effect of Egyptian clays e.g. montmorillonite (EM) and bentonite (EB) against aflatoxins-induced cytotoxicity, histochemical and biochemical changes in the Nile tilapia fish as a sensitive model for aflatoxins.

MATERIALS AND METHODS

Chemicals and kits: Aflatoxin B$_1$ was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Total protein, albumin and enzyme immunoassay for testosterone kits were purchased from BioMerieux, Laboratory of Reagents and Products (Marcy Létoile, France). All other reagents for micronucleus, chromosomal and DNA fragmentation assays were purchased from Invitrogen (USA).

Sorbent material: Egyptian bentonite (EB) and montmorillonite (EM) were kindly supplied from the Ceramic Department, National Research Center, Dokki, Egypt.

Fish: Sixty apparently healthy, two-month-old Nile tilapia fish (Oreochromis niloticus) with an average body weight of 90 ± 10g were purchased from El-Nobarya Fish Farm (El-Nobarya, Egypt) and transported alive in a large plastic water container supplied with battery aerators as a source of air. During transportation fishes were treated with lidocaine, CHNO$_2$, (5 mg/L) to reduce stress. Fish were maintained on a standard fish diet (free from AFs) at the Animal House, Veterinary Medicine Division, National Research Center (Dokki, Giza, Egypt). Feeding was done once daily using a pelleted diet (32% protein ration) at rate of 3% of the fish body weight. The water in aquaria was changed daily to avoid metabolite accumulations in glass aquaria (static system). After an acclimation period of one week, the fish were divided into six experimental groups (10 fish/group).
and each group was placed in a fully prepared aquarium containing de-chlorinated tap water, the average water temperature was 20 ± 3.7°C and the pH was in the range 7.17–8.19.

**Preparation of contaminated diets:**

Aflatoxin B₁ was dissolved in methanol and was mixed with the fish diet at a concentration of 1.5 mg AFB₁/kg diet. The diet was left in dark at room temperature for one week before administration to the fish to allow the methanol to evaporate. Sorbents were added to either AFB₁-contaminated diet or the basal diet at a concentration of 5 g/kg diet.

**Experimental design:**

Fish within different treatment groups were treated for 3 weeks according to their respective treatment as follows: group 1, untreated control; group 2, fed AFB₁-contaminated diet (1.5 mg/kg diet); group 3, fed basal diet supplemented with EB (5g/kg diet); group 4, fed AFB₁-contaminated diet supplemented with EB; group 5, fed basal diet supplemented with EM (5g/kg diet) and group 6, fed AFB₁-contaminated diet supplemented with EM. At the end of the experiment blood was collected from the caudal vein of all fish within different groups with a 5-cc heparinized syringe. Blood samples were centrifuged for 10 minutes at 3,500 rpm and 4°C. Plasma were collected and stored at -80 °C until analyzed (Lucky, 1977). Plasma was used for the determination of total plasma protein, albumin and testosterone. Globulin was calculated by subtract the total albumin from total protein according to Coles (1974).

**Micronucleus test:**

A drop of blood from the gills was mixed with a drop of foetal calf serum on a glass slide and air-dried. The slide was fixed in methyl alcohol for 5 min, and stained with 5% Giemsa for 7 min. Two thousands erythrocytes were examined for each fish for the determination of RBCs percentage that contained micronuclei (De Flora et al., 1993).

**Chromosomal preparation:**

Chromosomal preparation of kidney tissues was carried out according to the method described by (Al-Sabti, 1986) with some modification: In brief: the anterior kidney from each fish was excised and cut into fine particles in 5-7 ml of RBMI medium and 0.2 ml of 0.05 colchicine was added to each tube in vitro. Cultures were incubated at 37-38 °C for 1 h then the cells were centrifuged at 1000 rpm for 10 min and resuspended in pre-warmed (37°C) hypotonic solution (KCl 0.5 %) for 30 min at 37°C. The sample were centrifuged and fixed in cold mixture of 1:3 glacial
acetic acid and methyl alcohol. Two changes of the same fixative were applied with centrifugation and removal of the supernatant fluid each time then the sediment was suspended in a small amount of the fixative. The slides were produced by the conventional method and stained with Giemsa stain. Chromosome analysis was carried out in one hundred metaphase spreads for each fish.

**DNA Fragmentation assays for apoptosis protocol**

DNA fragmentation in liver and testes tissues was carried out according to Perandones et al. (1993). In brief, about 10-20 mg of liver or testes tissues were grinded in 400 μl hypotonic lysis buffer (10 mM Tris base, 1 mM EDTA and 0.2 % Triton X-10) and the cell lysate was centrifuged at 11,000 rpm for 15 min at 4°C. The supernatant containing small DNA fragments were separated; one-half the volume was used for gel electrophoresis and the other half, together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the Diphenyl amine. The samples were treated with equal volumes of absolute isopropyl alcohol and 0.5 M NaCl, to precipitate the DNA, stored at -20°C overnight and centrifuged at 11,000 rpm for 15 min at 4°C. The pellet was washed with 200 μl of 70 % ethanol and allowed to dry at room temperature. Extracted DNA was reconstituted in 12 μl of Tris-EDTA buffer and 3 μl loading buffer. The samples were incubated at 37°C for 20 min then electrophoresed on 1% agarose gels containing 0.71 μg/ml ethidium bromides. At the end of the runs, gels were examined using UV transillumination. The Diphenyl amine (DPA) assay reaction was modified by Perandones et al. (1993) from (Burton 1956). The percentage of DNA fragmentation was calculated in both tissues.

**Histochemical study**

After the collection of blood samples, all fish were sacrificed (by removing from water). Livers, testes and gills were dissected out and collected in Bouin’s solution for 24h and rinsed with 70% ethanol prior to embedding in paraffin wax. For Feulgen staining, 5-μm paraffin sections were cut and mounted on poly-lysine treated slides. The slides were dewaxed in xylene (2 x 5 min) followed by rehydration in decreasing ethanol series and demineralized water. Subsequently, the sections were rinsed in 1 M HCl for 1 min at room temperature and placed in 1 M HCl for 8 min at 60°C. The sections were then rinsed in 1 M HCl for 1min at room temperature and transferred to Schiffs reagent for 45 min. After washing the sections three times
for 2 min in a 10% bisulphite solu-
tion they were extensively rinsed
in demineralized water, dehydrated
through graded ethanol series of
70%, 95% and 100% for 5 min
each and brought to xylene
(Segers et al., 1996). The Feulgen
stain reaction specifically stains
nuclear DNA with a purple color.
DNA analysis was performed by
leica Qwin 500 image cytometry.
The interactive measurement menu
was used to detect optical density.
The optical density program was
used for the quantitative analysis
of DNA reaction. The intensity of
the color is directly proportional to
the DNA content within the nu-
cleus of the cell.

Statistical analysis
Data of biochemical analysis,
micronucleus test, chromosome
aberration and DNA damage were
analyzed using the General Linear
Model Procedure of the Statistical
Analysis System (SAS 1982). The
significance of the differences
among treatments was determined
by Waller-Duncan k-ratio (Waller
and Duncan, 1969). All statements
of significance were based on
probability of P < 0.05.

RESULTS
In the present study, Oreochromis niloticus fish treated
with AFB1 showed a significant
decrease in total protein, albumin,
globulin and testosterone level
compared to the control group
(Table, 1). Fish treated with EB or
EM alone were comparable to the
control except testosterone in the
group received EB which showed
a significant decrease compared to
the control group. The combined
treatment of AFB1 and EB or EM
resulted in a significant improve-
ment in TP, albumin and globulin.
Testosterone level showed insig-
nificant decrease in the group re-
ceived AFB1 plus EB however; it
was comparable to the control in
the group treated with AFB1 plus
EM.
Table (1): Plasma protein, albumin, globulin and testosterone hormone concentrations in fish treated with AFB$_1$ alone or in combination with EB or EM

<table>
<thead>
<tr>
<th>Parameter Groups</th>
<th>Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.937 ± 0.35$^a$</td>
<td>2.139 ± 0.04$^a$</td>
<td>2.918 ± 0.12$^a$</td>
<td>0.512 ± 0.07$^a$</td>
</tr>
<tr>
<td>AFB$_1$</td>
<td>3.090 ± 0.22$^b$</td>
<td>1.926 ± 0.04$^b$</td>
<td>1.164 ± 0.18$^b$</td>
<td>0.258 ± 0.01$^b$</td>
</tr>
<tr>
<td>EB</td>
<td>4.752 ± 0.33$^a$</td>
<td>2.111 ± 0.03$^a$</td>
<td>2.781 ± 0.21$^a$</td>
<td>0.360 ± 0.03$^c$</td>
</tr>
<tr>
<td>EM</td>
<td>4.605 ± 0.31$^a$</td>
<td>2.169 ± 0.06$^a$</td>
<td>2.537 ± 0.19$^a$</td>
<td>0.494 ± 0.07$^a$</td>
</tr>
<tr>
<td>AFB$_1$ + EB</td>
<td>4.116 ± 0.18$^a$</td>
<td>2.161 ± 0.05$^a$</td>
<td>2.128 ± 0.17$^c$</td>
<td>0.439 ± 0.02$^d$</td>
</tr>
<tr>
<td>AFB$_1$ + EM</td>
<td>4.818 ± 0.34$^a$</td>
<td>2.133 ± 0.04$^a$</td>
<td>2.786 ± 0.22$^a$</td>
<td>0.511 ± 0.02$^a$</td>
</tr>
</tbody>
</table>

Within each column, means superscript with different letters are significantly different at $P<0.05$

The results of the micronucleus test for fish in different treatment groups are presented in Table (2) and Fig (1). These results indicated that AFB$_1$ at the tested dose (1.5 mg/kg diet) resulted in a significant increase ($P \leq 0.01$) in the frequencies of micronucleated erythrocytes (MnRBCs) whereas; treatment with EB or EM alone had no significant effects on the number of MnRBCs compared to the control group. Fishes received the combined treatment of AFB$_1$ plus EB or EM showed a significant reduction in the frequencies of MnRBCs (36.8% and 55.6% respectively) although the number of MnRBCs was still significantly higher than those in the untreated control.
Table (2): Effect of EB and EM on the induction of micro-nucleated red blood cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of MnRBCs</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>3.20 ± 0.32a</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>149</td>
<td>29.80 ± 1.69&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EB</td>
<td>28</td>
<td>5.6 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>EM</td>
<td>22</td>
<td>4.40 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt; + EB</td>
<td>100</td>
<td>20.0 ± 0.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt; + EM</td>
<td>75</td>
<td>15.0 ± 0.45&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Within each column, means superscript with different letters are significantly different (P<0.05)

Fig (1): Normal and micronucleated red blood cell (RBC and Mn-RBC) in fish fed AFB<sub>1</sub>-contaminated diet alone or in combination with EB or EM

**Chromosomal aberrations**

The present study demonstrated that AFB<sub>1</sub> alone caused structural chromosomal aberration in the kidney tissues mainly, chromatid breaks and gaps. Structural aberrations included centromeric attenuations, deletions and fragments which scored as chromatid breaks. No significant differences
in chromosomal aberrations were observed in the groups treated with EB or EM alone compared with control group. Whereas, the combined treatment of AFB1 and clays were found to decrease significantly the total structural aberrations by 46.4 % in case of EB while it reached 64.3 % in case of EM compared to AFB1 alone treated group (Table 3).

Table (3): Effects of EB and EM on chromosomal aberrations in kidney of fish fed AFB1-contaminated diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chromosomal breaks</th>
<th>C.A.</th>
<th>C. F.</th>
<th>Gap</th>
<th>Total ab.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ch. Br.</td>
<td>Del.</td>
<td>Frag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.4 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0.8 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4.8 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EB</td>
<td>0</td>
<td>1.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>EM</td>
<td>0</td>
<td>1.2 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt; + EB</td>
<td>2.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.4 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AFB&lt;sub&gt;1&lt;/sub&gt; + EM</td>
<td>1.8 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.2 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.4 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Within each column, means superscript with different letters are significantly different (P<0.05)

Fig (2): Types of chromosome aberration in fish kidney treated with AFB<sub>1</sub> alone or in combination with EB or EM.

g = gap, b = break & d = deletion
DNA fragmentation
The current study revealed that treatment with AFB\textsubscript{1} resulted in a significant increase in the percentage of DNA fragmentation in liver and testes of fish by 39\% and 16.3\% respectively compared to the control group (Table 4 and Fig 3). Fishes treated with EB or EM alone were comparable to the control regarding DNA fragmentation. On the other hand, the combined treatment with AFB\textsubscript{1} and EB or EM succeeded to decrease the elevation in DNA fragmentation resulted from AFB\textsubscript{1}. This decrease was pronounced in the group treated with AFB\textsubscript{1} plus EM (54.9\%) than the group received AFB\textsubscript{1} plus EB (36.8\%).

![Agarose gel electrophoresis of DNA extracted from liver of fish treated with, AFB\textsubscript{1} plus EB or EM: Lane M: DNA molecular weight marker. Lane 1, control group; Lane 2, AFB\textsubscript{1}-treated group; Lane 3, EM-treated group; Lane 4, EB-treated group; Lane 5, AFB\textsubscript{1} + EM and Lane 6, AFB\textsubscript{1} + EB](image)

Fig (3): Agarose gel electrophoresis of DNA extracted from liver of fish treated with, AFB\textsubscript{1} plus EB or EM: Lane M: DNA molecular weight marker. Lane 1, control group; Lane 2, AFB\textsubscript{1}-treated group; Lane 3, EM-treated group; Lane 4, EB-treated group; Lane 5, AFB\textsubscript{1} + EM and Lane 6, AFB\textsubscript{1} + EB

Table (4): Effects of EB and EM on DNA fragmentation in testis and liver of fish fed AFB\textsubscript{1}-contaminated diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA fragmentation (%)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testis</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>5.6</td>
<td>10.3</td>
</tr>
<tr>
<td>AFB\textsubscript{1}</td>
<td>10.3</td>
<td>39</td>
</tr>
<tr>
<td>EB</td>
<td>6.0</td>
<td>11.8</td>
</tr>
<tr>
<td>EM</td>
<td>6.2</td>
<td>9.5</td>
</tr>
<tr>
<td>AFB\textsubscript{1} + EB</td>
<td>8.2</td>
<td>28.2</td>
</tr>
<tr>
<td>AFB\textsubscript{1} + EM</td>
<td>7.5</td>
<td>19.4</td>
</tr>
</tbody>
</table>
In the current study, DNA content was further confirmed in liver, testes and gills by the histochemical study using image analysis techniques. The results of optical density for DNA contents in different treatment groups are presented in Table (5). The density of DNA in liver indicated that treatment with AFB$_1$ resulted in a significant decrease in DNA content in the liver (Fig. 4), testes (Fig. 5) and gills (Fig. 6) compared to DNA content in the same organs of the control group. DNA content in these organs of fish treated with EM or EB were comparable to the control. The combined treatment with EM or EB with AFB$_1$ resulted in a significant improvement in DNA content. This improvement was pronounced in the group received AFB$_1$ plus EM.

Table (5): Optical density (O.D) of DNA in the testes, liver and gills of fish treated with AFB$_1$ alone and plus EM. or EB.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>AFB$_1$</th>
<th>EB</th>
<th>EM</th>
<th>AFB$_1$ + EB</th>
<th>AFB + EM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td>28.92 ± 2.54$^a$</td>
<td>19.06 ± 2.21$^b$</td>
<td>27.56 ± 2.45$^a$</td>
<td>26.32 ± 3.48$^a$</td>
<td>32.32 ± 2.12$^c$</td>
<td>38.54 ± 2.27$^d$</td>
</tr>
<tr>
<td><strong>Testes</strong></td>
<td>129.25 ± 2.84$^a$</td>
<td>86.27 ± 4.53$^b$</td>
<td>110.35 ± 4.07$^b$</td>
<td>122.46 ± 1.55$^d$</td>
<td>88.3 ± 4.63$^e$</td>
<td>93.68 ± 2.76$^f$</td>
</tr>
<tr>
<td><strong>Gills</strong></td>
<td>70.24 ± 0.93$^a$</td>
<td>62.47 ± 0.79$^b$</td>
<td>66.95 ± 0.73$^c$</td>
<td>68.22 ± 1.22$^a$</td>
<td>63.78 ± 1.12$^d$</td>
<td>74.89 ± 1.41$^a$</td>
</tr>
</tbody>
</table>

Within each raw, means superscript with different letters are significantly different (P<0.05)
Fig. (4): Sections of the liver of fish in: (A) control group showed normal reaction and DNA content; (B) Fish treated with AFB$_1$ showed a significant decrease in the reaction and DNA content; (C, D) Fish treated with EB or EM showed normal DNA content; (E) Fish treated with AFB$_1$ plus EB showed more or less normal DNA content and (F) fish treated with AFB$_1$ plus EM showed normal DNA content. (Feulgen stain X 400)
Fig. (4). Sections of the testes of fish in: (A) control group showed normal reaction and DNA content; (B) Fish treated with AFB₁ showed a significant decrease in the reaction and DNA content; (C, D) Fish treated with EB or EM showed normal DNA content; (E) Fish treated with AFB₁ plus EB showed more or less normal DNA content and (F) fish treated with AFB₁ plus EM showed normal DNA content. (Feulgen stain X 400)
Fig. (4). Sections of the gills of fish in: (A) control group showed normal reaction and DNA content; (B) Fish treated with AFB₁ showed a significant decrease in the reaction and DNA content; (C, D) Fish treated with EB or EM showed normal DNA content; (E) Fish treated with AFB₁ plus EB showed more or less normal DNA content and (F) fish treated with AFB₁ plus EM showed normal DNA content. (Feulgen stain X 400)
DISCUSSION

In the present study, we evaluated the ability of EB and EM to protect the fish from AFB$_1$ hazards. The selected doses of AFB$_1$, EB and EM were literature based (Cagauan et al., 2004; Abdel-Wahhab et al., 1998 and 2002). Our results indicated that treatment with AFB$_1$ resulted in a significant decrease in TP, albumin, globulin and testosterone concentrations. The decrease in TP, albumin and globulin reported herein may be indicate protein catabolism and/or kidney dysfunctions (Abdel-Wahhab and Aly, 2005; Abbès et al., 2006). The decrease in testosterone concentration was mainly due to the impairment of testicular tissues by AFB$_1$. Similar to the current observation, Agnes and Akbarsha (2003) reported that AFB$_1$ disturbs the testes function including spermatogenesis (Egbunike et al., 1980), Leydig cell function (Egbunike, 1982) and fertility (Ibeh et al., 1994).

Recently, Abou El-Saad and Mahmoud (2007) reported that AFB$_1$ decrease the male sex hormone levels and induced histopathologic alterations in the testis including a degeneration and highly mitotic division within the spermatogenic nuclei, in addition to some karyomegaly and nuclear pyknosis.

In a pervious work, Kenawy et al. (2009) reported that tilapia fish fed aflatoxin-contaminated diet showed severe histopathological changes included severe hyperplasia in the epithelial lining of the secondary lamellae of the gills as well as severe histological changes in the liver and kidney tissues. On the other hand, the cytogenetic results indicated that AFB$_1$ treatment induced a significant increase in the number of micronucleated red blood cells (Mn-RBCs) and chromosomal aberration as well as a significant increases the percentage of DNA fragmentation in the liver tissue. Previous studies on rat bone marrow and fish kidney revealed that AFB$_1$ induce DNA damage and chromosomal aberrations included chromatid gaps and chromatid breaks (Ferguson et al., 1986; Basaran et al., 1993; Abdel-Wahhab et al., 1998). The mutagenicity of AFB$_1$ arising from the toxin molecules which might be forming covalent-adducts resulting in the disturb of DNA replication (Bonnet and Taylor, 1989) consequently in the abnormality of the chromosomes (Sinha and Prased, 1990).

Another mechanism by which AFB$_1$ decrease DNA content may be as a result of decreasing the mitotic division of the cell. Previously, Legator and Withrow (1964) stated that there was an inhibitory effect of aflatoxin on mitotic division in cultured embryo-
monic lung cells. However, Smela et al. (2002) stated that the toxicity of AFB$_1$ is related to its metabolic fate in a complicated network of mutually competing pathways in the target tissue. Furthermore, AFB$_1$-induced oxidative perturbations and the interaction with DNA appears to be obligatory component of AFB$_1$-induced carcinogenesis (Abdel-Wahhab et al., 2007).

The action of AFB$_1$ on hepatic DNA is a multifaceted phenomenon. In the liver microsomes, AFB$_1$ is oxidised to its reactive epoxide forming AFB$_1$- 8, 9 epoxide (Busby and Wogan 1984). This subsequently links itself to DNA, through the N7 position of guanine and exhibits the mutagenicity through guanine to thymine transversions (Lasky and Magder 1997). AFB$_1$-DNA adduct destabilises the N-glycosidic bond of the nucleotide leading to depurination and DNA strand scission (Iyer et al., 1994). Formation of these adducts disrupts the normal working process of the cell and in the case of DNA adducts, can ultimately lead to a loss of control over cellular growth and division (Verma. 2004). This is consistent with several other findings, which report a direct correlation between AFB$_1$-induced oxidant generation and resultant DNA damage (Barraud et al., 2001; Meki et al., 2001). On the other hand, the effect of AFB$_1$ on DNA concentration in gills tissues may be a result of severe degenerative and necrotic changes associated with desquamation of the respiratory epithelial lining cells (Kenawy et al., 2009) resulted in the decrease in the cell number consequently decreased and DNA content.

A novel approach to aflatoxin management is the use of selective sorbents in diet that tightly bind and immobilize aflatoxin in the gastrointestinal tract. In a series of studies, we have demonstrated that phyllosilicates clay prevented aflatoxicosis in rats (Mayura et al., 1998; Abdel-Wahhab et al., 1998, 1999, 2002). The basic mechanism appears to involve sequestration of AFB$_1$ in the gastrointestinal tract and chemisorption (i.e. tight binding) to phyllosilicates, which results in reduction in toxin bioavailability (Phillips et al., 2002). In the present study, we evaluate the effect of AFB$_1$ on the DNA concentration in different tissues and the ability of montmorillonite (EM) and bentonite (EB) to inhibit AFB$_1$ effects.

Fish treated with AFB$_1$ alone or in combination with sorbents (EM or EB) was found to decrease the DNA content in liver, gills and testes compared to those of the control group. Moreover, the effect
of EM was more pronounced than EB in the prevention of DNA damage. Similar findings were reported by Abdel-Wahhab et al. (2002) who indicated that EM was effective in preventing the toxic effects of aflatoxins in rats. Moreover, these authors suggested that EM may posses three types of active binding sites: (1) those located at basal planes within interlayer channels, (2) those located on the surface, and (3) those located at the edges of clay particles. Previous reports indicated that EM 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 (Abdel-Wahhab et al., 1998, 1999). Furthermore, Abdel-Wahhab et al. (2002) suggested that EM induces its protective effect against AFs through the formation of adduct which is not affected by the gastrointestinal tract enzymes consequently reduce the toxin bioavailability.

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chromosomal aberrations."


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منع التغيرات الوراثية الخلوية والهستوكيميائية والبيوكيميائية في أسماك البلطي عن طريق التغذية بالمواد الرابطة

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الملخص العربي

تهدف هذه الدراسة إلى استخدام نوعين من السمى المصري (البنتونيت و المتموريلونيت) في الحماية من التغيرات البيوكيميائية والهستوكيميائية والوراثية الخلوية الناتجة عن الأفلاتوكسين ب. أجريت هذه الدراسة على ٥ مجموعات من أسماك البلطي النيلي شملت المجموعة الضابطة والمجموعة المغذة على ملوثة بالأفلاتوكسين ب، والمجموعات المغذية على علقاب مضار إليها البنتونيت أو المتموريلونيت مع أو بدون الأفلاتوكسين ب. لمدة مثلى ثلاثة أسابيع. في نهاية التجربة تم تجميع عينات الدم لتقدير هرمون التسترون والبروتينات و كرات الدم الحمراء المحتوية على نوويات. كما تم تجميع عينات من الأنسجة للدراسات الوراثية الخلوية (الحمض النووي الديسوكسي ريبوزي في الكبد والخصية والتستونات الكروموموسومية في الكلية) بالإضافة إلى الدراسات الهستوكيميائية على الكبد والخصية والخلايا. ظهرت النتائج وجود نقص ملحوظ في كل من البروتين والألبومين والغلوتينين وكذلك هرمون التستيروستيرون و كذلك في تركيز المادة الوراثية في أنسجة الكبد والخصية و الخصي صاحبها زيادة ممنوية في عدد كرات الدم المحتوية على نوويات وتشوهات الكروموموسومات في الكلية نتيجة التغذية على الملوحة بالأفلاتوكسين. لم تكن هناك تأثيرات ممنوية على جميع القياسات البيوكيميائية في الأسماك المغذية المواد الرابطة بتفريق ماهليا التستيرون في المجموعة المغذية على البنتونيت الذي حدث فيه نفسا ممنوية. كما أثبتت النتائج الوراثية الخلوية حدوث زيادة ممنوية في عدد كرات الدم المحتوية على نوويات والتستونات الكروموموسومية في الكلية وزيادة تكسر المادة الوراثية

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في الخصية وليس الكبد في المجموعات المغذاه على كلا من البنتونيت و
المنتموريلونيت. أدت التغذية على المواد الرابطة مع الأفلاتوكسين إلى منع أو تقليل
التغيرات الناتجة عن الأفلاتوكسين وقد كان هذا التحسن أوضح في المجموعة المعاملة
بالمنتموريلونيت. نستخلص من هذه الدراسة أن كلاً من البنتونيت و المتموريلونيت لهما
القدرة على الارتباط بالأفلاتوكسين وتكوين معقد غير قابل للتمثيل في الجسم وبالتالي
تقليل المتاح من الأفلاتوكسين في القناة الهضمية للأسماك ومن ثم يمكن استخدامهما
بنجاح في حالة الأغذية الملتوية بالأفلاتوكسين لحماية الأسماك من التأثيرات السامة لهذا
السم الفطري.

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