Pathological studies on effects of aflatoxin on *Oreochromis niloticus* with application of different trials of control

By

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**SUMMARY**

Aflatoxins (AFs) are among the most serious food contaminants that induce economic losses in livestock and fish industry. Sixty *Oreochromis niloticus* fish were divided into 6 equal groups. They were treated for 3 weeks according to their respective treatment as follows: group 1, untreated control; group 2, fed AFs-contaminated diet (3mg/kg feed); group 3, fed basal diet supplemented with EB (5mg/kg feed); group 4, fed AFs-contaminated diet supplemented with EB (AFs + EB); group 5, fed basal diet supplemented with EM (5mg/kg feed) and group 6, fed AFs-contaminated diet supplemented with EM (AFs + EM). At the end of the experimental period, all fish were sacrificed and dissected. Gills, skin, kidneys, Liver, spleen, brain and intestine specimens from all fish within different groups were collected for histopathological assay. Besides, liver specimens were collected for electron microscopical examination. Samples of musculature and livers were also taken for the determination of AFs residues. Gross pathological examination in fish treated with AFs-contaminated diet revealed that the liver was enlarged, pale, and friable with grayish patches. The kidneys were swollen, dark red in colour and friable. The spleen was congested. The gills were dark red in colour and gill cavity was filled with excessive mucous secretion. Histopathologically, severe degenerative and necrobiotic changes were seen in most organs of AFs-treated fish. Such changes were milder in (AFs + EM) treated group but increased in severity in (AFs + EB) treated group. Ultrastructural changes in liver were recorded and discussed in detail. The results of AFM1 residues revealed that, AFM1 is only detected in the liver and musculatures of AFs treated group. Whereas, AFM1 was not detected in the fish treated with (AFs + EB) or those in the group treated with (AFs + EM). It was concluded that, EM could effectively alleviate lesions of AFs and prevent presence of AFs residue in the fish tissue being safer for human consumption.
**INTRODUCTION**

Aflatoxins (AFs) are secondary metabolites of some strains of the molds *Aspergillus flavus*, *A. parasiticus* and *A. nomius* that grow on food and feed crops. Animals that consume AFs-contaminated feed develop various health problems including growth retardation, reduction of feed efficiency and liver and kidneys damage (Abdel-Wahhab et al., 1998, 1999, 2002; Bintvihok, 2002). AFs also cause immunosuppression and changes in relative organ weights (Hinton et al., 2003; Abdel-Wahhab et al., 2007), increased mortality and enhanced susceptibility to infectious diseases (Chang and Hamilton, 1991). AFs are known as a hepatocarcinogen in various animal species, including fish, birds, rodents, and nonhuman primates (Allameh et al., 2005). It is also a suspect human carcinogen and has been shown to play a role in human hepatocarcinoma (Wang et al., 2001). It has been shown that AFs especially AFB1 is activated by the hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB1-8, 9-epoxide, which subsequently binds to nucleophilic sites in DNA (Sharma and Farmer, 2004). The formation of AFB1-DNA adducts is regarded as a critical step in the initiation of AFB1-induced hepatocarcinogenesis (Preston and Williams, 2005; Abdel-Wahhab et al., 2006). Moreover, AFs toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H2O2) during the metabolic processing of AFB1 by cytochrome P450 in the liver (Abdel-Wahhab et al., 2005a; Preetha et al., 2006). AFs can not be eliminated completely from animal feed or the human food supply and represent a health concern for populations that cannot properly store agricultural commodities to limit mold growth or who have limited access to a wide variety of other foods (Roebuck, 2004).

Numerous strategies were elaborated to get rid of mycotoxin problems by detoxification or inactivation of mycotoxin-contaminating foodstuffs, such as γ-irradiation, thermal inactivation, physical separation, microbial degradation and treatment with a variety of chemicals (El-Nezami et al., 2004; Abbès et al., 2006a, b). Removing AFs from contaminated food and foodstuffs remains a major problem and there is a great demand for effective decontamination technology (Oguz et al., 2000). One strategy is to use clay minerals to bind aflatoxin in the animal's digestive tract and thus reduce toxin bioavailability. The aflatoxin/clay complex is then excreted in the feces (Abdel-
The basis of interest in the biological effects of montmorillonite (EM) concerns one or more of their physical and chemical properties, such as ion exchange capacity, adsorption and related molecular sieve properties. Several reports demonstrated that the administration of clay minerals at a level as high as 5 g/kg b.w. did not show any toxic effects that were measured using biochemical, haematological, and immunological parameters in mice (Abbès et al., 2006 a,b ; 2007a) and rats (Abdel-Wahhab et al., 1998, 1999, 2002). On the other hand, research is warranted to establish a sensitive animal model that can be used to compare the efficacy and safety of potential sorbents. The Nile tilapia, Oreochromis niloticus, may represent such a model, since this fish is highly susceptible to nutritional deficits and is extremely vulnerable to toxic insult from various chemicals including aflatoxins. The objective of the current study was to evaluate the ability of montmorillonite (EM) and bentonite (EB) collected from the Egyptian environment to protect against AFs toxicity in tilapia fish fed AFs-contaminated diet.

**MATERIALS AND METHODS**

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

**Fish:** Sixty apparently healthy, two-month-old Nile tilapia (Oreochromis niloticus) fish with an average body weight (90±10g) were collected 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. Fish were maintained on a standard fish diet (free from AFs) at the Animal House, Veterinary Medicine Division, National Research Center (Dokki, Giza, Egypt). After an acclimation period of one week, the fish were divided over six experimental groups (10 fish/group) and each group was placed in a fully prepared aquarium containing dechlorinated tap water, the average water temperature was 20 ± 3.7 °C, and the pH was in the range 7.17–8.19.

**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 AFs-contaminated diet (3mg/kg feed); group 3, fed basal diet supplemented with EB (5mg/kg feed); group 4, fed AFs-contaminated diet supplemented with EB; group 5, fed basal diet supplemented with EM (5 mg/kg feed) and group...
6, fed AFs-contaminated diet supplemented with EM. At the end of the experimental period, all fish were sacrificed and dissected. Liver, gills, skin, spleen, kidneys, intestine and brain samples from all fish within different groups were collected for histopathological assay. Liver samples were collected for transmission electron microscope (T.E.M). Samples of musculature and livers were also collected for the determination of AFs residues.

**Determination of aflatoxin M1 in tissues samples:**

Aflatoxin M1 (AFM1) is the major AFs metabolites which determined in the liver and musculatures. Determination of AFM1 in tissues samples (liver and musculatures) of each fish were collected and stored at -18 °C until the time of analysis. Each tissue sample was cut into small pieces and AFM1 residues was extracted and analyzed by the method of Jem-mali and Murthy (1976) using HPLC (model LC-10ADvp solvent delivery system; model SIL-10Advp auto injector, Shimadzu, Japan; ODS-5 C18 Brownlee reverse phase column (220 x 4.6 mm, particle size 5 µm) with a C18 guard column (Perkin Elmer, Norwalk, Conn.). The mobile phase consisted of water: methanol: acetonitrile 66:17:17, flow rate was 1 ml/min, oven temperature was 40°C and injection volume was 30 µl. Detection of AFM1 was carried out by excitation at 360 nm and emission at 440 nm (Fluorescence-10A XL, Shimadzu detector). The concentrations of AFM1 in tissue samples were estimated from a standard curve 0.04-10 ng/ml, prepared from AFM1 in chloroform (9.93 µg/ml) reference material RM 423 (LGC Promochem AB, Boras, Sweden). An AFM1 standard was injected every 10 injections as a quality control. AFM1 was stored at -20°C in a sylilated vial wrapped in aluminium foil. Since AFM1 is a possible carcinogen, care was exercised to avoid personal exposure and proper decontamination procedures with 10 % sodium hypochlorite were used.

**Histopathological studies:**

Tissue specimens from Liver, gills, skin, spleen, kidneys, intestine and brain were collected from all the experimental groups, fixed in 10% neutral buffer formalin, dehydrated in serial grades of ethyl alcohol, cleared by xylol, embedded in paraffin wax, sectioned at 3-5 microns, stained with Haematoxyline and Eosin (H&E) according to Roberts (2001) and then examined microscopically for recording the histopathological alterations.

**Ultrastructural study:**

Specimens (1x1 mm) were fixed in 5% cold cacodylate buffered gluteraldehyde for 2 h,
washed in cacodylate buffer, fixed in 1% osmium tetraoxide for 2 h and rewashed in cacodylate buffer, four times, for 20 minutes each. The fixed specimens were dehydrated using ascending concentrations of alcohol (30, 50, 70, 90 and 100%). Specimens were immersed in propylene oxide and epon for 30 minutes and finally in pure epon. Each tissue specimen was embedded in a capsule with embedding mixture (epon mixture and hardner) and kept in an oven for 2-3 days at 60°C. Using an LKB ultramicrotome, semi-thin sections were prepared, stained with toluidine blue and examined by a light microscope. Ultra-thin sections were prepared and stained by uranyl acetate and lead citrate, and examined under transmission electron microscope (Reynolds, 1963).

RESULTS

The results of AFM1 residues in liver and musculatures of fish in different treatment groups (Table 1) revealed that AFM1 is only detected in the liver and musculatures of the fish in the AFs alone group. Whereas, AFM1 was not detected in the fish treated with AFs + EB or those in the group treated with AFs + EM.

Table (1): AFM1 in livers and musculatures of fish within different treatments groups (µg/kg)

<table>
<thead>
<tr>
<th>Musculature</th>
<th>Liver</th>
<th>Group</th>
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<tbody>
<tr>
<td>ND</td>
<td>ND</td>
<td>Control</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>AFs</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>EB</td>
</tr>
<tr>
<td>ND</td>
<td>3</td>
<td>AFs + EB</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>EM</td>
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<tr>
<td>ND</td>
<td>2</td>
<td>AFs + EM</td>
</tr>
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ND: not detected

Histopathological investigations:

The macroscopical and microscopical examinations showed no obvious lesions in the control group (group 1).

Pathological changes in group(2) {AFs-treated group}:
Macroscopical examination revealed, enlargement of liver and kidneys. The liver was pale, friable and showed grayish patches on the hepatic surface. The kidneys were swollen, dark red in colour and friable. The spleen was also congested. The gill cavity filled with excessive mucous secretion and the gill filaments appeared dark red in colour. The histopathological changes in the gills revealed severe hyperplasia in the epithelial lining of the secondary lamellae associated with degenerative changes, necrosis and oedema (Fig. 1). Congestion of lamellar and branchial blood vessels was accompanied with telangectasis in some of secondary lamellae were also seen (Fig. 2). The gill rackers exhibited hyperplasia, hypertrophy of the goblet cells. Oedema in the gill arch also seen (Fig. 3). The skin revealed subdermal oedema with increase of the melanin carrying cells in the epidermal, dermal and subdermal layers as well as Zenker's necrosis of the myofibers, mononuclear inflammatory cells were also detected in between the musculature fibers (Fig. 4). The kidneys showed degenerative changes in the form of vacuolation and swelling of the tubular epithelium, periglomerular and peritubular oedema, degenerative changes and necrosis in the in the tubular epithelium and endothelial lining the glomerular tufts sometimes accompanied with atrophy of the tuft as well as congestion in the renal blood vessels and hemorrhages in between the interstitial tissue was also detected (Figs. 5 & 6).

The liver showed severe vacuolar degeneration accompanied with necrotic changes represented by pyknosis and karyorrhexis of the nuclei (Fig. 7), as well as vacuolation and necrosis of the pancreatic acinar cells and infiltration of mononuclear inflammatory cells were detected. The spleen showed oedema, severe congestion and hemorrhages along with lymphocytic depletion (Fig. 8). The brain showed focal gliosis and oedema in the cerebral cortex (Fig. 9). The intestine revealed slight desquamation and severe mucinus degeneration in the epithelial lining of the intestinal villi and few mononuclear cells infiltration in the lamina propria (Fig. 10).

**Pathological changes in group(3) {EB-treated group}:**

It was noticed that, the addition of the EB alone to fish diet revealed mild histopathological lesions.

**Pathological changes in group(4) {AFs+EB-treated group}:**

It was noticed that, the addition of the EB to AFS in group (4) increased the severity of the histopathological lesions in different organs, compared to those in groups (2) and (6) especially the
gills, where it showed severe degenerative changes which were represented by severe hyperplasia and necrosis of the lamellar epithelium leading to complete occlusion of the interlamellar spaces as shown in (Fig.11). The skin showed severe subdermal oedema and necrosis of the musculature fibers (Fig.12). The kidneys showed oedema, atrophy of the glomerulei and severe necrosis in the haemopiotic and renal tissues (Fig. 13). The liver revealed severe vacuolar degeneration and focal necrosis (Fig. 14). The spleen showed oedema, congestion and depletion of lymphocytes (Fig. 15). The brain showed severe oedema, neuronoal degeneration with swelling of nerve cells, focal gliosis, neuronophagia and satelletosis (Fig. 16). The intestine showed the same picture which appeared in the previous groups but in less extent (Fig. 20). The kidneys showed oedema and tubular nephrosis (Fig. 21). The liver revealed mild vacuolar degeneration (Fig. 22). The brain exhibited oedema in the cerebellum (Fig. 23). The spleen showed mild lymphocytic depletion. The intestine showed slight proliferation of goblet cells (Fig. 24).

**Pathological changes in group (5) {EM-treated group}:**

The current results revealed that fish treated with EM alone had neither macroscopical nor microscopical changes.

**Pathological changes in group(6) {AFs+EM treated group}:**

It was clear that the addition of EM to AFs in group (6) antagonize the effect of the aflatoxin to some extent in the different fish organs where the gills and gill rackers appeared nearly normal (Fig. 18 & 19). The skin showed the same picture which appeared in the previous groups but in less extent (Fig. 20). The kidneys showed oedema and tubular nephrosis (Fig. 21). The liver revealed mild vacuolar degeneration (Fig. 22). The brain exhibited oedema in the cerebellum (Fig. 23). The spleen showed mild lymphocytic depletion. The intestine showed slight proliferation of goblet cells (Fig. 24).

**Electron microscopic findings in the liver:**

The hepatocytes of the control fish showed an intact cell membrane with an obvious cytoplasmic organells and abundance of ribosomes. A numerous mitochondria of spherical or elongated shapes were scattered throughout the cytoplasm. Also, stacks of rough endoplasmic reticulum were arranged in several raws. A moderate amount of lipid was stored in the cytoplasm. A few proxisomes and lysosomes were seen. The nuclei appeared with prominent nuclear membrane and scattered heterochromatin (Fig. 25). The hepatocytes of the second group (AFs-treated group) showed vacuolation in the cytoplasm and degeneration as well as desorganization in the cytoplasmic and fat globules. Swelling of the rough and smooth endoplasmic reticulum and karyolyses of the nuclei were noticed (Fig.26).
Fig.(1): Gills of AFs-treated fish showing severe hyperplasia in the epithelial lining the secondary lamellae, degenerative changes, necrosis and oedema. H&E stain. X 250

Fig.(2): Gills of AFs-treated fish showing congestion of lamellar and branchial blood vessels accompanied with telangictasis in some of secondary lamellae. H&E stain. X 400

Fig.(3): Gill rackers of AFs-treated fish showing congestion, hyperplasia and hyper-trophy of the goblet cells. H&E stain. X 250

Fig.(4): Skin of AFs-treated fish showing subdermal oedema with increase of the melanin carrying cells, mononuclear inflammatory cells infiltration in between the musculature fibers as well as Zenker's necrosis of the myofibers, H&E stain. X 250

Fig.(5): Kidneys of AFs-treated fish showing vacuolation and swelling in the tubular epithelium and hemorrhages in between the interstitial tissue. H&E stain. X 400

Fig.(6): Kidneys of AF-treated fish showing congestion, oedema, necrosis in the tubular epithelium and endothelium of the glomerulai. H&E stain. X 400
Fig.(7): Liver of AFs-treated fish showing severe vacuolar degeneration and necrotic changes in the hepatocytes. H&E stain. X 400
Fig.(8): Spleen of AFs-treated fish showing oedema, severe congestion, hemorrhages and lymphocytic depletion. H&E stain. X 250
Fig.(9): Brain of AF-treated fish showing oedema and focal gliaosis. H&E stain. X 250
Fig.(10): Intestine of AF-treated fish showing slight desquamation, severe mucinus degeneration in the intestinal villar epithelium and few mononuclear cells infiltration in the lamina propria. H&E stain. X 400
Fig.(11): Gills of AFs+EB-treated fish showing severe hyperplasia and necrosis of the lamellar epithelium and complete occlusion of the interlamellar spaces. H&E stain. X 400
Fig.(12): Skin of AFs+EB-treated fish showing severe subdermal oedema and necrosis of the musculature fibers. H&E stain. X 250
**Fig. (13):** Kidneys of AFs+EB-treated fish showing, oedema, atrophy of the glomerulei and severe necrosis in the haemopiotic and renal tissues. H&E stain. X 400.

**Fig. (14):** Liver of of AFs+EB-treated fish showing severe vacuolar degeneration and focal necrosis. H&E stain. X 400.

**Fig. (15):** Spleen of AFs+EB-treated fish showing oedema, congestion and depletion of lymphocytes. H&E stain. X 250

**Fig. (16):** Brain of AFs+EB-treated fish showing severe oedema, satelletosis and degeneration and swelling of the nerve cells, H&E stain. X 400.

**Fig. (17):** Intestine of AFs+EB-treated fish showing goblet cells proliferation in the villar epithelium. H&E stain. X 250

**Fig. (18):** Gills of AFs+EM-treated fish appeared nearly normal. H&E stain. X 100
Fig. (19): Gill rackers of AFs+EM-treated fish appeared nearly normal. H&E stain. X 100
Fig. (20): Skin of AFs+EM-treated fish showing oedema in between the musculature fibers. H&E stain. X 250
Fig. (21): Kidneys of AFs+EM-treated fish showing oedema and tubular nephrosis. H&E stain. X 400.
Fig. (22): Liver of AFs+EM-treated fish showing mild vacuolar degeneration. H&E stain. X 100
Fig. (23): Brain of AFs+EM-treated fish showing, pericellular oedema in the cerebellum. H&E stain. X 250
Fig. (24): Intestine of AFs+EM-treated fish showing slight proliferation of goblet cells. H&E stain. X 100
**Fig.(25):** Electron micrograph of a hepatocyte from control fish showing an intact cell membrane with an obvious cytoplasmic organelles and abundance of ribosomes. Numerous mitochondria of spherical or elongated shapes were scattered throughout the cytoplasm. Also stacks of rough endoplasmic reticulum were arranged in several rows. A moderate amount of lipid was stored in the cytoplasm. Few proxisomes and lysosomes were seen. The nuclei appeared with prominent nuclear membrane and scattered heterochromatin. Uranyl acetate and lead citrate stain, X 6000.

**Fig.(26):** Electron micrograph of hepatocytes from AFs-treated fish showing vacuolation in the cytoplasm, degeneration, desorganization in the cytoplasmic and fat globules, Swelling of the rough and smooth endoplasmic reticulum and karyolyses of the nuclei. Uranyl acetate and lead citrate stain, X 2500.
**DISCUSSION**

Aflatoxins are among the most serious food contaminants that induce economic losses in livestock and fish industry (Mohamed and Mokhbatly, 1997). Actually, there are a variety of commercial adsorbents available on the market in the form of food additives. Many of these may be nonselective in their action and may pose significant hidden risks due to interaction with nutrients and other important feed borne chemicals (Mayura et al., 1998).

The present investigation revealed variable degenerative changes in gills, skin, kidneys, liver, brain, spleen and intestine in group 2 (AFs), group 3 (EB) and group 4 (AFs+EB). It was noticed that, the addition of the EB to AFS in group (4) increased the severity of the histopathological lesions in different organs, compared to those in groups (2) and (6).

In AFs-treated groups either alone or in combination with EB showed degenerative changes in the gills represented by hyperplasia and necrotic changes in the lamellar epithelium. These results are in accordance with that of Mohamed and Mokhbatly (1997). The skin revealed severe oedema, Zenker's necrosis and mononuclear cells infiltration in between the myofibers. The renal damage was represented by vacuolar degeneration and necrosis of the tubular epithelium, peritubular and periglomerular oedema, atrophy of the glomerular tuft as well as, necrosis of the haemopiotic elements. Nearly similar results were obtained by Mohamed and Mokhbatly (1997) and Mahmoud et al., (2004). Moreover, Jantrarotai et al. (1990) mentioned that, catfish intraperitoneally injected with aflatoxins showed necrosis of the haematopiotic and renal tissues.

In the present study, the hepatic damage was represented by severe vacuolar degeneration and focal necrosis of the hepatocytes and the pancreatic acini. Such lesions are in agreement with Chavez et al., (1994); Mohamed and Mokhbatly (1997); Boonyaratpalin et al., (2002) and Mahmoud et al., (2004). The spleen showed oedema, lymphocytic depletion, necrosis, congestion and haemorrhages. These changes agreed with El-Bouhy et al., (1993); Anjum, (1994); Ali et al. (1994); Mohamed and Mokhbatly, (1997) and Mahmoud et al., (2004) who mentioned that, these lesions developed as a result of immunosuppressive effect of aflatoxine. Also, Mona and Suzan (2005) recorded that, AFs causes a variety of biological effects including hepatotoxicity and immunosuppression with decrease in resistance to infectious diseases.
Neuronal damage observed in the brain included oedema, necrosis of some neurons and gliosis. The aforementioned lesions could be attributed to the neurotoxic effect of the AFs or its metabolites. Similar changes were recorded by Mohamed and Mokhbatly (1997) and Mahmoud et al. (2004).

It was clear that the addition of EM to the aflatoxin in group (6) antagonize the effect of the aflatoxin to some extent in different fish organs while addition of EB to the AFs in group (3) increased the severity of the histopathological lesions. The results of the current study revealed that EB posses a negative impact on fish as indicated by the severe changes in the histopathological study although it significantly decreased AFM1 in liver. In this concern, Mayura, et al. (1998) reported that, some sorbent materials may posses significant hidden risks due to interaction with nutrients and other important feed borne chemicals. On the other hand, EM showed a significant improvement in the histopathological lesions in all examined tissues as well as the AFM1 residues in liver. Similar result was reported by Abdel-Wahhab et al. (1998, 1999 and 2005) in rats. Moreover, Abdel-Wahhab et al. (2002) indicated that EM was effective in preventing the toxic effects of aflatoxins.

In general, 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., 2002), who suggested that, the 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.

It could be concluded that both EB and EM had the ability to bind aflatoxin and reduced AFM1 residues in tissues, however, the safety of these sorbents were different regarding their effects on the histopathological pictures of different organs. These results should be confirmed by further studies.

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دراسات بالأنثروبولوجيا عن تأثيرات الألفاتوكسين على أسماك البلطي النيلي
مع تطبيق محاولات مختلفة للتحكم فيها
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** قسم التلوث والتسمم الغذائي - المركز القومي للبحوث

الملخص العربي
إن الألفاتوكسين من أخطر ملوثات الطعام التي تسبب خسائر اقتصادية في الأسماك الحية وكذلك صناعة الأسماك، وقد أجريت هذه التجربة لمدة ثلاث أسابيع على عدد 100 سمكة من أسماك البلطي النيلي حيث قسمت إلى 3 مجموعات متساوية معاملة كالتالي: المجموعة الأولى وهي المجموعة الضابطة، المجموعة الثانية غذت على مادة ملوثة بالألفاتوكسين (1 مجم/كم غذية)، المجموعة الثالثة غذت على مادة ملوثة مواتزة مضاف إليها طمي البتونيت، المجموعة الرابعة غذت على مادة ملوثة بالألفاتوكسين مضاف إليها طمي البتونيت، المجموعة الخامسة غذت على مادة ملوثة مضاف إليها طمي المتموربولونيت، المجموعة السادسة غذت على مادة ملوثة بالألفاتوكسين مضاف إليها طمي المتموربولونيت، و في نهاية التجربة تم تشريح الأسماك و أخذ عينات من الخياشيم والجلد وكبد الطحال والمخ والأمعاء من جميع المجموعات للفحص الهسبتيولوجي بالميكروسكوب الضوئي وكذلك عينات من الكبد لفحصها بالميكروسكوب الإلكتروني وأيضاً أخذ عينات إضافية من الكبد وعضلات لقياس مستويات الألفاتوكسين بها.
و بالفحص الظاهري لمجموعة الأسماك المغذية على مادة ملوثة بالألفاتوكسين وجد تضخم بالكبد مع شحوب في لونه و سهولة تفتيتها، أما الكبد سميكة وذو ترقب وعامة في اللون والخياشيم، وكذلك احتقان بالطحال والخياشيم مع وجود افتراقات مخططة في التجويف الخيشومي.
و بالفحص الهستوتيولوجي كان هناك تغيرات انحلالية وتأثرات تشوهية ونخرية في معظم أعضاء الأسماك المغذية بالعليب الملوث بالألفاتوكسين وقد أصبحت هذه التغييرات دوريًا في الألفاتوكسين داخل الكبد وعظام الأسماك.
أقل شدة في الأسماك المشروعة على العلية الملوثة بالأفلاتوكسين المضاف إليها طمي المتموريلونيت وأكثر خطورة في الأسماك المغذاة بالعلكة الملوثة بالأفلاتوكسين المضاف إليها طمي البنيتونيت.

وقد تم فحص الكبد بالميكروسكوب الإلكتروني وسجلت نتائجه بالتفصيل. وقياس متبقيات الأفلاتوكسين في العضلات و الكبد فقد وجدت وجود هذه المتبقيات في عضلات كبد مجموعة الأسماك المغذاة على العلكة الملوثة بالأفلاتوكسين فقط ولا توجد في المجموعات الأخرى.

وقد خلصت الدراسة إلى أن طمي المتموريلونيت يستطيع أن يلطف من تأثير الأفلاتوكسين على أنسجة الأسماك وكذلك يمنع وجود متبقيات الأفلاتوكسين في الأنسجة فتصبح أكثر أمنًا للاستهلاك الأبدي.

المحموم:

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