



## Toxicity effects of aflatoxin B1 on growth indices and histopathological alteration in *Cyprinus carpio*

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**Abstract:** Aflatoxin B1 (AFB1) is a common contaminant of foods, the safety characteristics of feed used in fish aquaculture systems are an essential tool to assure the productivity of those animal exploitations. The aim of this study was conducted to evaluate the fungi contamination in 53 samples, which were randomly collected from private fish farms in Iraq. In addition, investigate the effects of aflatoxin B1 contamination in feeds on growth indices, total protein, serum albumin in blood, liver histopathology, as well as the AFB1 residues in the fish muscle, was also examined. To evaluate adverse effects of AFB1 toxicity on health status of the Common carp (*Cyprinus carpio*). Fish were randomly distributed into 15 polystyrene tank, within five experimental groups; (1) control fed with normal diet without solvent and AFB1, (2) positive control received feed with only solvent, and (3-5) fed on diets containing 0.5, 1 and 2 mg /kg of AFB1, respectively for 12 weeks. Growth indices, total weight gain (TWG) and average daily gain (ADG) were assessed; blood samples were collected to analyze serum total protein (TP) and serum albumin (SA). The toxin residues in the musculature and histopathological alteration in liver were also investigated. Molds were found in 31 samples (58.5%). *Aspergillus flavus* was the most frequent, found in all positive samples. Results indicated that AFB1 has a negative effect on *C. carpio* weight gain, average daily gain and decreased in serum TP, SA, as well as liver histopathology of the infected fish, indicated cloudy swelling of hepatocytes, cellular hypertrophy, the formation of vacuoles in the cytoplasm, and necrosis of liver parenchyma. Further, the AFB1 residues were detected in the musculature with high level only in fish fed 2 mg AFB1/kg for 12 weeks. Overall, the results indicate that feeding of common carp with diets contaminated with AFB1, even in low concentrations ( $\leq 2$  mg/ kg feed) can cause decreased in growth indices, histopathological damages and disturb their physiological balance.

**Key words:** Aflatoxin B1, Aflatoxicosis, *Cyprinus carpio*, feed poisoning, Tissue damage.

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### Introduction:

Common carp *Cyprinus carpio* is one of the major farmed freshwater species in the world, it exhibits a strong tolerance to environmental stress and has become a widely used model species (1). Aflatoxin - food contamination is a global problem especially in the world where warm temperatures and humidity favor the growth of the fungi, they are well

known carcinogens especially aflatoxin B1 (AFB1) to humans and animals (2). AFB1 is produced by some fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus*, under ideal temperatures and humidity, the International Agency for Research on Cancer (IARC) classified AFB1 as a Group I carcinogen (3). The increasing amount of herbal compounds in fish feed has enhanced chances of feed contamination with toxins, fungi metabolites and

mycotoxins (2). The major difficulty confronting the aquaculture industry in fish feeding is the occurrence of AFB1 due to its hepatotoxic and carcinogenic properties (4). Santacroce *et al.* (5), mentioned the target organ for aflatoxicosis is the liver. Aflatoxin metabolites negatively react with various cell proteins, inducing necrosis and tumor or cell death (4). The toxicology of AFB1 in aflatoxicosis, involves its biotransformation to the highly reactive AFB1-epoxide by the CYP 450 (6), leading to carcinogenesis and mutagenesis by forming adducts with DNA (7). AFB1 has been shown to pose a risk to aquatic organisms such as its effect on growth, feed conversion, apparent digestibility factors, physiological disorders and histological changes, especially on hepatocellular hepatic tissue and muscle (8). The accumulation of AFB1 in the fish muscles is increased with the increase of AFB1 concentration and duration of exposure in the feed. This is due to the inability of the fish to develop an effective mechanism for AFB1 metabolism with the development of time and thereby increase the retention of AFB1 (2). The histological changes caused by AFB1 on the liver include extensive vacuolation corresponding to relatively higher lipid contents, signs of degeneration and hepatocellular lipid deposition (6). So, this is the first study in Iraq that links the problem contamination of the feed farms of *Cyprinus carpio* fish with fungal that produced AFB1 as a result of poor storage or contamination of plant-derived materials that enter the feed industry, which can transmit the toxic effects of AFB1 to the consumer such

as human and lead to serious health problems, the most important is the Human Hepatocellular Carcinoma (HHC). Therefore, the current study investigated to detect the affected the growth indices and liver histological changes and the AFB1 residues in the fish muscle due to exposure to AFB1.

## **Materials and methods:**

### **Estimation of fungal load:**

Fifty-three aliquots of fish feed were randomly collected from farmed common carp. Fungi were isolated from feed using the dilution method according to the Food and Agricultural Organization (FAO) standard methods (9). Macroscopic examination of the colony to show hypha and spore of filamentous fungi.

### **AFB1 production and determination:**

The fungus *Aspergillus flavus* (accession no. bankIt1983439 KY 468968) was obtained from Mycotoxin Laboratory / College of Agriculture/ University of Baghdad and propagated on PDA. To produce AFB1 growing *on* sterile media which are Yeast Extract sucrose Broth medium with Peptone (YEB +P) under static conditions. The medium was prepared according to De Leon (8) with some modification as in Table (1). These substances were dissolved in 1 liters of distilled water with pH 5.6 and then 100 ml of the prepared medium was placed in Erlenmeyer flasks with a capacity of 500 ml, autoclaved and inoculated by 10 ml of spore suspension ( $10^6$  spores/ml) and incubated at  $25 \pm 2^\circ\text{C}$  for 21 days.

**Table (1): Components of semi Synthetic medium**

Ingredients	Grams / Liter
yeast extract	22
Sucrose	200
Peptone	10
Final pH	5.6± 0.2
Distill water	1 L

After the incubation period, the AFB1 was extracted from culture according to method in previous study (10). Using High-performance liquid chromatography (HPLC) for quantitative estimation of AFB1. Aflatoxin B1 is then used as a stock solution to prepare experimental concentrations.

#### **Control Diet:**

All the ingredients of commercial food in Table (2) were powdered, sieved, blend and extruded through a kitchen noodle maker with a 3 mm, dried at 45 °C overnight and stored in freezer, the percentage of protein in fish feed about 26 %.

**Table (2): The ingredients of control fish feed**

Ingredients	% of diet
Fish Meal	15
Soybean	30
Yellow corn	18
Local barley	15
Wheat bran	15
Sun flower oil	5
Vitamins	1
Mineral mix	1

#### **Experimental diet:**

The experimental diet had similar composition as that of the control diet to which varying quantities of the toxin added from the AFB1 stock solution. Three experimental diets with (0.5 ,1and 2) mg of AFB1 /kg of basal diet were prepared by adding the required quantities from the AFB1 stock solution into the oil portion of the diet before blending and the methanol and acetone was allowed to evaporate. The ingredients were mixed with water, extruded and then dried (11).

#### **Experiment design:**

A total of 300 common carp with average 45±5 g body weight brought

from Mahaweel Fish Farm in Babil governorate which were transferred to Animal and Fish Resource Research Center. Fish were acclimated for two weeks in polystyrene tank with de-chlorinated tap water, at a pH of 7.4 ± 0.2, temperature 24 ± 2 °C, photoperiod: 16 h light: 8 h dark and with continuous aeration enough for keeping the dissolved oxygen always higher than 6 mg /l, the water was renewed (50% rate) every 24 h. After the acclimation period, one hundred fifty healthy common carp were randomly divided into the five experimental groups with three replicates for each group and added AFB1 from stock solution. Fish were fed on diet with 26% total protein. Fish

fed twice daily at a feeding ratio of 2% of body weight during experiment periods (12). Initial and final weights were recorded for all the experimental fish. Fish were deprived of food, 24 h before sampling. After 12 weeks, fish were captured from each group, blood collected and then anesthetized by MS-222. Fish were sacrificed by decapitation and dissected to remove the liver and muscles.

### **Growth indices:**

Growth calculations were estimated 12 weeks of treated with AFB1. All calculations were performed for each fish individually. Growth indices were assessed in terms of total weight gain (TWG), average daily gain (ADG) according to the following (13):

$TWG = \text{final body weight (g)} - \text{initial body weight (g)}$

$ADG = \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{number of days in the feeding experimental period}}$

### **Serum biochemical parameters:**

Using the Automated Biochemistry analyzer (Kenza, Biolabo, France) for the estimation of the enzymes activities, this includes Total Protein (TP) and Serum Albumin (SA) in the blood using Micro Plate Kite (Cohesion Biosciences Limited, United Kingdom).

### **Histopathological Examination:**

Portion of liver samples were used for a histopathological examination according to the method of Suvarna *et al.* (14) using paraffin sections technique, the fragments were fixed in

10% formaldehyde solution, embedded in paraffin, segmented using microtome, and then stained with haematoxylin / eosin. Morphological examination of the samples was studied using light microscopy and Photographed using ICC50 HD camera (12).

### **AFB1 residue in muscles:**

Measurement the AFB1 accumulation in fish muscle after 12 weeks of experiment by HPLC method according to Bbosa *et al.* (2).

### **Statistical Analysis:**

The Statistical Analysis System-SAS (2012) program was used to effect of difference factors in study parameters. Multiple range test (ANOVA) was used to significant compare between means in this study.

### **Results and Discussion:**

#### **Estimation of fungal load:**

Thirty-one samples of feedstuffs for farmed common carp revealed to be contaminated with molds. The total number of fungi varied from 1 to 3.3  $\log_{10}$  CFU·g<sup>-1</sup> (colony forming units per gram). The most common fungi isolated were *Aspergillus*, *Penicillium*, *Cladosporium* and *Fusarium*. *Aspergillus flavus* was the most frequently found 31 (58.5%), presenting a mean value of 2.7  $\log_{10}$  CFU·g<sup>-1</sup>, ranging between 2.0 and 3.2  $\log_{10}$  CFU·g<sup>-1</sup> (Table 3). The presence of *A. flavus* in some samples has been pointed to as a potential risk factor to Aflatoxins produced in the feed during storage (2).

**Table (3): Number and average of mold genera account in feed for farmed common carp**

Mold	No. of + ve samples	+ ve %	contamination ( $\log_{10}\text{CFU}\cdot\text{g}^{-1}$ )*	Range ( $\log_{10}\text{CFU}\cdot\text{g}^{-1}$ )*
<i>Aspergillus flavus</i>	31	58.5	2.7	2 - 3.1
<i>Aspergillus niger</i>	28	52.8	2.2	1 - 2.6
<i>Aspergillus glaucus</i>	24	45.3	1.9	1 - 2.3
<i>Penicillium spp.</i>	22	41.5	2.0	2 - 2.8
<i>Cladosporium spp.</i>	22	41.5	1.9	2 - 3.3
<i>Fusarium spp</i>	19	35.8	1.8	1 - 2.3

Note: (\*) CFU-Colony Forming Units per gram.

*A. niger* was found in 28 samples (52.8%) at a mean value of  $2.2 \log_{10} \text{CFU}\cdot\text{g}^{-1}$ , ranging from 1 to  $2.6 \log_{10} \text{CFU}\cdot\text{g}^{-1}$ . *A. glaucus* was found in 24 (45.3%), showing colonies levels ranging from 1 to  $2.3 \log_{10} \text{CFU}\cdot\text{g}^{-1}$ . *Penicillium spp.* and *Cladosporium spp.* were both found in 22 (41.5%) in levels that ranged from 2.0 to  $2.8 \log_{10} \text{CFU}\cdot\text{g}^{-1}$  and 2.0 to  $3.3 \log_{10} \text{CFU}\cdot\text{g}^{-1}$ , respectively. *Fusarium spp.* Were found in 19 (35.8%), showing levels of contamination that varied from 1 to  $2.3 \log_{10} \text{CFU}\cdot\text{g}^{-1}$  (Table 3). The contamination of feedstuffs by yeast and mold varies according to the geographical area, moisture, temperature, and hygienic conditions (15). Almost all fungi identified in this study can be categorized as normal mycoflora. However, this does not mean that they cannot cause disease, they can rather be considered opportunistic fungi, as many of them have virulence factors, which enable them to cause diseases, particularly under favorable predisposing conditions. Therefore, there is a mandatory need to develop new strategies against aflatoxicogenic strains of *A. flavus* in fish (2). In the present study identified and enumerated spoilage fungi in more than half of the samples of feed for farmed common carp. Jakić-Dimić *et al.* (16) tested 43 samples of feedstuffs and found high levels of mold contamination (*Aspergillus*, *Penicillium* and

*Fusarium*). Results of surveys published in 2007, in crops and feeds for livestock, showed high numbers of positive samples, with levels of contamination that ranged from  $1.7 \log_{10}$  to  $4.7 \log_{10} \text{CFU}\cdot\text{g}^{-1}$  (17), this is consistent with the results of the present study. Another study characterized the mycobiota of 130 samples of poultry feed, those samples were contaminated with levels that ranged from 3 to  $4.9 \log_{10} \text{CFU}\cdot\text{g}^{-1}$  for *Aspergillus spp* and 3.0 to  $5.3 \log_{10} \text{CFU}\cdot\text{g}^{-1}$  for *Penicillium spp* (18). These results were similar to other study screening of feed for livestock showed lower fungi contamination (17).

### Producing AFB1:

The isolated *A. flavus* was found to produce AFB1 at a concentration of 55.6 ( $\mu\text{g}/\text{ml}$ ) at pH 5.6 and  $25 \pm 2 \text{ C}^\circ$ . The media developed a yellowish orange hue probably due to the production of secondary metabolite after 21 days of incubation. According to FDA, the maximum safe level of AFB1 in human and animals feeds is 0.5- 15 ppb (FDA Regulatory Guidance). The pathological states arising from the consumption of feeds contaminated with aflatoxins (AFs) are termed Aflatoxicosis. According to International Agency for Research on Cancer (IARC), AFB1 confirmed as a potential carcinogen for human and is classified as Group 1(19).

### Growth indices:

The results of the growth indices shown in Table (4). During the experiment, no mortality was observed in AFB1-exposed groups. The negative control group appeared healthy, this group showed a significant ( $P < 0.01$ ) increase in growth indices as indicated by the total weight gain (TWG) and Average daily growth (ADG)  $0.96 \pm 10.72$  g and  $0.61 \pm 0.07$  g/d, respectively. In the second group, fish were fed contaminated diet with solvent without AFB1 recorded  $29.56 \pm 2.04$  g and  $0.35 \pm 0.03$  g/d for TWG and ADG respectively after 12 weeks. In the

third group, fish were fed the contaminated diet with AFB1 0.5 mg, the fish recorded the significant decrease in growth indices relative to group 1. For example, TWG and ADG recorded  $9.30 \pm 0.17$  g and  $0.11 \pm 0.02$  g/d after the 12 weeks of toxin exposure, respectively. In the fish fed 1 mg AFB1, recorded  $10.56 \pm 0.20$  g and  $0.12 \pm 0.02$  g/d for TWG and ADG, respectively after the same exposure periods. Either group fed on the higher AFB1 concentration (2mg) which suffered from the significant decrease in TWG and ADG  $2.73 \pm 0.72$  g and  $0.03 \pm 0.005$  g/d, respectively.

**Table (4): Growth indices for *C. carpio* fed on contaminated diets with different concentrations of AFB1 in 12 weeks (Mean  $\pm$  SE).**

Groups	Initial weight (g)	Final weight (g)	Total weight gain (g)	Average daily gain (g/day)
C-	$50.00 \pm 0.58$ a	$100.96 \pm 10.42$ a	$50.96 \pm 10.72$ a	$0.61 \pm 0.07$ a
C+	$46.17 \pm 1.58$ a	$75.73 \pm 0.62$ b	$29.56 \pm 2.04$ b	$0.35 \pm 0.03$ b
T1	$44.80 \pm 0.46$ a	$54.10 \pm 0.63$ c	$9.30 \pm 0.17$ c	$0.11 \pm 0.02$ c
T2	$45.00 \pm 0.57$ a	$55.56 \pm 0.20$ c	$10.56 \pm 0.20$ c	$0.12 \pm 0.02$ c
T3	$48.50 \pm 1.20$ a	$51.23 \pm 0.82$ c	$2.73 \pm 0.72$ d	$0.03 \pm 0.005$ d
Level of significances	NS	**	**	**

Mean with the different superscripts with each column differed significantly ( $P < 0.01$ ) \*\*.

AFB1 causes bleeding in tissues due to effects on the endothelial cells of the circulatory system, endothelial cells are apparently sensitive to AFB1 and most pathologic damages were observed in these cells (20). Major pathologic intestinal changes in fish fed diets contaminated with different levels of AFB1 were atrophy and necrosis of mucous cells, exfoliation of the mucous layer epithelium, as well as bleeding and rupture of intestinal capillaries. Damage to the intestine can negatively affect absorption of nutrients and lead to malnutrition, reduced growth rate and reduced physiological regeneration in AFB1-treated fish (17). Pathological

changes, reduced digestion and absorption of food in the intestine, decreased enzyme activity and malnutrition are all aflatoxins' effects on the digestive system in the fish (21). In addition to, the effect of mycotoxins especially AFB1 on carbohydrate metabolism is due to reduced hepatic glycogen and increased blood glucose levels. It interferes with the cellular metabolism of glucose (17). They both inhibit glycogen synthesis by decreasing glycogen syntheses and transglycosylase enzyme activities that catalyze elongation and rearrangement of the glycogen molecules (18). It also reduces hepatic glycogen by

accelerating glucose-6-phosphate oxidation which affects the metabolism of carbohydrates and therefore on growth indicators (16). Differences may be attributed to fish species, experimental conditions or duration of AFB1 exposure. Similar results were demonstrated for the effect of AFB1 in Beluga (*Huso huso*) (21) and broiler chickens (22). Due to human consumption of contaminated food, dietary contamination of AFB1 poses a big risk to human health in different regions of the World Particularly Asian and African countries (17).

### Biochemical parameters:

As shown in Table (5), Total Protein (TP) and Serum Albumin (SA) in the serum of *C. carpio* fed with AFB1-contaminated dietary were significantly ( $P < 0.01$ ) decreased compared with control group. The rates of TP in control groups (negative and positive) was  $37.5 \pm 0.45$  (mg/ml) and  $34.9 \pm 0.61$  (mg/ml) respectively. The SA value in control groups was  $18.5 \pm 0.27$  (mg/ml) and  $12.61 \pm 0.19$  (mg/ml), respectively. TP and AS showed the drastic decrease in the T3 groups, that were fed with the diet containing high-level of AFB1.

**Table (5): Total protein and serum albumin in common carp fed on contaminated diets with concentrations of AFB1 during 12 weeks (Mean  $\pm$  SE)**

Groups	Total protein (mg/ml)	Albumin (mg/ml)
C-	$37.51 \pm 0.45$ a	$18.53 \pm 0.27$ a
C+	$34.90 \pm 0.61$ ab	$12.61 \pm 0.19$ b
T1	$30.64 \pm 0.25$ b	$8.60 \pm 0.24$ c
T2	$24.70 \pm 0.28$ c	$5.82 \pm 0.31$ d
T3	$18.91 \pm 0.36$ d	$3.10 \pm 0.16$ d
Level of significance	**	**

Mean with the different superscripts with each column differed significantly ( $P < 0.01$ ) \*\*.

TP and SA concentrations were significantly lesser ( $P < 0.01$ ) in *C. carpio* fed on AFB1 containing rations as compared with the control group. Similar results were recorded by Sahoo and Mukherjee (23) who noticed reductions of TP and SA levels in Indian major carp (*Labeo rohita*) fish exposed to AFB1. The decrease in plasma total proteins and albumin in *Oreochromis niloticus* fed AFB1 containing ration (24). Blood proteins are used for energy production during toxicity and the protein catabolism is induced by stress (22). In addition, aflatoxin hepatotoxicity leads to

alterations in protein synthesis and cellular integrity of the liver (6). AFB1 also proved to be immunosuppressive in fish (25). The TP and SA are involved mainly in nutrition, acid-base balance, water distribution as well as immunity and metabolic needs (26). The decrease in TP was correlated with damage severity of hepatopancreas organ. On the other hand, AFB1 disrupts endoplasmic reticulum and reduces the RNA biosynthesis, attachment of polyribosomes to the endoplasmic reticulum, and disruption in ribosomes functions also (25), thus severely affecting protein biosynthesis.

Significant reductions in total proteins by AFB1 were recorded in sea bass (*Dicentrarchus labrax*), *O. niloticus* and *Labeo rohita* (27). The reduction of TP leads to a reduction in growth in affected fish and indicative of impaired nutrient absorption, impotence, and weight loss. Also, the reduction of protein content in body tissues like in skeletal muscle, heart, liver, and kidney have been associated with the increased liver and kidney necrosis due to the damage caused by the accumulated AFB and its metabolites in the body following the aflatoxin exposure (28). SA helps in maintenance of osmotic balance and transporting many exogenous chemicals endogenous metabolites and some immune parameters against infections (25). In our study, AFB1 caused significant reductions ( $P<0.01$ ) in total serum protein and albumin. These may be due to metabolic products of AFB1 that binds with cellular macromolecules and inhibits protein synthesis in the liver (2).

#### AFB1 Residue in fish tissues:

Following 12 weeks of feeding period, the residues of AFB1 were measured in the control groups and affected fish (C-, C+, T1, T2, and T3) as shown in Table (6). The residues in muscle were significantly higher

( $P<0.01$ ) in T3 group ( $9.3\pm 3.4\text{mg/kg}$ ), being considered as high level according to the Zohri *et al.* (29). Presence of AFB1 residues in fish meat is the very dangerous problem for food safety (23). The accumulation of AFB1 is increased significantly with increased AFB1 concentration, fish species and exposure time (European Commission, EC (30). The higher AFB1 residual levels were measured in affected fish muscles currently. Similar to these findings were recorded in Nile tilapia and sea bass (27). Another showed that after feeding tilapia with different levels of AFB1, no residue revealed in fish meat (25). Other studies showed lesser muscle AFB1 residual level (31). On the other hand, AFB1 residues (5 ppb) in fish muscles tilapia were detected after 12 weeks in high toxin dietary concentration (100 ppb), although its level (5 ppb) was below the AFB1 safe/permissible level for human consumption (20). Muscle AFB1 residual level was also reported in previous studies (32). In spite of some studies, the mechanisms of toxic substance elimination from the fish body are not obviously proved (33). The maximum levels for AFB1 in various foods are in the range of 0.5 to 15  $\mu\text{g/kg}$ . To prevented and reduce the risk of AFB1 in food and feed (25).

**Table (6): The accumulation of AFB1 in muscle of *C. carpio* after exposure to different concentration of AFB1 in 12 weeks (Mean  $\pm$  SE)**

Groups	AFB1 (mg/kg)
C-	-
C+	-
T1	$1.6 \pm 1.7$ c
T2	$5.3 \pm 2.9$ b
T3	$9.3 \pm 3.4$ a
Level of significance	**

Mean with the different superscripts with each column differed significantly ( $P<0.01$ ) \*.

In this respect, Magouz *et al.* (34) detected the residue of AFB1 in the

group fed on AFB1 contaminated diet (150 ppb). In addition, Abdelhamid *et*



*al.* (35) reported that AFB1 residues in the *O. niloticus* fish showed accumulative effect related to the levels of dietary AFB1 and feeding period. In addition, the significant increase of AFB1 residues was observed in *O. niloticus* fish after 6 months (35). Other studies recorded residues of AFB1 in the whole body of *O. niloticus* at the end of the experiment (35).

Many studies revealed that animals have AFB1 residues in their tissues after exposure to AFB1 that causes poor health effects after ingestion (34). El-Sayed and Khalil (28) concluded that delayed exposure of sea bass with small amounts of AFB1 (0.0018 mg/kg) resulted in poor health performance and the accumulation of the AFB1 residues in the edible tissues of the fish which ultimately have the threat for the consumers. In agreement with these results, Han *et al.* (36) reported that when gibel carp exposed to more than 0.01 mg/kg of AFB1 in the diet, European Union detected the accumulation of AFB1 residues in the muscles and ovaries above the safety levels of 0.002 mg/kg as set. In Nile tilapia, AFB1 residues in the liver regions were noted exposed to as low as 2 mg/kg (25).

In another study, high concentrations of AFB1 residue (0.005 mg/kg) were detected in sea bass muscles. So, sea bass was a highly susceptible species to AFB1 and it was found that consumption of contaminated fish leads to negative health effects in humans (24). Other study observed the absorption and excretion of AFB1 by rainbow trout (*Oncorhynchus mykiss*) on exposure of 21 days (37). AFB1 accumulation in ovaries and muscles for fish which significantly increased with the concentration of AFB1 in the diets and the effect on the growth and liver

function of gibel carp fish, and cause damage to the fertility of fish (36). So it is concluded that the toxicity caused by AFB1 have detrimental effects on humans and animal's health (35).

Therefore, can link the exposure AFB1 caused to exert a bio-accumulative effect throughout the food chain down to the humans (37). To protect consumer's safety, rules and safety limits for several mycotoxins in certain foodstuffs are contained in certain laws or regulations by governmental agencies of different countries.

#### **Histopathological examination to liver tissue:**

Histopathological alternations observed in the liver of the exposed fish to different concentrations of AFB1 were illustrated in following Figures (1 to 5).

#### **Liver of C- group:**

The microscopical examination of liver tissue exhibited normal structural finding hexagonal hepatocytes round nuclei and uniform cytoplasm (Figure 1).

#### **Liver of C+ group:**

Figure (2) showed extensive vacuolation noticed in liver parenchymal, hydropic swelling in nature accompanied with central nuclei (2A) together with sinusoidal dilation and congest (2B). Also central lobular MNCs aggregation with mild vacuolation of hepatic parenchymal (2C). The central vein showed mild vasodilation with moderate mononuclear cells (MNCs) as shown in Figure (2D).

### Liver of T1 group:

The principal hepatic manifestation characterized by pronounced fatty changes of hepatocyte with moderate MNCS infiltration (3A) mainly seen around central vein (3B), together with scatter appearance of MM associated with ductal dilation reported in other section (3C).

### Liver of T2 group:

The majority of liver section showed vasodilation with MM hyperplasia (4A), as well as moderate cellular swelling with acidophilic cytoplasm resulting in sinusoidal narrowing accompanied with multifocal MNCS mainly in the portal region (4B).

Similar to previous T1, evidence of ductal dilation with eosinophilic secretion seen in (4C). Other section showed mild vacuolation of hepatic parenchyma with central vein and sinusoidal congestion (4D).

### Liver of T3 group:

The relative increase of bleeding and liver discoloration was the main apparent changes in T1 group. Showed severe vacuolation and lesions in the form of central veins surrounded by inflammatory cells (5A), focal areas of necrosis between the hepatocytes (5B), severe hemolysis between clearly necrotic hepatocytes and some of the hepatocytes (5C), even showed pyknosis (5D).

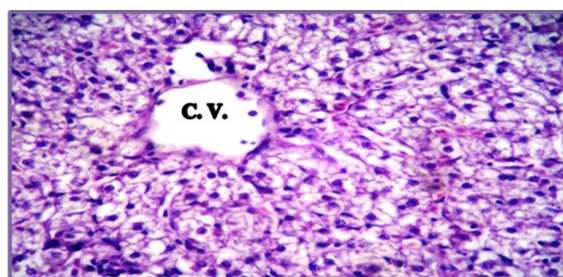


Figure (1): Liver histopathological section of C- group H and E stained showing normal structural limit (Central Vein (C.V.), hepatocytes, sinusoids (H &E stain  $\times$  40).

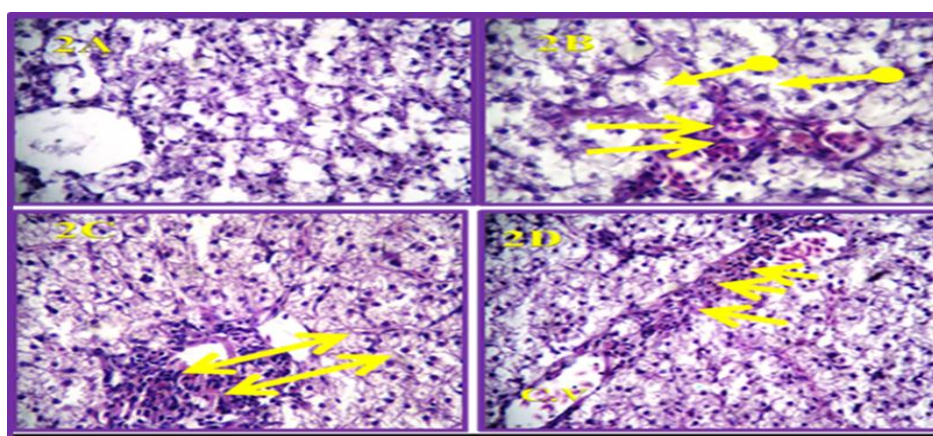


Figure (2): Liver histopathological section of C+ group H and E stained showing hydropic swelling in nature accompanied with central nuclei (2A), sinusoidal dilation and congestion (2B), central lobular MNCs aggregation with mild vacuolation of hepatic parenchymal (2C), central vein showed mild vasodilation with moderate mononuclear cells (2D).



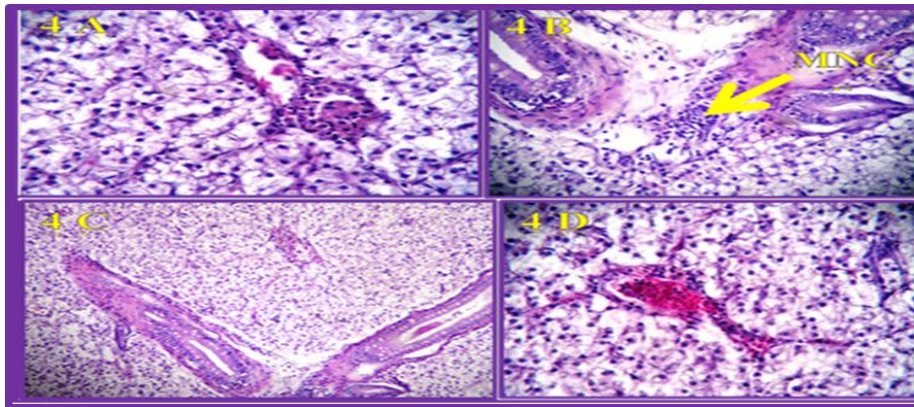
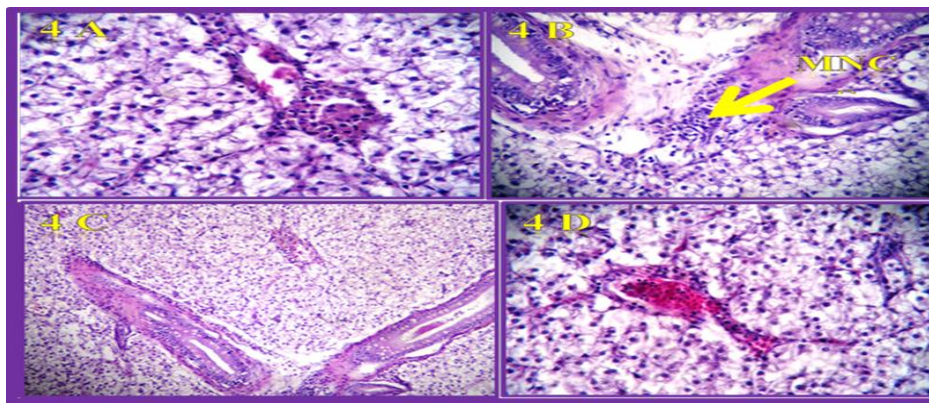


Figure (3): Liver histopathological section of T1 group H and E stained liver specimen showing pronounced fatty changes of hepatocyte with moderate MNCS infiltration (3A), ) mainly seen around central vein (3B), together with scatter appearance of MM associated with ductal dilation reported in other section (3C).



Figure(4): Liver histopathological section of T2 group H and E stained showing mild C.V dilation with MM hyperplasia (4A), multifocal MNCS infiltration mainly in the portal region (4B), ductal dilation with eosinophilic secretion (4C), mild vacuolation of hepatic parenchyma with C.V. and sinusoidal congestion (4D).

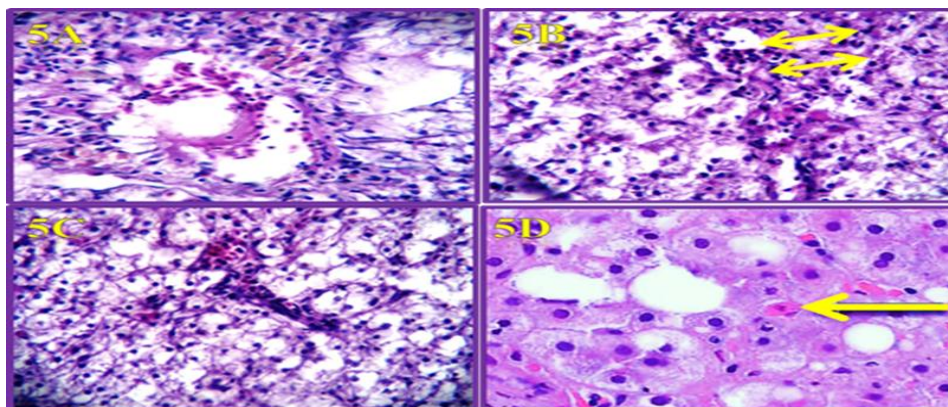


Figure (5):Liver histopathological section of T3 group H and E stained showing evidence of endothelial damage of C.V lining with large vacuoles formation of adjacent parenchymal accompanied with MNCs infiltration (5A), intense hydropic swelling with liver parenchymal necrosis  $\longleftrightarrow$  were recognized in major liver section resulting sever disruption of liver architecture (5B), some of canaliculi become more slender accompanied with slight sinusoidal congestion (5C), some of the hepatocytes showing pyknosis  $\leftarrow$  (5D).

Study the alterations and histopathological changes may provide direct evidence of the toxic effects of AFB1 on fish. Hence, in the present study found pathological alterations in liver of *C. carpio* treated with AFB1 (fat changes in hepatocytes, cloudy swelling, formation of cytoplasmic vacuoles, necrosis of liver cells, tissue fibrosis of portal veins, excessive proliferation of bile duct cells and necrosis of pancreatic cells).

Aflatoxicosis can have an economically significant effect on the production of farmed fish. Liver most important target organ in fish that is susceptible to aflatoxicosis (6). Bleeding in tissues indicates AFB1 effects on the endothelial cells of the circulatory system. Endothelial cells are apparently sensitive to AFB1 and most pathologic damages were observed in these cells (18). AFB1 is hepatotoxic and hepatocarcinogenic in several animal species at a very low concentration (7). The microscopic finding indicated that AFB1 induced severe histopathological changes in the hepatic tissues for the liver of *C. carpio*, the mitochondrial swelling, endoplasmic reticulum dilation and a lot of lipid droplets were observed in primary hepatocytes, therefore, this combination was classified as an additive response of the AFB1(38).

Similar histopathological damages of AFB1 on fish which reported in liver of tilapia (35), rainbow trout (39), Penaeid shrimps (40), *Labeo rohita* (24), *Clarius lazara* (41), *channel catfish* (42), and *Acipenser ruthenus* (32), rohu (*Labeo rohita*) (24) and Red drum (7). In addition, livers of *O. niloticus* injected with 6 mg AFB1 /kg body weight showed severe lesions such as thrombosis in blood vessels, focal

areas of necrosis between the hepatocytes, which had prominent vacuolization with pycnosis (42).

This was in agreement with El-Barbary and Mehrim (43) where *O. niloticus* injected with AFB1 (9mg /kg body weight) showed severe hemolysis, congestion and thrombosis in the blood vessels along with an accumulation of melanomacrophages.

Vacuolar degeneration was also observed in the liver of tilapia injected with AFB1 (38).

AFB1-contaminated diet caused destruction of cell membranes and necrosis of tissues. Nucleus hypertrophy, hyper chromosome, widespread biliary hyperplasia, liver focal necrosis and cellular inflammation are reported in hybrid sturgeons treated with AFB1-contaminated feed (30). Other study showed atrophy of hepatopancreatic tubules was the first histopathological sign of aflatoxicosis in the farmed white shrimp (*Litopenaeus vannamei*), cellular inflammation, and infiltration of hemocytes, necrosis and infiltration of fibroblastic cells between the tubules of the hepatopancreas which depends on concentration of AFB1 and the duration of feeding (44). In addition, experimental studies on various species of rodents, birds and fish indicated that AFB1 are capable to induce oxidative damage in the cells and produce reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals and hydrogen peroxides in hepatocytes (45). Other study reported that the liver of tilapia fed with 638 and 793  $\mu\text{g}$  AFB1 / kg diet were infiltrated by many inflammatory cells (25). The pathological changes of liver investigation may be due to the primary site of metabolism of ingested AFB1 as well as the primary laceration of residues and lesions (2). In return, no

histopathological lesions were showed in the liver of gibel carp (*Carassius auratus gibelio*) treated with AFB1 (38), another study which indicated that gibel carp is a less susceptible species to AFB1 exposure up to approximately 1000 ppb, at least for 12 weeks.

In conclusion, this work provides the sensitivity of *C. carpio* exposure to doses 2 mg /kg feed of AFB1 in the long term. The impairment in fish health was evidenced by reduced growth indices, decreased total protein and serum albumin and AFB1 residual deposition in the muscles at the increased concentration of AFB1 due to the inability of the metabolism system to expulsion of AFB1 or its metabolic products. Also, the severity of tissue damage in fish treated with different levels of AFB1 rises with an increase in dosage. Tissue damage in fish treated with different levels of AFB1 can be used as a clinical device in diagnosing aflatoxicosis. The results of the current study show that the significant histopathological alterations in the hepatocytes tissue of fish exposed to different levels of AFB1.

Many methods should be found to reduce the level of molds contamination in food products or ingredients through physical, chemical or biological methods, consumption of molds contaminated foods might be inevitable, especially in regions with high growth of mycotoxin-producing fungi, and mechanism-based preventive or interceptive measure to reduce the *in vivo* toxicity of molds might be strategy worthy of further investigating.

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