IMPACT OF AFLATOXIN B1 ON GROWTH PERFORMANCE AND HISTOPATHOLOGICAL CHANGES IN *CYPRINUS CARPIO*

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ABSTRACT

This study aimed to identify the impact of aflatoxin B1 (AFB1)-contaminated diet on growth performance and histopathological changes of the kidney and muscle tissues in *Cyprinus carpio* . A total of 300 fish with an average of weight 45±5 g, were randomly distributed into 15 plastic tanks, and were divided into five experimental groups; (group 1) served as control , fish fed normal diet without solvent and AFB1, (group 2) positive control fish were fed with only solvent, and (groups 3-5) fish were fed diets containing 0.5, 1 and 2 mg AFB1/kg of feed, respectively. Results showed the negative effect for AFB1 on weight gain, average daily gain of fish. Histopathological changes of kidney and muscles in AFB1-treated fish showed devastation of the renal tract, glomerular atrophy with necrosis, expansion of Bowman's space, necrosis in urinary tubules, peeling and degradation in the epithelium of tubules, vascular congestion, and interstitial hemorrhage associated with mononuclear cells (MNCs) infiltration with tubules, increased of urinary lumen space, and increase of melano-macrophage centers (MMC). The results also showed a significant damage to the structure of muscles as a result of the disintegration of muscle fibers, which increased with the increase in the AB1 concentrations.

Key words: carp, growth,kidney, muscles, mycotoxin, life below water

تأثير الافلاتوكسين B1 على النمو والتغيرات المرضية النسجية في اسماك الكارب الشائع *Cyprinus carpio*

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المستخلص

هدفت الدراسة الحالية للتحري عن تأثير الاعلاف الملوثة بالافلاتوكسين B1 (AFB1) على انسجة الكلى والعضلات لاسماك الكارب الشائع *Cyprinus carpio*. استخدمت 300 سمكة بمعدل وزن 45± 5غم . وزعت الأسماك عشوائيا في 15 حوضاً بلاستيكياً، مع خمس مجموعات تجريبية (المعاملة 1) السيطرة السالبة المغذاة على النظام الغذائي العادي بدون مذيب و AFB1 , (المجموعة 2) السيطرة الموجبة المغذاة على النظام الغذائي مع المذيب فقط ، و المعاملات 3-5 تم تغذيتها على اعلاف التي تحتوي على AFB1 بتراكيز 1,5 و1 و1,5 ملغم/كغم على التوالي . أشارت النتائج إلى أن AFB1 له تأثير سلبي على الزيادة الوزنية للاسماك ، ومعدل الوزن اليومي. أظهر الفحص النسجي المرضي للكلى والعضلات في الأسماك المصابة تلف القنوات البولية وضمور وتنخر الكبيبات وتمدد في محفظة بومان مع تنخر النبيبات البولية ونزف وتحلل لبطانة النبيبات البولية فضلا عن احتفان الاوعية الدموية بالاضافة الى نزيف خلالي مع ارتشاح للخلايا وحيدة النواة (MNCs) داخل القنوات البولية. كذلك وجود خلايا بلعمية واسعة وكبيرمع زيادة بالهيموسايدرين (MMC). كما اظهرت النتائج وجود ضرر كبير في البنية التركيبية للعضلات نتيجة لتفكك الالياف العضلية والذي يتناسب طرديا مع تركيز AFB1 .

الكلمات المفتاحية: كارب ، نمو، كلية ، عضلات ، سموم فطرية، الحياة تحت الماء

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INTRODUCTION

Common carp *Cyprinus carpio* which are substantial species of freshwater cultured and is widely distributed all over the world and is able to tolerant various environmental conditions (9). Moldy feed toxicosis distinguished as a serious fish farms problem, especially in hot and humid areas, in addition to, use of plant protein sources in feed production such as corn, peanuts and most vegetable products that enter the feed industry that increase fungal growth such as *Aspergillus flavus*, which produced aflatoxin B1 which poisonous carcinogens to humans and animals (7, 12). AFB1 is classified from the World Health Organization (WHO) as a class A carcinogen and there is no safe dose even if it is in small quantities. AFB1 poses a risk to aquatic organisms where it affects growth, feed conversion, physiological disorders and histological changes of various organs (10). AFB1 accumulates in fish organs and muscles in a concentration-dependent manner and duration of exposure due to the inability of fish to develop an effective mechanism of aflatoxin B1 metabolism (3). Histopathology is the fastest, critical, and relatively credible tool to evaluate damages in various tissues of fish exposed to toxic compounds. Therefore, studying pathological changes may provide direct evidence of the toxic effects of AFB1 in fishes. Thereby, the -current study aimed to investigate the effect of different concentrations of AFB1 on growth performance and histopathalogical changes in kidney and muscles of *Cyprinus carpio*.

MATERIALS AND METHODS

***Aspergillus flavus* isolate:** This study was conducted in the Ministry of Science and Technology/ Agricultural Research Department/ Animal and Fish Resource Center. *Aspergillus flavus* (accession no. KY468968.1) was obtained from Mycotoxin Laboratory, College of Agriculture, University of Baghdad. The fungal culture was maintained on Potato Dextrose Agar (PDA).

Maintaining of fungal cultures and preparation of spore suspensions: A proximately 39g. of (PDA) was added to one liter of distilled water.The medium was autoclaved at 121 °C and 1.5 kg /cm2 for 15 minutes, after the sterilization period, cooled out to 45 °C, and then tetracycline (250mg/l) was added to prevent bacterial growth. The medium was poured in petri plates, and universal tubes, then was left to harden and stored in the refrigerator until use (14). The fungal isolate was grown on PDA medium at 25±2°C for 7 days; mature spores were harvested on 10 ml of sterile normal saline. The universal tubes inoculated with fungal spores and incubated slanted at 25±2°C for 17 day (12).Inoculation the Yeast Extract sucrose Broth medium and Peptone (YEB +P) with 5 ml of spore suspension (1 x10^6 spore/ ml), mix well and incubated at 25 ± 2 ° C for 21 days (Table1).

Table 1. Components of semi synthetic medium

Contents	G / l
Yeast extract	22
Sucrose	200
Peptone	10
pH	5.6± 0.2
Distilled water	1 L

After incubation period, the AFB1 were extracted from culture media (5), using, High-Performance Liquid Chromatography (HPLC) to measure the concentration of AFB1 (18). To prepare the experimental concentrations stock solution of extracted AFB1 were used. A total of 300 of common carp (45±5g.) were obtained from Al- Mahawil fish farm in Babylon province and were transferred to Animal and Fish Resource Research Center, fish were acclimated for two weeks. After the acclimation period, fish randomly divided into the five experimental groups with three replicates for each group. Group 1 fed the normal diet as a negative control group (C-), whilst second group fed diet containing extraction solution (methanol, acetone, and diluted water) served as a positive control (C+). Experimental diets which are containing 0.5, 1 and 2 mg AFB1/ kg diet from the stock solution of AFB1. Fish were fed twice daily with a feeding ratio of 2% of body mas during experiment periods (1, 14). Fish were forbid of food 24 h before assemblage. At the end of the experimental trial (12 weeks) fish were picks up from each group and anesthetized by MS222. Then, fish were dissected to remove the kidney and muscles for studying the histological alterations in these tissues. Histopathological examination were done according to the method of Suvarna *et al.* (16,

11) using paraffin sections technique, the tissues were fixed in 10% formaldehyde solution, embedded in paraffin, segmented using microtome, and then stained with hematoxylin and eosin. Morphological examination of the specimens was studied using light microscopy and Photographed using ICC50 HD camera (16). Statistical Analysis System- SAS program was used to identify the effect of AFB1 in study parameters. ANOVA and multiple rang test were used to study significant differences between means in this study.

RESULTS AND DISCUSSION

Results of the present showed that the growth parameters for common carp were affected by the different concentrations of AFB1 in diets (Tab. 2), No mortality was observed during the experimental study. Results showed highly negative correlation between AFB1 and final weight.

Table C 2. Growth performance (Mean ± SE) for *C. carpio* fed on diets with different concentrations of AFB1 for 12 weeks

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

Mean with the different superscripts with each column differed significantly (P≤0.01) **

In negative control group, fish appeared healthy, showed a significant (P < 0.01) increase in growth indices as indicated by the Total Weight Gain (TWG) and Average Daily Growth (ADG) which reached 50.96 g and 0.61g/d, respectively. While, AFB1-treated groups recorded a significant decreased in growth indices, such as the group that fed on the higher AFB1 concentration (2mg/Kg) revealed significant decreasing in TWG and ADG compared with the negative control group. The aflatoxins' effects on the digestive system in the fish appear as bleeding in tissues due to effect on the endothelial cells for blood vessels (8). Pathological changes, reduced digestion and absorption of food in the intestine, decreased enzymes activity and malnutrition (4). In addition, the effect of mycotoxins especially AFB1 on carbohydrate metabolism is due to reduced hepatic glycogen and increasing in blood glucose levels. It interferes with the cellular metabolism of glucose (1).

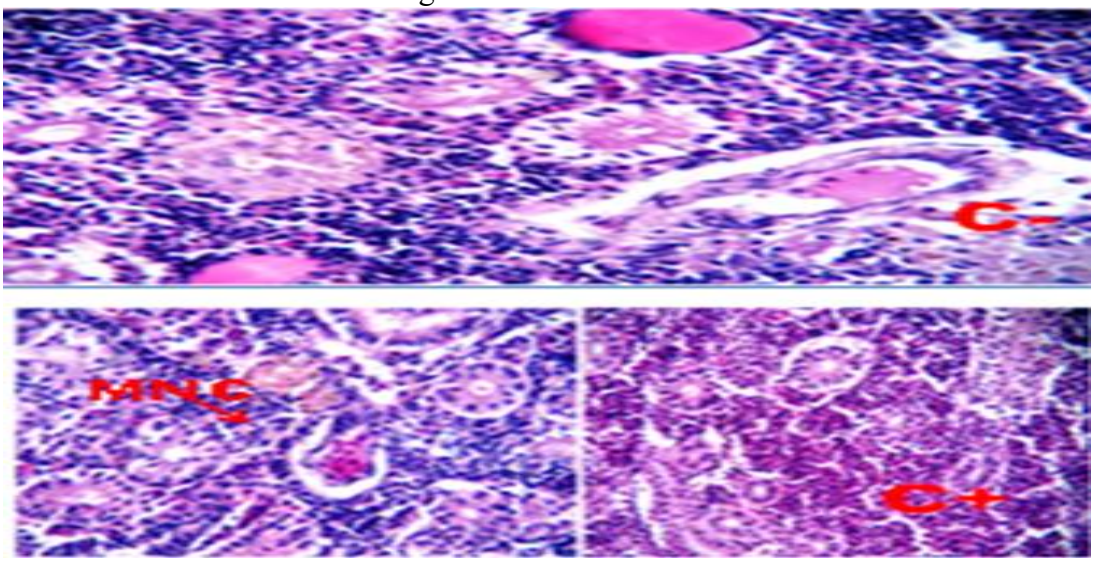

Figure 1. Histopathological changes of kidney which exposed to different concentrations of AFB1: (C-) negative control, (C+) positive control, (MNCs) Mononuclear cells. × 40, H&E

Histopathological changes for the kidney and muscles of the treated fish with different concentrations of AFB1 are shown Fig.1&2.The microscopical examination showed normal structure of kidney tissue in the negative control (Fig. 1: C-), infiltration of MNCs accompanied with mild congestion of glomerular tuft in the positive control (Fig. 1: C+). While the kidney section in AFB1-treated groups showed subversion of the renal tract, glomerular atrophy and necrosis, enlargement of Bowmans space, urinary tract necrosis, peeling and degeneration tubular epithelum (Fig.2:T2,T3). Vascular congestion, interstitial hemorrhage associated with MNCS infiltration with tubules (Fig.2:T1). Increased urinary lumen space, dilation of urinary space, and increase in melanomacrophage centers (MMC) (Fig.2:T1-T3). Melanin-microphages or melanomacrophages (MMs) are pigmented phagocytes found primarily in poikilotherm lymphoid tissues. MMs appear as a darkly pigment due to high lipofuscin, melanin, and hemosiderin content, so it can be histologically distinguishable by light microscopy (Fig.1).This may be due to MMCs represent a primitive site of adaptive immune system activation (12,15).=

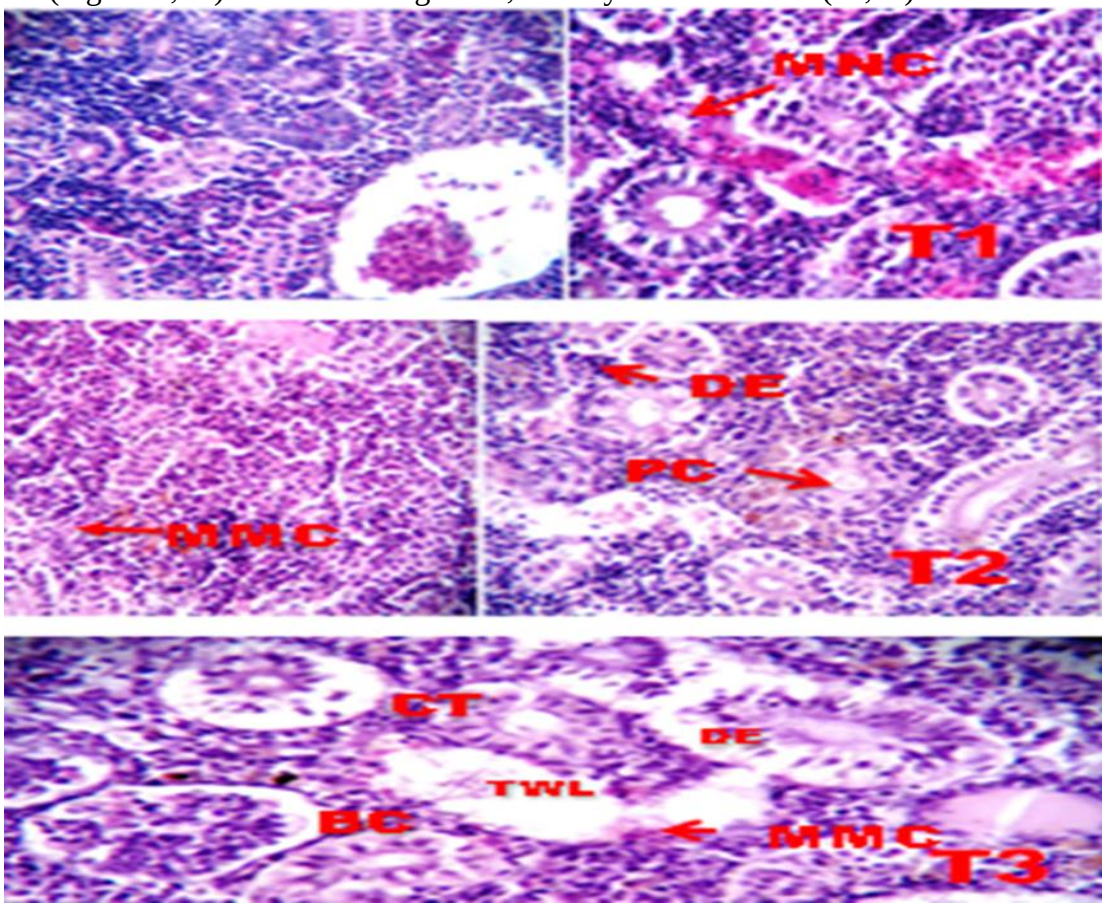

Figure 2. Histopathological changes of kidney which exposed to different concentrations of AFB1: (T1) 0.5 mg AFB1, (T2) 1 mg AFB1, and (T53) 2 mg AFB1, collecting tubule (CT), tubules with dilation lume n (TWL), Bowmans capsule (BC), degenerated epithelium (DE), proximal convoluted tubule (PC), Melanomacrophage centers (MMCs), × 40, H&E

One of the negative effects of AFB1 on fish increase kidney filtration and loss of large amounts from amino acids, proteins, glucose, electrolytes and water due to glomerular atrophy, necrosis, Bowman's capsule expansion, and increased urinary space (17). Decadent urinary tubules and necrosis of urinary tract epithelial cells were reported in AFB1-treated fish *Labeo rohita* (6). In another study, the result showed necrosis, blood clots and atrophy of glomeruli, in addition to melanosis coli were other alterations found in *Oreochromis niloticus* fed with AFB1-contaminated diet (14). In muscular tissue, histological changes in Fig. 3: C- and C+ showes normal texture, myotomes and myoseptum which clearly appeared. Individual myotomes separated by myoseptum. Histologically. In AFB1 treated groups muscle showed gradual damage in the muscle texture

dependent on increasing concentrations of the AFB1. Abnormality in muscle fibers, separations among muscular fibers were obviously observed (Fig. 4: T1, T2 and T3).

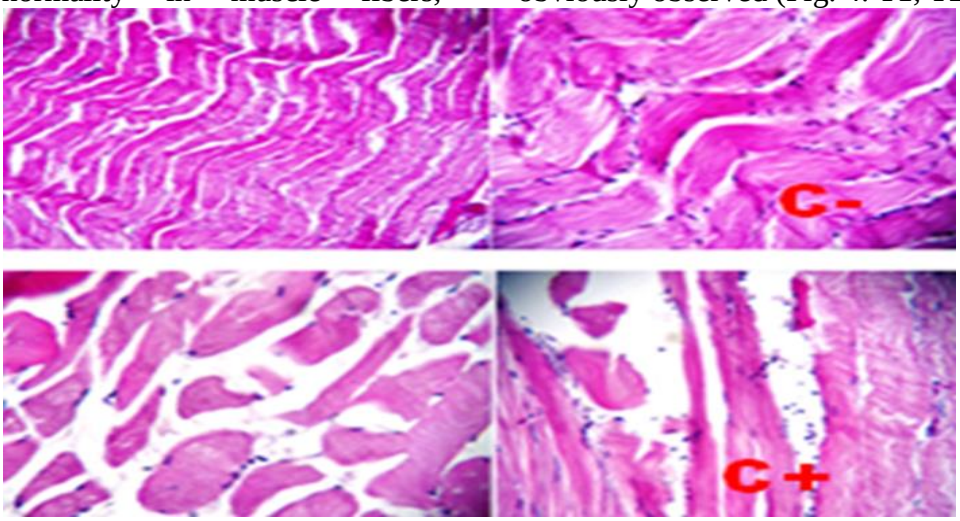

Figure 3. Histopathological changes of muscles which exposed to different concentrations of AFB1: (C-) the negative control and (C+) positive control, × 40, H&E

Ramesh and Nagarajan (11,13) has observed necrosis, mild lesion, inflammation and cellular degeneration in the muscle tissue for the *Clarias batrachus* exposed to different concentrations of AFB1, in addition, observed the atrophy, degeneration of muscle bundles with infiltration of inflammatory cells between them and focal areas of necrosis in fish exposed to different pollutants (2, 4,13). The degree and severity of histological alternations in the kidneys and muscles of common carp were associated with a decrease in final weight, due to the increase in the concentration of AFB1. The final weight of fish decreased in 1 and 2 mg of AFB1 as a result of damage to the muscle structure and this is due to the decrease in glycogen content in muscle fibers with an increase in AFB1 (12). From these results indicated that the texture of kidney and muscle were a sensitive tissues that affected by AFB1.

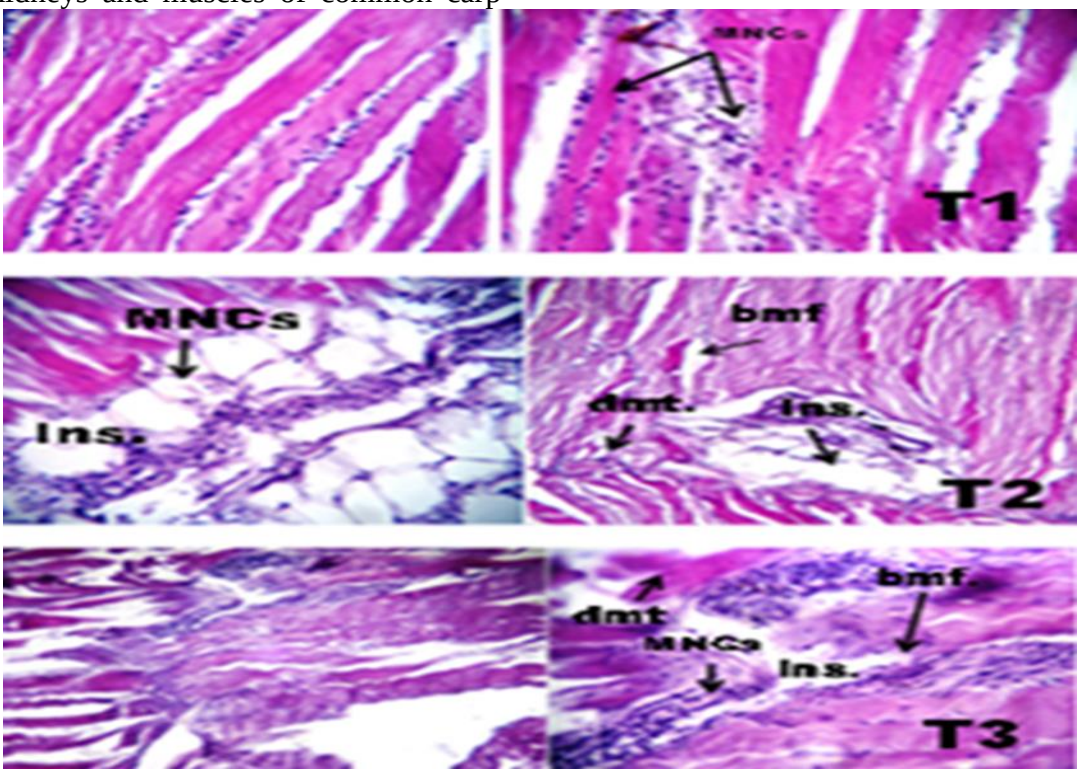

Figure 4. Histopathological changes of muscles which exposed to different concentrations of AFB1: (T1) 0.5 mg AFB1, (T2) 1 mg AFB1, and (T3) 2 mg AFB1, mononuclear cells (MNCs), broken myofibrils (bmf), disintegrated myotomes (dmt), lesion (lns). × 40, H&E

As far as our knowledge, this is the first study in Iraq to investigate the effects of AFB1 on kidney and muscles for *C. carpio*, and the possibility of transmission of this damaging through the food chain to the human. Usually, AFB1 are accumulation in the liver and in the internal guts of the fish, this affects the muscle growth of fish. Therefore, it is advised to get rid of the internal organs before eating. However, the accumulations of these toxins, even if they are in low concentrations in the muscles, have a harmful effect to the consumer.

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