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Detection of Aflatoxin B1 and Assessment of Pathological Effects in Fresh *Cyprinus carpio* Marketed for Human Consumption in Diyala Province, Iraq

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ABSTRACT

Aflatoxins are highly toxic, carcinogenic fungal secondary metabolite that contaminate food and can have harmful effects on both humans and animals if consumed. The current study was designed to detect the presence of Aflatoxins B1 (AFB1) in the muscles and liver of common carp (*Cyprinus carpio*) sold in Baqubah markets in Diyala Province, as well as in fish feed used by fish farmers, in order to assess the bioaccumulation of AFB1 in fish and the potential risk to human health associated with its consumption. Twenty fresh fish samples of *Cyprinus carpio* weighing 1- 2.75kg were collected randomly. High performance liquid chromatography (HPLC) was used to estimate the AFB1 concentration in the muscles, liver of collected fish and in fish feed. Histopathological sections were made to assess the toxic pathological effects of AFB1 on organs of *Cyprinus carpio*. The HPLC analysis showed that 35% of muscles samples were contaminated with AFB1 and 100% of liver and feed samples were contaminated with AFB. Histopathological findings of the liver revealed cloudy swelling of hepatocytes, slight edema with severe blood vessels congestion and inflammatory cells infiltration, in addition to the presence of slight necrosis and congestion in central vein. Histopathological findings of the spleen section showed congestion, hemosiderin precipitation with severe depletion in splenic parenchyma and infiltration of inflammatory cells. Intestinal section showed closing of villi with infiltration of inflammatory cell; moreover, there were congestion, increase in the number of goblet cells together with slight infiltration of mononuclear cells and complete damage of villi. In conclusion, AFB1 is present in *Cyprinus carpio* sold at Baqubah markets and in fish feed, which appears to be the primary source of contamination in fish. Thus, it is important to make sure that the materials used to feed fish are of a high quality.

INTRODUCTION

Aflatoxins are secondary metabolite that are highly toxic, carcinogenic and are produced mostly by fungi that are a member of *Aspergillus* genus **(Khlangwiset *et al.*,**

2011; Muayad *et al*., 2025). These fungus organisms pose a high threat to food and feed stock, especially in times of low soil humidity and drought pressure. High temperatures may promote these fungi, and also the high values of humidity **(Pitt and Hocking, 2009; Muayad *et al*., 2025)**. There are at least 13 kinds of aflatoxin, the most imporant of which are B1, B2, G1 and G2, with B1 being the most toxic and the most widespread **(Alinezhad *et al*., 2011)**. One such poison is known as aflatoxin **(AL-Ezzy & Abdulameer, 2021; Al-Shammari *et al*., 2025)**. Aflatoxins, which are frequently found in grains (such as maize and wheat), nuts, and seeds, represent a serious threat to food safety and public health **(Al-Khalidi *et al*., 2020; Dinkissa & Hailu, 2022)**. The aflatoxins have been known to recover potential harmful effects on the people as well as the livestock **(Kensler *et al*., 2011; Najem *et al*., 2020)**. Ingestion is the major way in which aflatoxin gets into the human system. When it enters the body, it is metabolized by microsomal mix-function oxidase enzyme in liver to form a reactive epoxide intermediate. Microsomal mixed-function oxidase belongs to a group of CYP450 enzymes. Mutagenic alterations to DNA are associated with the epoxide intermediate (8,9-epoxide) **(McMillan *et al*., 2018)**. The prevalent mutation found is the GT transversion at codon 249 of p53 tumor suppress gene. Moreover, this epoxide has a potential to react with other macromolecules including RNA and proteins, which results in cellular dysregulation. It also plays a part in suppressing protein, DNA and RNA production. Another pathways to toxicity is the depletion of glutathione that can result in the development of toxicity via reactive oxygen species. Patients having aflatoxin toxicity can also show non-specific symptoms and clinical signs; however, most prominent ones are the signs of hepatotoxicity. As a rule, adult are more resistant to aflatoxin as compared to children who have higher mortality rates. The exposure of aflatoxin is known to cause developmental delays, aflatoxicosis, and hepatocellular carcinoma **(Abbas, 2005)**, immune suppression, growth retardation **(Khlangwiset *et al*., 2011; Sabah *et al*., 2019)** and severe malnutrition in children **(McMillan *et al*., 2018; Qusai *et al*., 2024)**. The primary route for the transmission of this toxin is to animals, and consequently to humans, through contaminated animal feed. Aflatoxins infiltrate food chains during ingestion of tainted food products **(Bryden, 2012)**. One of the significant challenges faced by the aquaculture sector concerning fish nutrition is the prevalence of AFB1, owing to its carcinogenic and hepatotoxic characteristics **(Wild & Turner, 2002; Salam *et al*., 2020)**. The histopathological alterations induced by AFB1 in the hepatic tissue encompass marked vacuolation linked to relatively elevated lipid levels, indications of degeneration, and lipid accumulation within hepatocytes **(Madhusudhanan *et al*., 2004; Bashar *et al*., 2025)**. Aflatoxins have been associated with immunosuppression, growth abnormalities, alterations in gene expression, changes in biochemical markers within fish and impairment of tissues **(Mohapatra *et al*., 2011; Vaziriyan *et al*., 2018)**. Moreover, aflatoxins have the potential to interfere with DNA replications, inhibit RNA polymerase activity and mRNA transcriptions, obstruct amino acid transport and proteins

synthesis, consequently resulting in suboptimal growth rates, alongside inducing both gross and microscopic lesions in fish **(Mahfouz & Sherif, 2015)**.

This work is significant because eating fish contaminated with AFB1, even at low concentrations, over a long time can cause toxic mycotoxin accumulated in organs and constitute a danger risk to human health. The purpose of present study was to detect the presence of AFB1 in the muscles of *Cyprinus carpio* sold in Baqubah markets, Diyala Province and in fish feed, in order to assess the bioaccumulation of AFB1 in fish consumed and the potential risks to humans.

MATERIALS AND METHODS

Samples collection

Twenty fresh *Cyprinus carpio* fish samples weighing 1- 2.75kg were collected randomly from Baqubah markets in Diyala Province, Iraq during May 2025. Fish raised in earthen ponds and cages in the Tigris River of the province of Diyala are the primary source of fish for Baqubah markets. The samples were shipped in an ice box to Pathology Lab. in the College of Veterinary Medicine/University of Diyala. The fish were then dissected; fragments of dorsal muscle and liver were collected, cleaned by distilled water, stored in plastic bags of -20°C until analyzed by HPLC. For histopathological examination, liver, spleen and intestine were collected, preserved in 10%formaldehyde solution and then prepared for examination. Fish feed samples were collected from offices that sell fish feed in Baqubah market. Each sample were ground in a mill and stored in paper bags of room temperature until analysis by HPLC.

Detection of AFB1 in the samples

The test was conducted in laboratories of Scientific Research Authority-Environment and Water Department, according to method provided by **Liu *et al*. (2012)** utilizing a device with a high-performance liquid chromatography (HPLC), (SYKAMN) model, made in Germany, where carrier phase was used: Acetonitrile: Distilled water (70:30). Additionally, separation column was C18-ODS 25cm*4.6mm to separate the AFB1, and the fluorescence detector (ex=365nm, em= 445nm) was used to detect the AFB1, where flow rate of carrier phase was 0.7 ml/min.

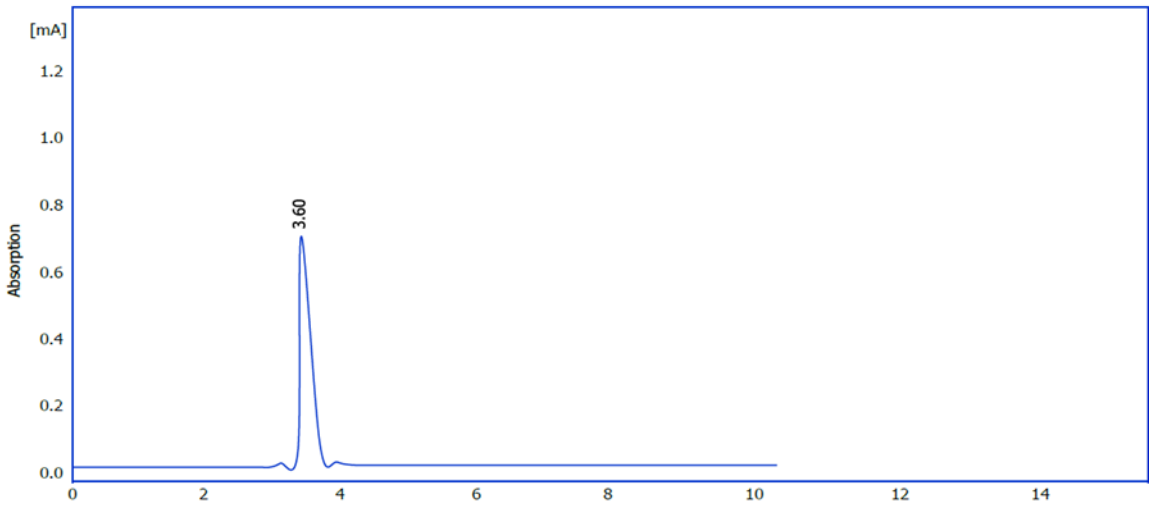

Fig. 1. Standard curve for AFB1

Histopathological examination

Samples of liver, spleen, and intestine were cleaned with tap water before being processed for histopathological analysis in accordance with **Bancroft (2008)**.

Statistical analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS version 18.0). T-test and ANOVA tests were used to determine the presence of significant difference at $P< 0.05$.

RESULTS

Detection of AFB1 concentration by HPLC in muscles of *Cyprinus carpio* samples

The results of present study for levels of AFB1 concentration (ppb) in muscles of *Cyprinus carpio* samples are shown in Table (1). The HPLC analyses for muscles samples revealed that seven muscle samples were positive for AFB1 in 35% and thirteen muscle samples were negative for AFB1 in 65%. The minimum concentration of AFB1 was 0.00 ppb and the maximum concentration of AFB1 was 9.11ppb and the Mean ± SE was 2.3045± 0.75843ppb.

Table 1. Levels of AFB1 concentration (ppb) in muscles of *Cyprinus carpio* samples

No. of Examined muscles of *Cyprinus carpio* samples (S)	**AFB1 concentration (ppb)**	**Minimum AFB1 concentration (ppb)**	**Maximum AFB1 concentration (ppb)**	**Mean±SE AFB1 concentration (ppb)**
S 1	0	0.00	9.11	2.3045 ± 0.75843
S 2	5.09			
S 3	6.77			

S 4	0			
S 5	0			
S 6	0			
S 7	0			
S 8	8.7			
S 9	4.11			
S 10	0			
S 11	0			
S 12	0			
S 13	0			
S 14	0			
S 15	0			
S 16	7.08			
S 17	5.23			
S 18	9.11			
S 19	0			
S 20	0			

Detection of AFB1 concentration by HPLC in liver of *Cyprinus carpio* samples

The findings of the present investigation for AFB1 concentration in the liver of *Cyprinus carpio* samples are shown in Table (2) and Fig. (2).

HPLC analyses for liver samples (Top five AFB1 concentrations in contaminated *Cyprinus carpio* muscles) revealed that all liver samples were positive for AFB1 in 100%. The minimum concentration of AFB1 was 0.25ppb and the maximum concentration of AFB1 was 0.99ppb and the Mean ± SE was 0.5280± 0.14534ppb.

Table 2. Levels of AFB1 concentration (ppb) in liver of *Cyprinus carpio* samples

No. of *Cyprinus carpio* liver samples	AFB1 concentration (ppb)	Minimum AFB1 concentration (ppb)	Maximum AFB1 concentration (ppb)	Mean±SE AFB1 concentration (ppb)
S3	0.25	0.25	0.99	0.5280±0.14534
S8	0.33			
S16	0.75			
S17	0.32			
S18	0.99			

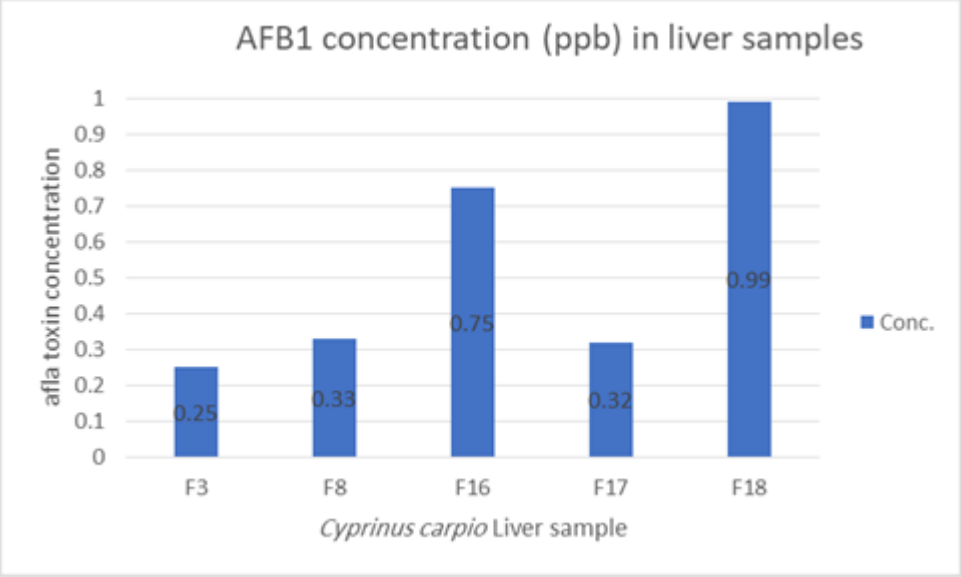

Fig. 2. The levels of AFB1 concentration (ppb) in liver of *Cyprinus carpio* samples

Detection of AFB1 concentration by HPLC in feed samples of *Cyprinus carpio*

The results of present study for AFB1 concentration in feed samples of *Cyprinus carpio* are shown in Table (3) and Fig. (3).

HPLC analyses for feed samples showed that all feed samples (imported feed and local feed) were positive for AFB1 in 100%. The minimum concentration of AFB1 of imported feed was 12.09ppb and the maximum concentration of AFB1 was 14.08ppb and the Mean ± SE was 13.0850± 0.99500ppb, while the minimum concentration of AFB1 of the local feed was 11.08ppb and the maximum concentration of AFB1 was 22.08ppb and the Mean ± SE was 16.7133± 3.17822ppb with no significant difference (*P*-value = 0.448226) between imported and local feed.

Table 3. The levels of AFB1 concentration (ppb) in feed samples of *Cyprinus carpio*

Source of feed	No. of feed samples	Minimum AFB1 concentration (ppb)	Maximum AFB1 concentration (ppb)	Mean±SE AFB1 concentration (ppb)	T -test *P*-value
Imported feed	2	12.09	14.08	13.0850±0.99500	0.448226
Local feed	3	11.08	22.08	16.7133±3.17822	

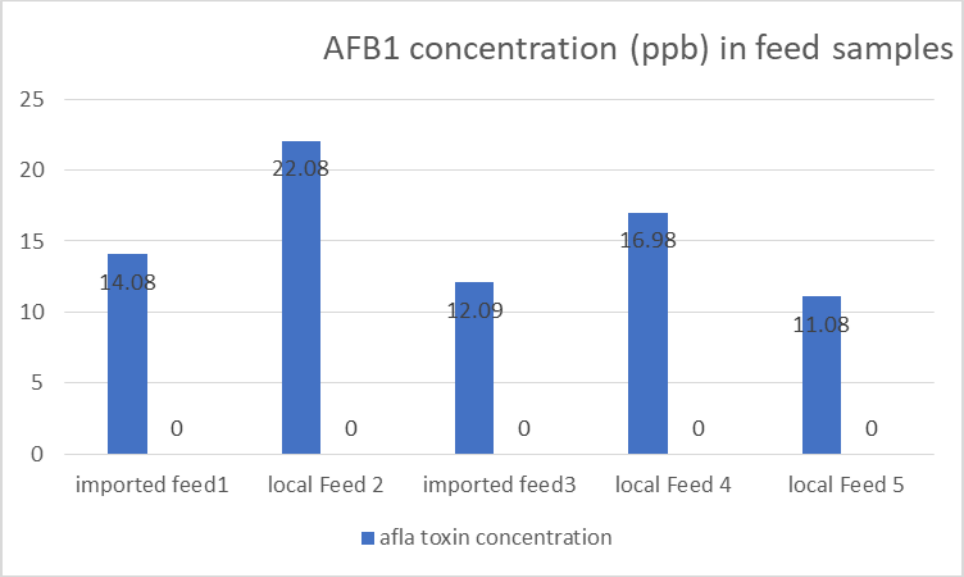

Fig. 3. The levels of AFB1 concentration (ppb) in feed samples of *Cyprinus carpio*

Histopathological examination

Histopathological alterations of the liver section of *Cyprinus carpio* revealed cloudy swelling of hepatocytes, slight edema with sever blood vessels congestion and inflammatory cells infiltration (Mononuclear cells MNCs) (Fig. 4A). Moreover, there were slight necrosis and congestion in central vein (Fig. 4B).

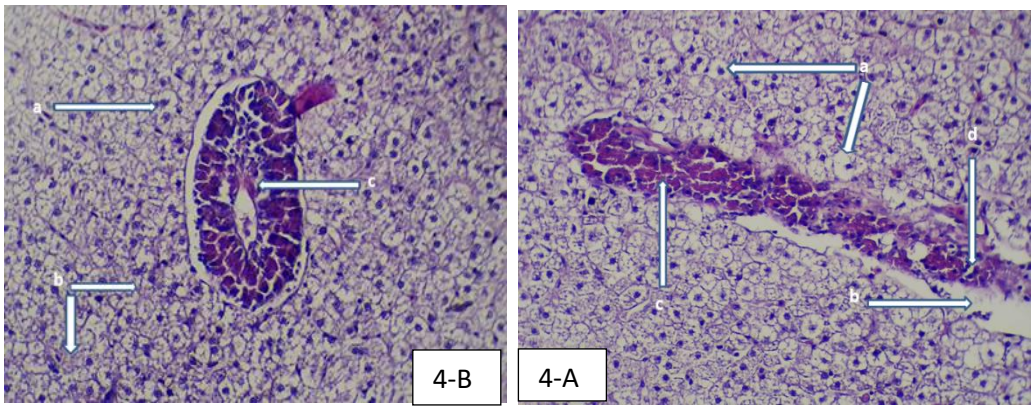

Fig. 4. (A) Histopathological section of the liver of *Cyprinus carpio* showing cloudy swelling **(a)** slight edema **(b)** with sever blood vessel congestion **(c)** and inflammatory cells infiltration **(d)** H&E ×20; **(B)** Histopathological sections of the liver of *Cyprinus carpio* showing cloudy swelling **(a)** slight necrosis **(b)** and congestion in central vein (c). H&E ×20

Histopathological findings of the spleen section of *Cyprinus carpio* showed congestion, hemosiderin precipitation with sever depletion in splenic parenchyma and infiltration of inflammatory cells primarily perivascular cuffing cells (Fig. 5A). Moreover, there were severe infiltration of inflammatory cells (MNCs) and hemosiderin laden in the nucleus (Fig. 5B).

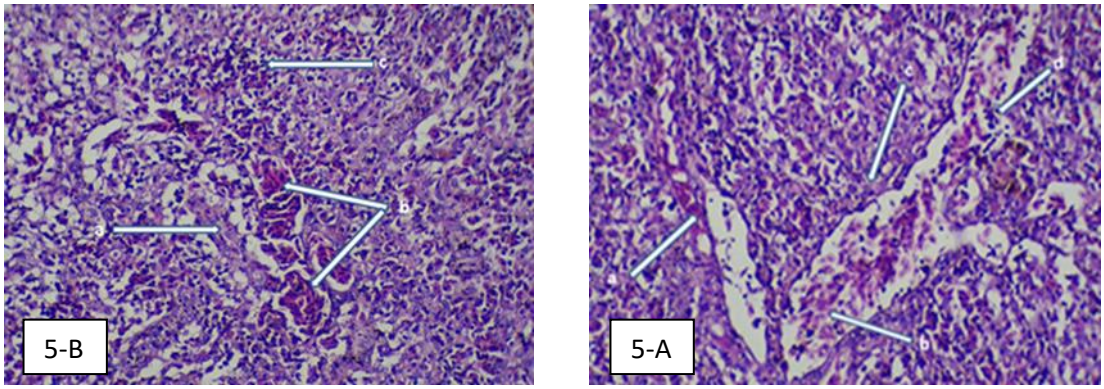

Fig. 5. (A) Histopathological section of the spleen of *Cyprinus carpio* showing congestion **(a)** hemosiderin precipitation **(b)** with severe depletion **(c)** and infiltration of inflammatory cells **(d)** H&E ×20 **(B)** Histopathological sections of the spleen of *Cyprinus carpio* showing slight depletion **(a)** hemosiderin laden in nucleus **(b)** and sever infiltration of inflammatory cells **(**MNCs) **(c)** H&E ×20

Intestinal section of *Cyprinus carpio* showed closing of villi with infiltration of inflammatory cells (Fig. 6A), also there were congestion, increase number of goblet cells together with slight infiltration of mononuclear cells and complete damage for villi (Fig. 6B).

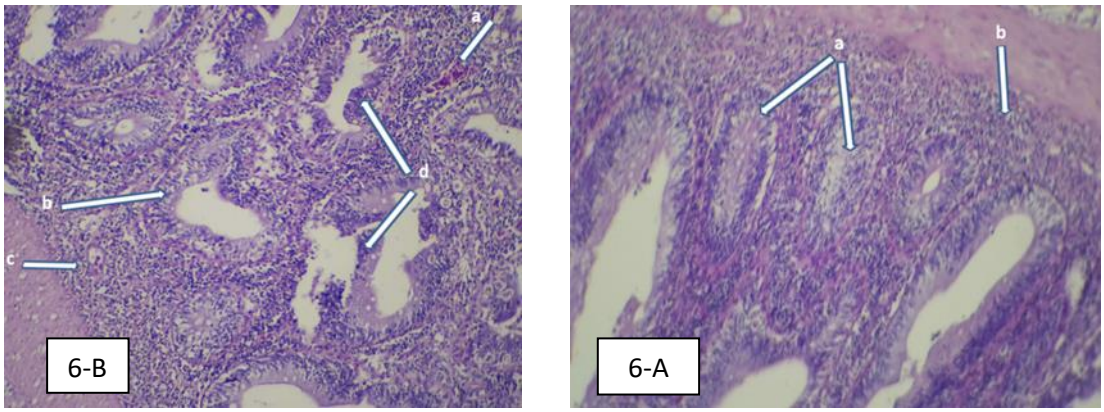

Fig. 6. (A) Histopathological section of the intestine of *Cyprinus carpio* showing closing of villi **(a)** with infiltration of inflammatory cells **(b)** H&E ×20 **(B)** Histopathological sections of the intestine of *Cyprinus carpio* showing congestion **(a)** increase number of goblet cells **(b)** together with slight infiltration of mononuclear cells **(c)** and complete damage for villi **(d)** H&E ×10

DISCUSSION

The results of HPLC analyses for AFB1 concentration in *Cyprinus carpio* showed that, the toxins were present in seven muscle samples and all examined liver samples which suggests that they might have come from feeds. These results are in accordance with **Al-Rubaiy *et al*. (2018)**. Similar findings were observed in sea bass and the Nile tilapia **(Ghaednia *et al*., 2013)**. Further research revealed that AFB1 was accumulated throughout *O. niloticus's* body once the experiment was finished **(Abdelhamid *et al*., 2004)**. Another study reported that no residue was found in the fish meat after feeding tilapia with varying amounts of AFB1 **(Selim *et al*., 2014)**. The presence of AFB1 residues in fish meat poses a serious risk to food safety **(Manafi *et al*., 2014)**. Additionally, the presence of AFB1 in food, even at low amounts, is detrimental to humans due to the possibility of bioaccumulation into tissue **(Shephard, 2008)**. The present study's findings demonstrated that all examined feed samples were contaminated with AFB1. These results are in line with **Marijani *et al*. (2017)**, who noticed that concentration of AFB1 in fish feed was 2-806 ppb. Moreover, **Santacroce *et al*. (2008)** found that the level of AFB1 ranges from 0.46 -68.5ppb in fish feeds. On the other hand, **Barbosa *et al*. (2013)** reported 1.83- 67.35ppb AFB1 level in fish feeds. The presence of AFB1 in samples of fish feed may have resulted from the preparation of these feeds using materials that had previously been contaminated with AFB1, or from unsuitable storage conditions that have encouraged fungal development and the subsequent production of mycotoxins **(Marijani *et al*., 2017)**.

AFB1's toxic effects on fish may be directly demonstrated by examining the histopathological alterations. The present investigation discovered pathological change in intestine, spleen liver and of *Cyprinus carpio* contaminated with AFB1. The impact of aflatoxins on the circulatory system's endothelial cells is shown by congestion of blood vessels in tissues. Endothelial cells appear to be sensitive to aflatoxin, since these cells exhibit the majority of pathologic damage **(Mehrim *et al*., 2006)**. The same alterations were noted in Rohu as well (*Labeo rohita*) **(Sahoo *et al*., 2001)**. The liver is the main organ in fish that is susceptible to aflatoxicosis **(Madhusudhanan *et al*., 2004)**. According to the current study's microscopic findings, AFB1 caused moderate histo-pathological changes in the liver of *Cyprinus carpio*. The necrosis of hepatocytes indicates AFB1 effect in cell membranes destruction and tissues necrosis. Damage to the liver caused by AFB1 can disturb physiological balance and homeostasis of fish. Other studies revealed severe histopathological change in liver of *Cyprinus carpio* treated with aflatoxin **(Abdelhamid *et al*., 2004; Shahafve *et al*., 2017; Al-Rubaiy *et al*., 2018)**. In contrast, the liver of the gibel carp (*Carassius auratus gibelio*) treated with aflatoxin showed no histopathological changes **(Nomura *et al*., 2011)**. In the current study, microscopical examination revealed that AFB1 has pathological effect on spleen tissue,

including congestion, hemosiderin precipitation, severe depletion in the spleen parenchyma and infiltration of inflammatory cells. It suggested that, there might be immune system dysfunction or suppression due to the structural abnormality of the spleen. The results of the current study showed AFB1 induced histopathological changes in the intestine of *Cyprinus carpio*. Intestinal damage can impair nutrients absorption and result in reduced growth rate, and malnutrition decreased regeneration of physiologically in fish exposed to aflatoxin **(Mehrim *et al.*, 2006)**. According to **Sahoo *et al*. (2001)**, histopathological changes in rohu treated with aflatoxin were intestinal hemorrhage and exfoliation of mucous layers epithelium. The effects of aflatoxins on fish digestive systems include pathological alterations, decreased intestinal food digestion and absorption, decreased enzyme activity, as well as malnutrition **(Applegate *et al*., 2009)**.

CONCLUSION

The results of present study revealed the presence of AFB1 in the muscles of *Cyprinus carpio* sold in Baqubah markets in Diyala Province. Although the AFB1concentration in fish muscles was lower than the permissible limit (20ppb), it can accumulate in many organs and constitute a danger risk to human health when consumed. Additionally, AFB1 was found in the fish feed, which seems to be the primary source of contamination for fish. Thus, it is important to make sure that the materials used to feed fish are of a high quality.

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