Arab Universities Journal of Agricultural Sciences
(2023) 31 (2) 231-245
Website: http://ajs.journals.ekb.eg

Role of Vitamin C Supplementation in Alleviation of Aflatoxin-Contaminated Feed of Nile tilapia (*Oreochromis niloticus*)

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https://doi.org/10.21608/AJS.2023.223804.1529

Received 18 July 2023; Accepted 25 October 2023

Keywords:

Aflatoxin B1,
Nile tilapia,
Vitamin C,
Blood biochemistry,
Contamination

Abstract: The purpose of the research was to illustrate the effects of feeding Nile tilapia fingerlings (*Oreochromis niloticus*) with low doses of aflatoxins (AFs) with and without vitamin C supplementation to investigate the capacity of vitamin C in detoxification. Ten experimental diets were formulated and divided into three categories. The first category included: T1, T4 and T7 feedings included 20, 40 and 80 µg AFs kg^{-1} feed respectively, while both the second category T2, T5 and T8 and the third category T3, T6 and T9 were treated with contaminated feed with AFs for 57days then both categories shifted to different regime till the conclusion of the experimentation. The second category was fed uncontaminated feed while the third category was fed a supplemented diet with 100 mg Kg^{-1} of vitamin C. For 113 days the experiment was conducted. The results showed that shifting from a contaminated diet to an uncontaminated diet (category 2) or adding vitamin C to the contaminated diet (category 3) improved the deterioration that occurred in the values of growth performance, biochemical parameters and histological disorders caused by AFs. Furthermore, the results from the control group were superior to all the treatments.

1 Introduction

Egypt is notable for being the largest country in Africa and the sixth-largest country globally in terms of aquaculture production (FAO, 2022). Fish feed is one of the main pillars in the aquaculture industry that consumes the highest portion of the running cost during the production cycle (Ahmed et al 2021). Hence, spoilage and contamination of feeds with mycotoxins may occur, during storage in the farm and/or during the raw material supplying chain before processing the feeds. This is one of the elements affecting the development and general health of fish. Additionally, it may lead to fish mortality resulting in tremendous financial losses to aquaculture producers (Oliveira and Vasconcelos 2020). Once synthesized, mycotoxins are challenging to get rid of because they are resistant to heat and some production processes (Cortés-Sánchez et al 2020). There are over 20 different varieties of aflatoxins (AFs), the four main types being B1, B2, G1 and G2. The extremely dangerous secondary metabolites of the genus *Aspergillus* are known as AFs (Abdel Rahman et al 2017). Aflatoxin B1 (AFB1), a significant category of mycotoxins, poses

the greatest threat to human health due to its high toxicity among the mycotoxin families. (Bedoya-Serna et al 2018). They are classified as carcinogenic, mutagenic and immunosuppressive agents (Abdel-Daim et al 2020). It also results in physiological imbalance and behavioral modifications including weariness and lack of balance. (Anater et al 2020), as well as histological damage induction. AFB1 is the most hepato-carcinogenic natural substance, exposure to AFB1 results in poor growth, anemia, liver and organ damage and higher mortality (Oliveira and Vasconcelos 2020). Additionally, the hepatic ability to produce clotting factors is compromised, including lower levels of both prothrombin and fibrinogen leading to higher blood clotting duration (Doerr et al 1974). The limit permit for AFs in feeds is 20 µg kg^{-1} (B1 + B2 + G1 + G2) (Mazumder and Sasmal 2001, Alvarado et al 2017, Cortés-Sánchez et al 2020). Based on this recommendation, the doses of AFB1 in the present study were calculated. Vitamin C is a vigorous antioxidant, which helps prevent and scavenge reactive oxidants produced during immune response and cell metabolism. This protects important biomolecules such as nucleic acids, carbohydrates, lipids and proteins in host cells from damage caused by external sources (Ponnampalam et al 2022). Additionally, studies have suggested that vitamin C can enhance macrophage function by facilitating apoptosis and clearance of dead cells. Based on these findings, it is believed that vitamin C could act as a powerful immunity booster to lessen the biological harm brought on by exposure to AFs. In order to completely comprehend the possible advantages and disadvantages of employing vitamin C as a preventive agent in fish farming, more research is necessary (Chen et al 2022). The present study objective was to examine the role of vitamin C in detoxification by feeding Nile tilapia fingerlings low doses of AFs.

2 Materials and Methods

Fingerlings were purchased from a private hatchery at Al-Abassa, Abu-Hammad, Sharkia Governorate, Egypt. Before the experiment initiation, the fish underwent a three-week acclimatization period.

2.1 Experimental tanks

The experiment was conducted in a static water system using quadrate fiberglass containers that were 60 cm length × 60 cm wide × 40 cm deep. A plastic net was placed over the tanks to prevent fish from jumping out. Using standard siphon hoses in all tanks, feed leftovers and solid wastes were removed every day and the siphoned water was compensated for by fresh dechlorinated water.

2.2 Experimental diets

A commercial diet comprising 30 percent crude protein (CP) was fed to the fish in the current study. A chemical component of the experimental diet was analyzed, as revealed in **Table 1**. Daily fish feed allowances (5% of fish biomass) were divided into three portions (six days a week). The ingredients of a commercial feed were corn gluten - yellow corn - wheat brane - fish meal - wheat middling - soybean meal - calcium diphosphate - soy oil - fish oil and vitamin minerals premix.

Table 1. Chemical analysis of the commercial diet used in present study

Chemical analysis	on DM basis
Dry matter (DM)	88.7%
Crude protein (CP)	30.2%
Ether extract (EE)	5.3%
Crude fiber (CF)	4.8%
Ash	6.1%
NFE*	53.6%
GE** (kcal/kg diet)	4000

Vitamins and minerals are included kg^{-1}: 48×10^5 I.U (vit. A), 6×10^2 mg (vit. B6), 20 mg (Biotin), 8×10^5 I.U. (vit. D3), 144 mg (vit. E), 400 mg (vit. B1), 1600 mg (vit. B2), 4×10^3 mg (Pantothenic acid), 4 mg (vit. B12), 4×10^2 mg (Niacin), 2×10^5 mg (Choline chloride), and 400 mg (folic acid). Minerals: 12×10^3 mg Iron, 16×10^3 mg Manganese, 12×10^2 mg Copper, 120 mg Iodine, 80 mg Cobalt, 40 mg Selenium, and $16x10^3$ mg Zinc).

* NFE = Nitrogen free extract (100 – [CP + EE + CF + Ash)].

** GE = Gross Energy was calculated as 5.65, 9.45, and 4.12 Kcal as calorific values of gram crude protein, lipid and carbohydrates, respectively, according to NRC (2011).

2.3 Aflatoxins

Difuranocumarin derivatives known as AFs are synthesized by specific *Aspergillus* species and categorized into B1, B2, G1 and G2 as the main four types (Vaamonde et al 2003, Pietsch 2020). The liquid aflatoxin was obtained from the Food Safety Laboratory, Agriculture Research Center, Egypt. The toxins (AFB1) contained B1 (97.25%), B2 (1.62%) and G2 (1.13%); AFB1 was prepared in concentrations (0, 20, 40 and 80 µg kg^{-1} diet).

2.4 Experimental design

Within 4.7 ± 0.5 g fish^{-1} as initial body weight, triplicate groups of 14 fish /replicate were fed one of the four experimental prepared diets containing different concentrations of AFB1 0, 20, 40 and 80 µg AFB1 kg^{-1} diets for 113 days. The treatments were divided into three categories as shown in **Fig 1**. The first category included treatments T1, T4 and T7, which were fed contaminated diets with 20, 40 and 80 µg AFB1 kg^{-1} diet respectively for the whole experimental period (113 days). The second category included treatments T2, T5 and T8, which were fed AFB1 (by the same levels used in the first category) for 57 days, then shifted to a typical 30% CP commercial diet for the remaining 56 days of the experimental period as a recovery period. The third category included treatments T3, T6 and T9, which had the same treatment procedure as in the first category but starting on day 58, the AF-contaminated diets received 100 mg of vitamin C kg^{-1} of diet until the completion of the experiment; the reference control was group T0 which received the normal diet throughout the experiment (113 days).

2.5 Water quality

An oxygen meter (Lutron model Do-5509, Taiwan) was used to test the water temperature and dissolved oxygen (DO) daily. A digital pH meter (Hanna model PHEP, Romania) was used to assess the pH; the levels of ammonia and nitrite were disregarded because of the constant water changes. A 0.5 HP air blower was used to regularly aerate the fish-rearing tanks.

2.6 Fish sampling intervals

Throughout the 113-day trial, the experimental fish were weighed biweekly to adjust feeding allowances and examine the growth performance characteristics. The growth performance indices such as total weight gain (TWG), average daily gain (ADG), specific growth rate (SGR), as well as feed efficiency parameters such as feed conversion ratio (FCR), and protein efficiency ratio (PER) were measured. The survival rate (SR) of fish was recorded daily in each treatment through the experimental period. At the end of the experiment, the biochemical parameters of blood such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, hemoglobin, hematocrit and histological sections of the liver and kidneys were collected.

2.7 Fish growth performance and feed utilization parameters

The parameters used to assess fish growth performance and feed utilization were calculated as described by El-Nahal et al (2019) as follows:

TWG (g) = Final weight (g fish^{-1}) – initial weight (g fish^{-1}).
ADG (g fish^{-1} day $^{-1}$) = AWG (g fish^{-1}) / experimental period (day).
SGR (% day^{-1}) = 100 (ln final weight - ln initial weight) / experimental period (day).
SR (%) = number of fish at the end of the experiment × 100 / total number of fish at the start of the experiment.
FCR = Feed intake (g) / body weight gain (g).
PER = Gain in weight (g) / protein intake (g).

2.8 Blood sampling and analysis

At the end of the experiment, six samples of *O. niloticus* fish (n=6) were taken randomly from each treatment and anesthetized by using 0.1 mL clove oil per liter of water (Fernandes et al 2016). Through a caudal venous puncture from each individual, blood was collected with the use of a 1ml syringe. Samples were collected in two types of tubes, one without anticoagulant to assess AST, ALT and creatinine in serum and the other containing K2EDTA as an anticoagulant to assess the complete blood count (CBC) parameters (Faggio et al 2014, Ahmed et al 2020).

2.9 Hematology

Some hematological parameters such as ALT, AST and creatinine were measured using an auto-hematology analyzer (Rayto model RT7200, China). Other hematological measurements including hemoglobin (Hb), packed cell volume (PCV or Hct), total red blood cells (RBCs) and total white blood cells (WBCs) were determined also. The samples of clear serum were obtained by centrifuging the blood for 20 minutes at 3500 rpm after it had been collected. Then, the serum ALT, AST and creatinine were assessed using the spectrophotometric commercial kits (Biomed Diagnostic, Egypt) following the manufacturer's instructions.

2.10 Hepatosomatic index

The liver was weighed after being removed from the body cavity and dried on filter paper. The hepatosomatic index (HSI) was determined using the following formula:

$$HSI\% = (\text{Liver weight} / \text{Total body weight}) \times 100$$

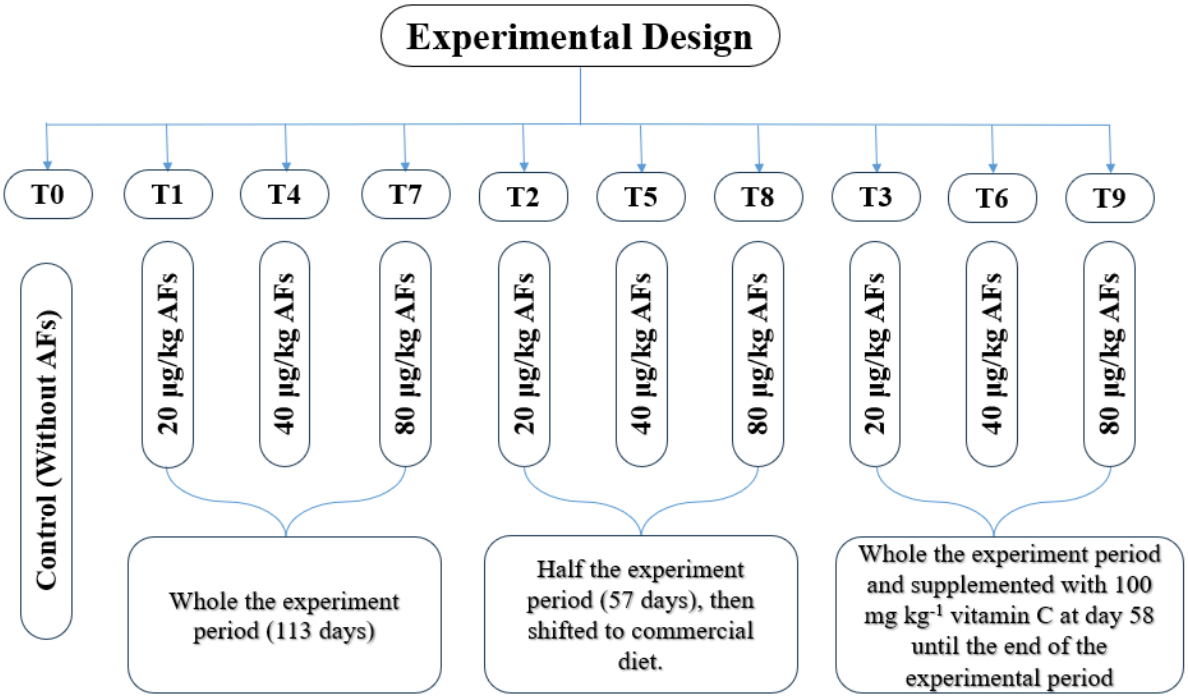


Fig 1. Details of the experimental design

2.11 Histological examination

Small sections of the experimental fish's liver and kidneys were dissected, after which they were fixed for 24 hours in a 10% formalin solution. Later, 70% ethanol was used to preserve fragments, dehydrated by ascending alcohol concentrations, embedded in paraffin wax, and finally blocked in paraffin wax. According to Genten et al (2009), 5 µ fine transverse sections were cut and hematoxylin (H) and eosin (E) were to stain the slices. By using light microscopy, the tissue slides were examined and the photo was captured by fluorescence Leica (DM2500, Germany).

2.12 Statistical analysis

Using analysis of variance (ANOVA) as a one-way analysis, by the General Linear Models (GLM) of the SAS program (model 2005), all numerical data was statistically analyzed. In each instance, significance values were accepted at $P < 0.05$. Using Duncan's multiple range test, the means of all the different treatments were compared (Duncan 1955).

The model of the statistical analysis was as follows:

$Y_{ij} = \mu + T_i + E_{ij}$

μ = the overall mean.

T_i = the effect of treatment.

E_{ij} = the random error.

3 Results and discussion

3.1 Water quality parameters

During the experimental period the parameters were within the normal ranges sufficient for rearing *O. niloticus*, where water temperature was maintained at 29 ± 2°C, pH at 7.9 ± 0.2 and DO at 5.5 ± 0.5 mg L^{-1} which are in line with those reported by El-Sayed (2006).

3.2 Growth performance

The results showed that both AFB1 and Vitamin C addition significantly reduced weight gain, feed intake and FCR in a dose-dependent manner. The overall trends of the final body weight, TWG, ADG, SGR and FCR values were significantly affected ($P<0.05$) by the experimental diets in the first category, T1, T4 and T7, where the fish continued to consume contaminated feed throughout the whole experimental period (113 days). Compared to the control group (T0), fish in the first category were fed the lowest AFB1 concentration (20 µg

kg^{-1} diet, T1). The actual values obtained for the parameters of growth performance are shown in **Table 2**. It is noted that TWG, ADG, PER and SR percentages were lowered by 36.4, 36.0, 27.3 and 15.8% respectively, while FCR increased by 31.9% compared to the control group. Fish fed a 40µg kg^{-1} diet (T4) showed a reduction in TWG, ADG, PER and SR percentages by 41.4, 44.0, 22.3 and 18.4% respectively, and an increase in FCR by 24.6%. Fish fed the highest level of AFB1, 80 µg kg^{-1} diet (T7), recorded reductions of 36.4, 40.0, 23.1 and 7.9% for TWG, ADG, PER and SR respectively. The second category treatments, T2, T5 and T8, were fed AFB1 for 57 days and then shifted to uncontaminated feed. Fish-fed T2 (20 µg kg^{-1} diet for 57 days) reduced TWG, ADG, PER and SR percentages by 27.3, 28.0, 18.2 and 5.3% respectively, but increased FCR by 18.9% compared to the control group. While fish-fed T5 (40 µg AF kg^{-1} diet) showed decreased TWG, ADG, PER and SR percentages by 28.3, 32.0, 31.4 and 15.8% respectively. Fish-fed T8 (80 µg AF kg^{-1} diet) reduced TWG, ADG, PER and SR percentages by 35.4, 28.0, 21.5 and 10.5% respectively, and increased FCR by 23.9% compared to those fed T0 diet. For the third category, fish were fed the contaminated diet supplemented with 100 mg vitamin C kg^{-1} diet (T3, T6 and T9). Fish fed T3 (20 µg AF kg^{-1} diet + vit. C) declined TWG, ADG, PER and SR by 30.3, 32.0, 17.1 and 13.0% respectively and increased FCR by 17.4 %. Fish-fed T6 (40 µg AF kg^{-1} diet + vit. C) reduced TWG, ADG, PER and SR by 33.3, 28.0, 28.9 and 7.9% respectively, while increased FCR by 24.2 %. Fish-fed T9 (80 µg AF kg^{-1} diet + vit. C) reduced TWG, ADG, PER and SR by 29.3, 32.0, 19 and 13.0% respectively, and increased FCR by 21 % compared to the fish-fed T0 diet. It is noted from these values that shifting from a contaminated diet to an uncontaminated diet (category 2) or adding vitamin C to the contaminated diet (category 3) improved the deterioration that occurred in the values of growth performance caused by AFB1, although the control diet (T0) remains the best. This observation agrees with that of Ayyat et al (2018) who mention that the addition of dietary vitamin C in tilapia feed showed positive effects, where the harmful effects like growth retardation, cirrhosis and hepatocellular carcinoma of aflatoxin toxicity were reduced**.** Similarly, Barany et al (2021), showed that aqua feeds contaminated with AFB1even with low levels of AFB1 would have negative effects on tissue integrity, metabolism and growth of treated sea bream, *Sparous aurata.*

AF contaminants are tolerated differently by aquatic species, *O. niloticus* is considered highly sensitive to AFB1 (Mohamed et al 2017, Kaleem and Sabi 2021). From the data on fish growth rates and feed consumption throughout the experiment, treatment with levels of AFB1 up to 80 g kg^{-1} did not appear to have an impact on growth rates when compared to other AF-contaminated diets. These results correspond to those reported by Bedoya-Serna et al (2018), who revealed that different species responded differently to lower AFB1 levels (below 50 µg kg^{-1} diet). These results are consistent with Pietsch (2020) who reported that early aflatoxicosis in fish feed is frequently characterized by poor growth, but doses of 20 to 200 µg kg^{-1} diet had no adverse effects on the efficiency of protein and feeding utilization. Low levels of AFB1 may impair animal performance by inhibiting nutritional digestion, absorption, and metabolic and physiological processes (Xu et al 2022).

3.3 Hematology

In the first category treatments T1, T4 and T7, which were treated with AFB1 for 113 days, the actual values obtained for the CBC parameters are shown in **Table 3**. It is noted that fish fed higher AFB1 diet T7 (80 µg kg^{-1} diet) had a reduction in RBCs, Hb and Hct by 30.7, 12.1 and 23.9% respectively, but increased WBCs by 43.7% compared to those fed uncontaminated diet (T0).

In the second category treatments, T2, T5 and T8, fish were fed an AFB1-contaminated diet for 57 days and then shifted to an uncontaminated diet for the subsequent 56 days of the experimental period. The results showed that fish fed the lowest AFB1 diet T2 (20 µg kg^{-1} diet) reduced RBCs, Hb and Hct by 25.0, 5.0 and 19.6% respectively but increased WBCs by 6.3 % compared to fish fed T0 diet. Fish in T5, fed 40 µg kg^{-1} diet, reduced RBCs, Hb and Hct by 22.0, 20.0 and 14.4% respectively, but increased WBCs by 18.2% compared to the control group (T0). On the other hand, fish fed the highest AFB1- contaminated diet T8 (80 µg kg^{-1} diet) reduced Hb by 8.2 % but increased RBCs, WBCs and Hct by 8.2, 44.3 and 14.9% respectively compared to fish in the control group (T0). In the third set of treatments (T3, T6 and T9), the same feeding procedure as the first set (category 1) was followed, but starting from day 56 of the experimental period, 100 mg of vitamin C kg^{-1} diet was added to the feeding regime until the end of the study. The results showed that fish fed the lowest AFB1 diet T3 (20 µg kg^{-1} diet + vit. C) reduced RBCs, WBCs, Hb and Hct by 26.4, 5.0, 10.9 and 16.0 % respectively compared to those in T0; while fish fed

Table 2. Effects of aflatoxin B1-contaminated diet on growth performance, feed efficiency, and survival rate parameters of *O. niloticus*

Treatment	Exposure Period (day)	Initial weight (g)	Final weight (g)	TWG (g)	ADG (g fish^{-1} day^{-1})	SGR (% day^{-1})	FCR	PER	SR (%)
Control (T0)	113	5.33 ± 0.33	38.66^{a} ± 3.75	33.00^{a} ± 3.46	0.25^{a} ± 0.02	1.49^{a} ± 0.07	2.85^{a} ± 0.39	1.21^{a} ± 0.18	90.50^{a} ± 2.40
20 µg (T1)	113	5.00 ± 0.00	26.00^{bc} ± 2.88	21.00^{b} ± 2.88	0.16^{b} ± 0.02	1.25^{b} ± 0.06	3.76^{b} ± 0.15	0.88^{b} ± 0.03	76.20^{b} ± 6.29
40 µg (T4)	113	5.00 ± 0.00	24.66^{c} ± 0.33	19.33^{b} ± 0.33	0.14^{b} ± 0.00	1.20^{b} ± 0.04	3.55^{ab} ± 0.21	0.94^{b} ± 0.05	73.83^{bc} ± 9.53
80 µg (T7)	113	4.33 ± 0.33	25.66^{c} ± 1.45	21.00^{b} ± 1.15	0.15^{b} ± 0.00	1.27^{b} ± 0.02	3.57^{ab} ± 0.10	0.93^{b} ± 0.02	83.33^{ab} ± 2.37
20 µg (T2)	57	5.00 ± 0.57	28.66^{bc} ± 1.20	24.00^{b} ± 0.00	0.18^{b} ± 0.00	1.31^{ab} ± 0.07	3.39^{ab} ± 0.28	0.99^{ab} ± 0.07	85.73^{ab} ± 4.13
40 µg (T5)	57	5.33 ± 0.57	32.00^{b} ± 1.15	23.66^{b} ± 0.88	0.18^{b} ± 0.00	1.38^{ab} ± 0.03	4.05^{b} ± 0.26	0.83^{b} ± 0.05	76.20^{b} ± 6.29
80 µg (T8)	57	4.67 ± 0.33	25.66^{c} ± 0.88	21.33^{b} ± 0.88	0.16^{b} ± 0.00	1.30^{ab} ± 0.01	3.53^{ab} ± 0.20	0.95^{b} ± 0.05	80.97^{b} ± 2.37
#20 µg + Vit. C (T3)	113	4.6 ± 0.33	27.66^{bc} ± 0.33	23.00^{b} ± 0.00	0.17^{b} ± 0.00	1.31^{ab} ± 0.04	3.34^{ab} ± 0.14	1.00^{ab} ± 0.04	78.60^{b} ± 8.26
#40 µg + Vit. C (T6)	113	5.00 ± 0.57	26.66^{bc} ± 0.88	22.00^{b} ± 0.57	0.16^{b} ± 0.00	1.23^{b} ±0.04	3.54^{ab} ± 0.22	0.86^{b} ± 0.05	83.33^{ab} ± 2.37
#80 µg + Vit. C (T9)	113	4.33 ± 0.33	27.33^{bc} ± 2.02	23.33^{b} ±2.02	0.17^{b} ± 0.00	1.37^{ab} ± 0.10	3.45^{ab} ± 0.32	0.98^{ab} ± 0.09	78.60^{b} ± 8.26
Significance		NS	*	*	*	*	*	*	*

Means with different superscripts in the same column are significant at *P<0.05*.
#: Supplemented by 100 mg vitamin C kg^{-1} diet on day 58 until the end of the experimental period.
TWG: total weight gain, ADG: average daily gain, SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio, SR: survival rate.

Table 3. Effect of aflatoxin B1-contaminated diet on hematological parameters of *O. niloticus*

Treatment	Period (day)	RBC (10^6 µL^{-1})	Hb (g dL^{-1})	Hct (%)	WBC (10^3 µL^{-1})
Control (T0)	113	1.40^{ab} ± 0.38	17.87^{a}± 0.88	18.30^{ab}± 5.80	31.80^{de}± 0.08
20 µg (T1)	113	1.19^{ab}± 0.28	15.33^{ab}± 1.33	10.94^{b}± 3.62	35.10^{d}± 0.23
40 µg (T4)	113	1.09^{ab}± 0.21	15.47^{ab}± 0.63	16.16^{ab}± 3.00	37.95^{bc}± 0.77
80 µg (T7)	113	0.97^{b}± 0.33	15.70^{ab}± 0.78	13.93^{ab}± 4.99	45.70^{a}±1.73
20 µg (T2)	57	1.05^{ab}± 0.29	16.97^{ab}± 0.54	14.72^{ab}± 4.40	33.80^{e}± 0.51
40 µg (T5)	57	1.08^{ab}± 0.15	14.27^{b}± 1.17	15.67^{ab}± 2.37	37.60^{bc}± 0.00
80 µg (T8)	57	1.43^{ab}± 0.27	16.40^{ab}± 0.62	21.03^{ab}± 3.58	45.90^{a}± 1.02
#20 µg + Vit. (T3)	113	1.03^{ab}± 0.30	15.93^{ab}± 1.13	15.38^{ab}± 5.07	30.20^{e}±0.92
#40 µg + Vit. (T6)	113	1.18^{ab}± 0.24	14.63^{b} ± 0.67	17.41^{ab}± 3.80	35.05^{cd}±0.00
#80 µg + Vit. (T9)	113	1.91^{a}± 0.20	16.70^{ab}± 0.15	25.97^{a} ± 2.42	40.40^{b}±0.57
Significance		*	*	*	*

Means with different superscripts in the same column are significant at *P<0.05*.
#: Supplemented by 100 mg vitamin C kg^{-1} diet on day 58 until the end of the experimental period.
RBCs: red blood cells, Hb: hemoglobin, Hct: hematocrit, WBCs: white blood cells.

the T6 diet (40 µg kg^{-1} diet + vit. C) had a lower decline percentage in RBCs, Hb and Hct by 15.7, 18.1 and 4.9 % respectively but increased WBCs by 10.0 % compared to fish in T0. Fish fed the highest AFB1 diet T9 (80 µg kg^{-1} diet + vit. C) reduced Hb by 6.6 % and increased RBCs, WBCs and Hct by 36.4, 27.0 and 41.9% respectively compared to fish in the control diet (T0). It is noted from these values that shifting from a contaminated diet to an uncontaminated diet (2^{nd} category) or adding vitamin C to the contaminated diet (3^{rd} category) improved the deterioration that occurred in the values of hematological parameters caused by AFB1, although the control diet remains the best. This result could be attributed to that vitamin C can enhance macrophage function by enhancing phagocytosis to kill microbes, boosting migration in response to chemotaxis, clearance of dead cells and facilitating apoptosis (Chen et al 2022). Moreover, our findings are consistent with those reported by Selim et al (2014) who revealed that AFB1 causes anemia and decreased hematological indices in *O. niloticus*. Moreover, according to Dana et al (2018), dietary vitamin C supplementation decreased the negative effects of AFB1 on blood biochemical markers including ALT and AST and enhanced fish development performance. Even though there was no significant difference in WBCs across groups in the current investigation, AFB1-contaminated meals decreased WBC values when consumed alone or in combination with vitamin C. Peng et al (2021) revealed that dietary AFB1 up to 100 µg kg^{-1} elevates immune response. The actual values obtained for the serum biochemical parameters and hepatosomatic index (HSI) are shown in **Table 4**, where, in the first category treatments T1, T4 and T7, which were treated with AFB1 for 113 days, showed that fish-fed higher AFB1 diet T7 (80 µg kg^{-1} diet) increased ALT, AST, HSI and creatinine by 69.7, 57.1, 106.9 and 15% respectively compared to fish fed the control diet (T0). The second category treatments T2, T5 and T8 were fish-fed AFB1-contaminated diets for 57 days then shifted to uncontaminated feed for 56 days. The results showed that fish fed the lowest AFB1 diet T2 (20 µg kg^{-1} diet) reduced creatinine by 35%. ALT, AST and HSI were increased by 26.1, 28.5 and 98.0 % respectively compared to those fed the control diet (T0). A fish-fed diet containing 40 µg kg^{-1} diet (T5) reduced creatinine by 20%. ALT, AST and HSI were elevated by 34.9, 57.1 and 33.3 % respectively compared to those in the control group (T0) while fish fed the highest AFB1- contaminated diet T8 (80 µg kg^{-1} diet) reduced creatinine by 20%, but increased ALT, AST and HSI by 56.7, 57.1 and 49.0 % respectively compared to those in the control group (T0). In the third category treatments (T3, T6 and T9), fish fed T3 diet (20µg AFB1 Kg^{-1}diet + Vit. C) increased ALT, AST and HSI by 24.0, 7.1 and 41.2 % respectively compared to those in the control group (T0). Fish fed of T6 diet 40µg AFB1 Kg^{-1} + Vit. C (T6) increased ALT, AST and HSI by 37.0, 14.2 and 22.6 % respectively compared to those in the control group (T0). Fish fed the diet containing 80µg AFB1 Kg^{-1} + Vit. C (T9) increased ALT, AST and HSI by 50.1, 14.2 and 66.7 % respectively compared to those in the control group (T0). However, serum creatinine did not significantly differ among treatments T3, T6 and T9 in the third category and those in the control group (T0) as indicated in **Table 4.** These results are consistent with Pietsch (2020) findings that showed that elevated AFB1 levels (for example, at levels 250 µg kg^{-1} diet) in *O. niloticus* can affect blood biochemical markers. Similarly, Abdel Rahman et al (2017) noted that AFB1 led to notable increases in serum ALT and creatinine. Additionally, according to Varior and Philip (2012), the lysosomal membrane's constancy was significantly altered by AFB1, which resulted in pathological disturbances and a disorder of hepatocyte permeability in *O. mossambicus*, which were supported by increased levels of the AST and ALT enzymes. As a result, the increased blood levels of AST and ALT showed that these cellular enzymes have been released into the blood, which may indicate probable liver necrosis and altered hepatocyte membrane permeability. According to Dana et al (2018), vitamin C supplementation increases development performance and lessens the negative impacts of AFB1 on biochemical markers including ALT and AST.

3.4 Histological examination

The liver and kidney of *O. niloticus* fingerlings were examined for the presence of anomalies. The results showed that while the control has normal structures, a direct correlation with the rate of deterioration in liver and kidney structure was observed in treatments with AFB1 that continued for 113 days. While in treatments T2, T5 and T8 in which fish shifted from an AFB1-contaminated diet to a non-contaminated diet showed improvement in liver and kidney structure. Similar improvement in liver and kidney structures was also observed in third-category treatments T3, T6 and T9, in which fish were fed the AFB1-contaminated diet and supplemented with vitamin C. Despite fish in those treatments continued to feed contaminated diets,

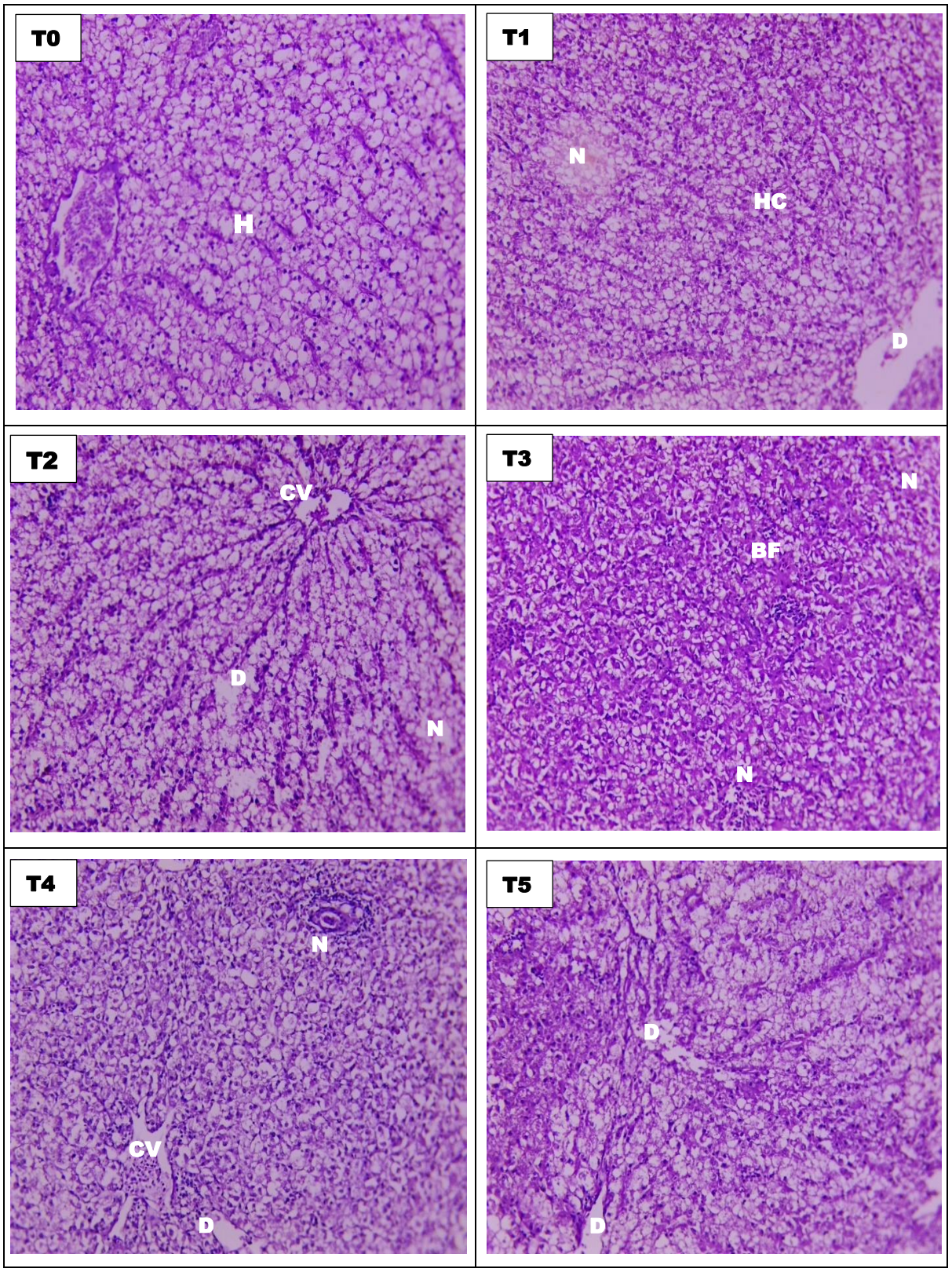
Table 4. Effect of aflatoxin B1-contaminated diet on serum biochemical parameters and hepatosomatic index of *O. niloticus*

Treatments	Period (day)	ALT(GPT) (IU L^{-1})	AST(GOT) (IU L^{-1})	HSI (%)	Creatinine (mg dL^{-1})
Control (T0)	113	$7.66^{b} \pm 1.33$	7.0 ± 0.86	$1.02^{d} \pm 0.06$	$0.20^{b} \pm 0.06$
20 µg (T1)	113	$11.0^{ab} \pm 0.57$	9.0 ± 0.33	$1.26^{cd} \pm 0.09$	$0.40^{a} \pm 0.11$
40 µg (T4)	113	$11.66^{a} \pm 0.86$	11.0 ± 0.57	$1.78^{abc} \pm 0.21$	$0.16^{b} \pm 0.06$
80 µg (T7)	113	$13.0^{a} \pm 0.86$	11.0 ± 1.00	$2.11^{a} \pm 0.37$	$0.23^{ab} \pm 0.03$
20 µg (T2)	57	$9.66^{ab} \pm 0.33$	9.0 ± 0.57	$2.02^{ab} \pm 0.22$	$0.13^{b} \pm 0.10$
40 µg (T5)	57	$10.33^{ab} \pm 0.86$	11.0 ± 1.15	$1.36^{bcd} \pm 0.13$	$0.16^{b} \pm 0.03$
80 µg (T8)	57	$12.0^{a} \pm 0.57$	11.0 ± 0.66	$1.52^{abcd} \pm 0.18$	$0.16^{b} \pm 0.07$
#20 µg + Vit. (T3)	113	$9.50^{ab} \pm 0.86$	7.5 ± 0.86	$1.44^{abcd} \pm 0.18$	$0.20^{b} \pm 0.03$
#40 µg + Vit. (T6)	113	$10.5^{ab} \pm 0.86$	8.0 ± 1.15	$1.25^{cd} \pm 0.10$	$0.20^{b} \pm 0.06$
#80 µg + Vit. (T9)	113	$11.5^{a} \pm 0.86$	8.0 ± 0.54	$1.70^{abcd} \pm 0.31$	$0.20^{b} \pm 0.06$
Significance		*	NS	*	*

Means with different superscripts in the same column are significant and at $P < 0.05$.
#: Supplemented by 100 mg vitamin C kg^{-1} diet on day 58 until the end of the experimental period.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, HSI: hepatosomatic index.

supplementation of vitamin C mitigated the damage caused by AFB1. As stated by Anater et al (2020), the presence of AFB1 contamination may result in focal necrosis, inflammation of cells and hypertrophy or sporadically large multinucleated hepatocytes, pleomorphic nuclei and hyperchromatic, which are indicators of hepatoma. The current findings in **Figs 2 and 3** showed that the effect was progressive with increasing levels of dietary AFB1.

It is well-recognized that the liver is the primary organ affected by fish aflatoxicosis. AFB1-8,9 epoxide, which is produced by the liver as a response to aflatoxins may be activated to be genotoxic and carcinogenic, and it negatively interacts with many cellular proteins to cause tissue and cellular damage (Anater et al 2020, Souza et al 2020) as an indication of liver damaged and aberrant hepatocyte membrane permeability (Gonçalves et al 2018). According to Annabi et al (2011) and Anater et al (2020), lesions of the caudal kidney of fish exposed to toxic substances frequently included necrosis, glomerular blood vessel enlargement, and an increase or decrease in Bowman's capsule space. In the current investigation, fish exposed to AFB1 showed renal tissue abnormalities when compared to the control group. These observations are consistent with the findings obtained by Zeng et al (2019) in grass carp, *Ctenopharyngodon idella* that exhibited vacuolar degeneration and cell necrosis due to exposure to AFs. They also confirmed that as the intake of AFB1 in the diet rises from 59 to 147 µg kg^{-1}, the severity of these findings worsens. They partly explained these results by the way AFs interact with the nucleic acids DNA and RNA.


T0
H
T1
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T5
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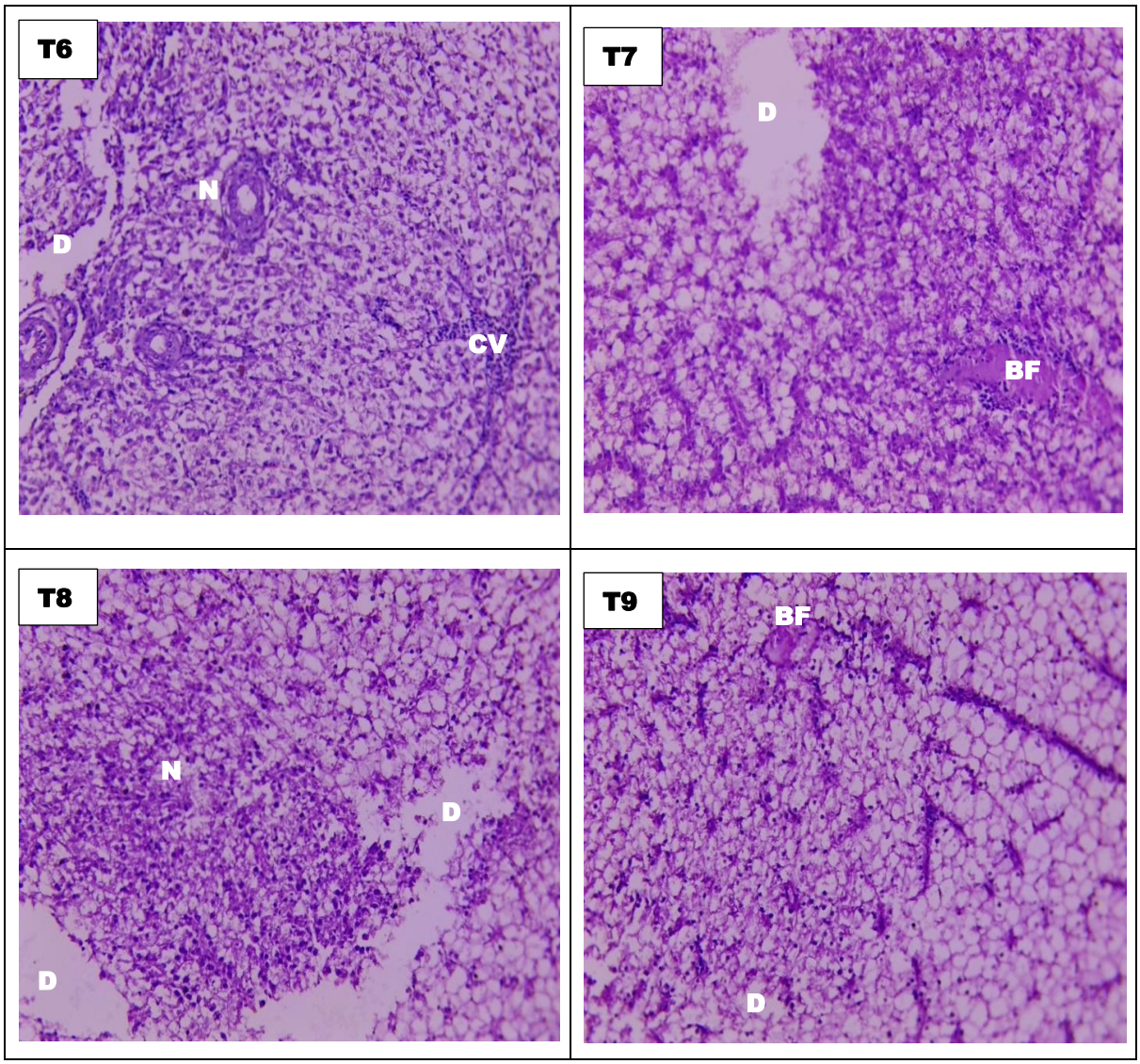
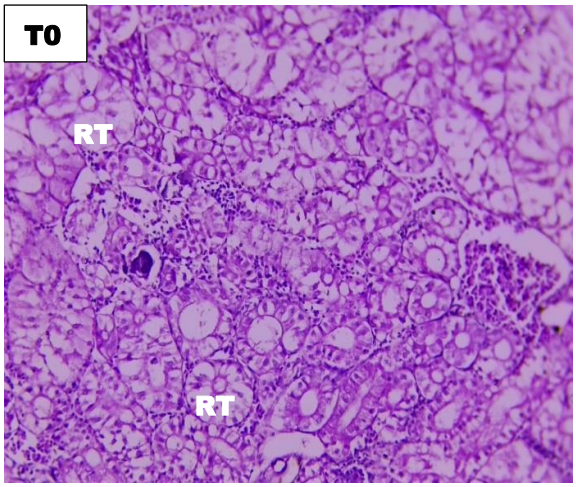
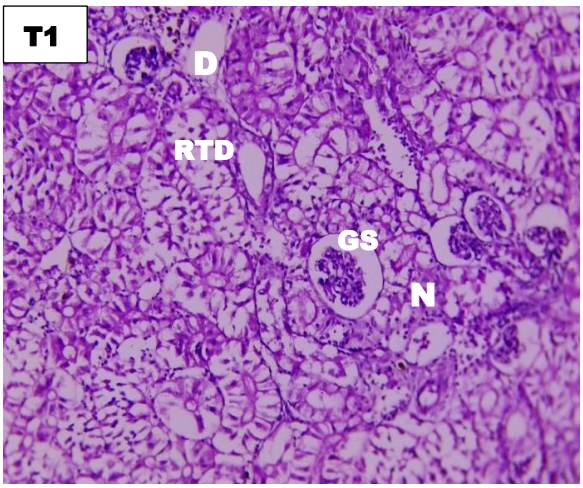
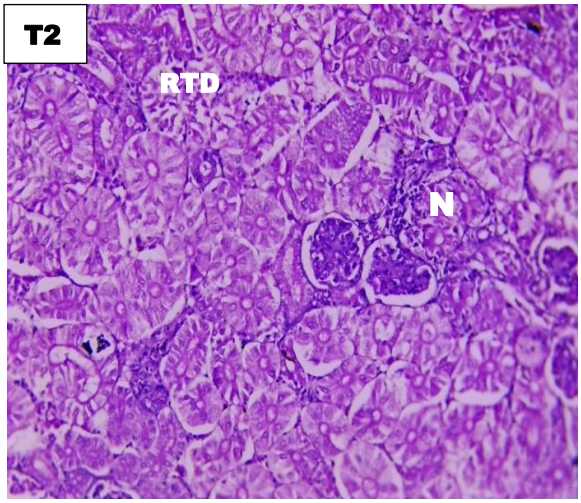
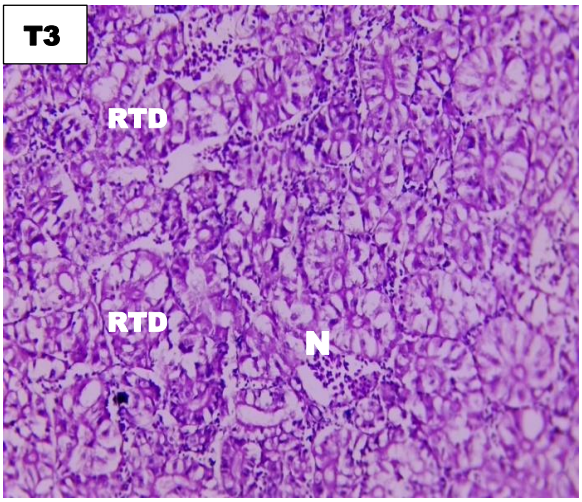


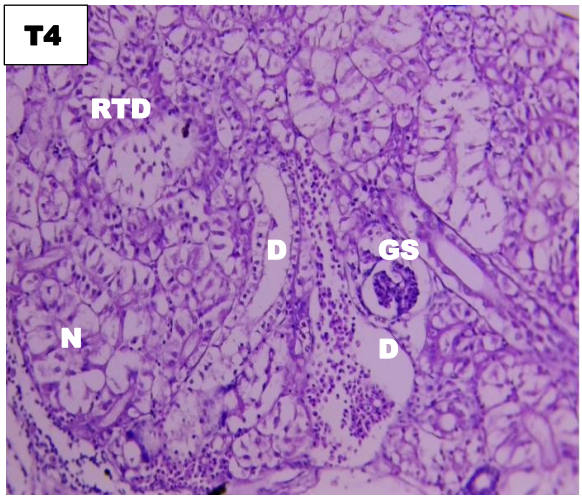
Fig 2. Cross sections of *O. niloticus* liver stained with H&E (X 20 ×0.45) showing the difference in hepatic structure between non treated group, (T0) and other AFB1-treated groups. Treatments T1, T4 and T7, fish fed different AF contaminated diets (20, 40 and 80 µg kg^{-1} diet), respectively throughout the experiment period. Treatments T2, T5 and T8, fish fed AFB1-contaminated diets for 57 days then shifted to uncontaminated diets for the remaining 56 days of the experimental period. Treatments T3, T6 and T9, fish fed AFB1-contaminated diets for 57 days then shifted to contaminated diets supplemented with 100 mg kg^{-1} diet of vitamin C until the end of the experimental period. Section shows hepatic structures; HP, hepatopancreas; HC, hepatic cells; CV, central vein; D, degeneration; BF, blood infiltration and N, necrosis.


T0
RT
RT


T1
D
RTD
GS
N


T2
RTD
N


T3
RTD
RTD
N


T4
RTD
D
GS
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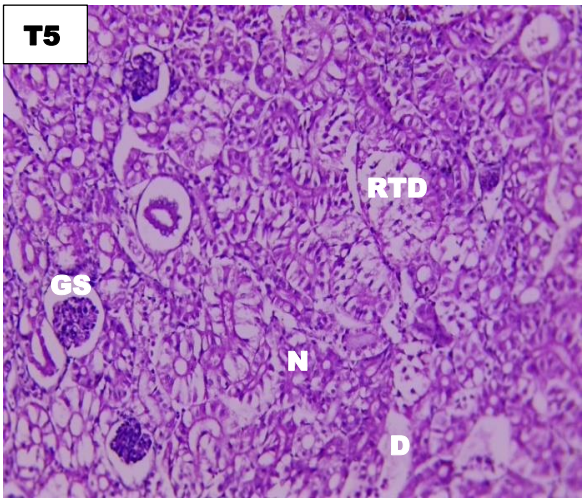
D


T5
RTD
GS
N
D

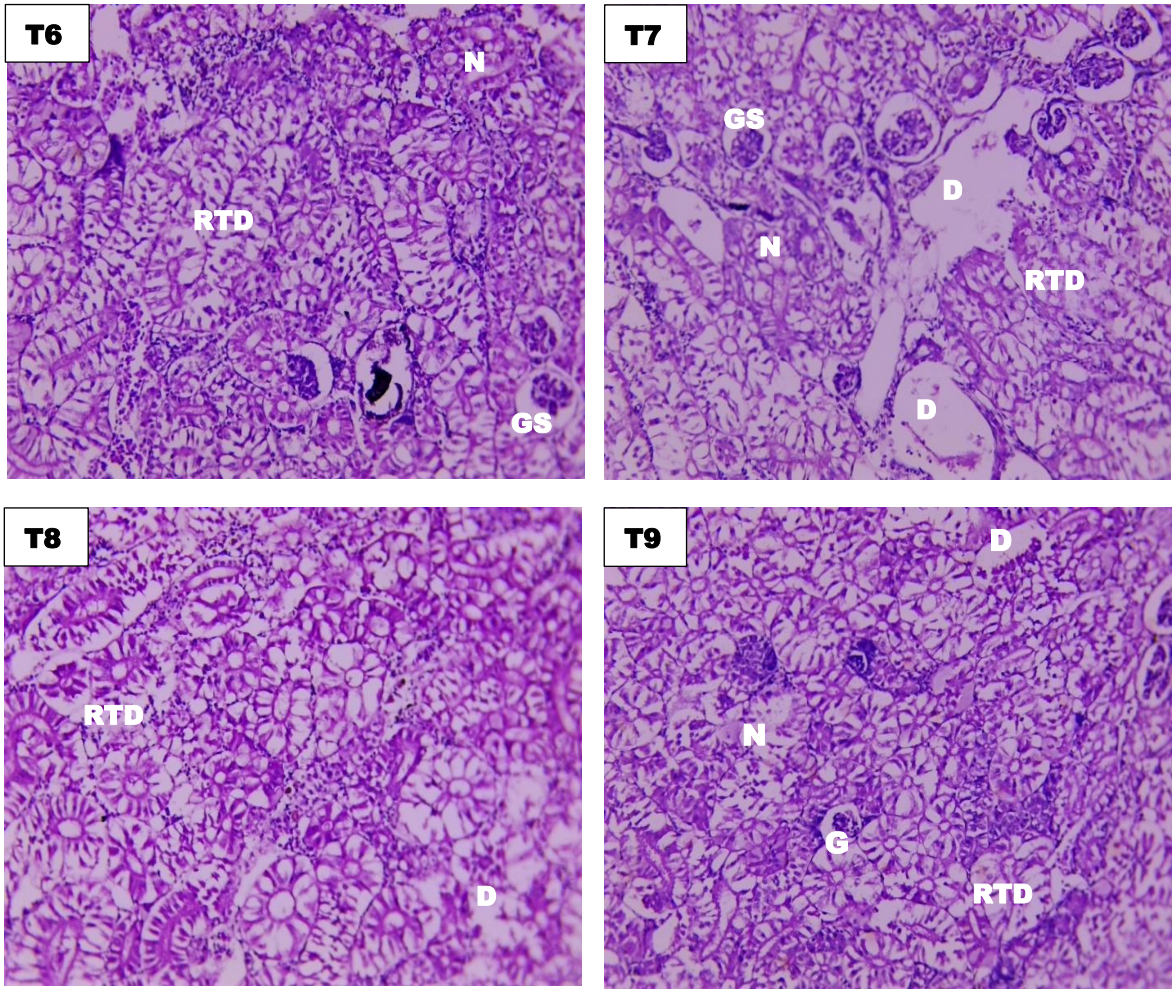


Fig 3. Cross sections of *O. niloticus* kidney stained with H&E (X 20 × 0.45) showing differences between non-treated fish, (T0) and other AFB1-treated groups. Treatments T1, T4 and T7, fish fed different AFB1-contaminated diets (20, 40 and 80 µg kg^{-1} diet), respectively throughout the experiment period. Treatments T2, T5 and T8, fish fed AFB1-contaminated diets for 57 days then shifted to uncontaminated diets for the remaining 56 days of the experimental period. Treatments T3, T6 and T9, fish fed AFB1-contaminated diets for 57 days then shifted to contaminated diets supplemented with 100 mg kg^{-1} diet of vitamin C until the end of the experimental period. The section shows: RT, renal tubules; RTD, renal tubules degeneration; GS, glomerular shrinkage; N, necrosis; and D, degeneration

4 Conclusions

The outcomes of the current experiment revealed that the feed contaminated with AFB1 (*Aspergillus flavus*) at concentrations up to 80 µg kg^{-1} diet showed significant differences compared with the uncontaminated feed in terms of the productive performance of fish represented in growth performance (TWG, ADG and SGR), feed efficiency parameters (FCR and PER), and SR of fish, as well as hematological and serum biochemistry parameters (ALT, AST and creatinine), and HSI. In addition, the kidney and liver tissues suffered damage from AFB1-contaminated diets. Therefore, the existing data indicates that vitamin C supplementation may improve fish health and productivity when AFB1 is present. However, it is important to note that the optimal dosage and duration of vitamin C supplementation may vary depending on the specific conditions of the aquaculture system and the degree of AF contamination. Finally, further attempts are seriously required regarding determining the suitable level of vitamin C and the optimum recovery period as protective strategies against aflatoxicosis not only for *O. niloticus* but also for other fish species.

Acknowledgment

The authors are thankful to Fish Branch, Faculty of Agriculture, Ain Shams University, for providing laboratory facilities for research work.

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