Study on toxicity of *Oreochromis niloticus* with aflatoxinB1

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ABSTRACT

The purpose of this study was to determine the effect of aflatoxin B_1 (AFB_1) on immunity and hematological profile, RBC, WBC, Hb, serum protein, albumin-A, globulin-G, A/G ratio, phygocytic activity, nitro blue tetrazolium reduction and challenge test against *Aeromonas hydrophilla* of apparently healthy 150 Nile tilapia *Oreochromis niloticus* weighed 23.42±1.2 gram. After 84 days of exposure to AFB_1 100 and 20 µg/kg fish samples were collected then *O. niloticus* were treated with biological antitoxin for 3 days then samples were taken after stoppage the treatment by 7 days. Immune activities and hematological profile were significantly ($P \leq 0.05$) influenced in dose and duration of exposure dependent manner this was reflected in the health and growth performance of *O. niloticus*. Results indicated that improvements had achieved by lowering the AFB_1 compained with biological treatment.

Keyword: Aflatoxin B_1, *Oreochromis niloticus*, immunity, blood, liver enzyme.

INTRODUCTION

Aflatoxins are mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *A. parasiticus* (Cheeke and Shull, 1985). In Egypt, aflatoxins and other mycotoxins are frequently detected in feedstuffs such as cottonseed, peanut, corn, milo, rice, dried fish, shrimp, and meat meals (Hassan *et al*., 2002). Problems associated with mycotoxins tend to be worse in the tropics like Egypt where high humidity and temperature create optimal conditions for fungal growth. Hagazy (1988) reported that 32% of examined cereal grains and 6% of examined concentrates and fish meals contained 1-50 ppb of aflatoxin; 9% of cereal grains and 3% of concentrates and fish meal contained 51-200 ppb and 8% of cereal grains and 16% of concentrates and fish meal contain 201-2000 ppb.

The ingestion of aflatoxin contaminated diets led to hazard effects on fish production and health (Shehata *et al*., 2003 and Zaki *et al*., 2008). The principle aflatoxin, AFB_1, is one of the most toxic of all naturally occurring carcinogens. A major epidemic of liver tumors (hepatomas), which struck US trout hatcheries in the early 1960s, was traced to contaminated cottonseed meal in the feeds (Wolf and Jackson, 1963). Aflatoxin causing damages of different organs lowering immunoglobuline production leading to increase susceptibility to infectious diseases. Aflatoxin interferes with vaccine-induced immunity in livestock and lowers the resistance to infectious diseases (Diekman and Green, 1992). Aflatoxin extraction or detoxification has proven practical for only a few feed ingredients, e.g., ammoniation of cottonseed meal has been reported to effectively detoxify the aflatoxin contaminant (Ahmad *et al.,* 1996). So the aims of this work were to investigate the effects of

aflatoxin B1 on immune status of *O. niloticus* and to evaluate the effectiveness of biological antitoxin.

MATERIALS AND METHODS

1- Experimental design:

A total number of 150 apparently healthy of *O. niloticus* were obtained from private fish farms at El-Hamol -Kafr El-Sheikh Governorate- weighing 23.42±1.2 gram. *O. niloticus* acclimated in fiberglass tanks for 15 days to laboratory conditions. Fish randomly distributed in glass aquarium (50 x 40 x 40 cm) containing about 60 liters of dechlorinated water and aquarium water temperature was adjusted at 25±2.5 ^{o}C as well as continuous oxygen supply by air pump. *O. niloticus* were fed pelleted ration (purchased from local market) with daily percentage 3% of body weight six days per week. Experiment had lasted for 94 days divided into two phase 84 and 10 days.

Source of AFB_1:- AFB_1 was produced through pellets fermentation using *Aspergillus parasiticus* NRRL 2999 according to the method described by Abdelhamid and Mahmoud (1996).

O. niloticus were divided into 5 treatments T1, T2, T3, T4 and T5, each treatment had 3 replicates

T1-fed AFB_1 at level 100 µg/kg for 84 days.

T2-fed AFB_1at level 100 µg/kg for 84 days then 20 µg/kg for 10 days.

T3-fed AFB_1 at level 100 µg/kg for 84 days then 20 µg/kg +biological antitoxin 2g/kg diet for 3 days then20 µg/kg for 7days.

T4-fed AFB_1 20 µg/kg for 84 days.

T5- fed $AFB_1$20 µg/kg + biological antitoxin 2g/kg diet for 3 days then AFB_1 20 µg/kg 7 days.

Biological antitoxin:- A blend of biological substances trade name syner-tox (purchased from local market) consists of citric acid 80 ml, phosphorus acid 60 ml, malic acid 5ml, tartaric acid 5 ml, disodium EDTA 15 ml, propylene glycol 100 ml, lactic acid 80 ml, calcium lactate 25 ml, dried *bacillus subtilis* fermentation extract 260 ml, sodium citrate 40 gram, papain 40 gram, distilled water (180 ml) upto 1 liter.

Determination of AFB_1 in ration:- The quantitative determination of AFB_1 by quantitative thin layer chromatography TLC was carried out according to the method of Eppley (1968).

Growth performance was calculated according to the following equations:-

a-Average daily gain (ADG) = (W1-W0)/T Where, W0 and W1 were the initial and final body weight per (g), and T is the number of days in the feeding experimental period (Castell and Tiews, 1980).

b-Total weight gain (TG) = Wt1-Wt0 Where wt1 is the final body weight (g) and wt0 is the initial body weight (g) (Castell and Tiews, 1980).

c-Feed conversion ratio (FCR) = Mass of feed intake (g)/ body mass gain (g) Where, the weight gain is (the biomass of fish at the start + the biomass of the dead fish- the biomass of the fish at the end) (Tacon, 1987).

d-Relative Growth Rate (RGR %) = 100(final weight–initial weight)/Initial weight.

e-Survival rate % = No. of live fish in specific period / Total population during that period x 100.

2- Clinical, post mortem examination and survival rate of *O. niloticus*:

The collected fish were clinically examined according to Amlacher (1970). They were examined for any abnormalities exophthalmia, skin, erosion, ulcers,

hemorrhages and detachment of scales. The collected fish were opened according to method described by Amlachar (1970). Internal organs were examined by making three cuts the first from infront of anus through abdominal cavity toward the head then the second perpendicular to the first behind the bronchial cavity and the third cut ran from anus to head parallel to the lateral line. Then the abdominal wall was removed and internal organs were investigated.

3-Haemogram analysis of *O. niloticus*:

Blood samples were taken two times, the first after 84 days and the second after 10 days. Blood film was prepared according to the method described by Lucky (1977). Red blood cell (RBCs) and White blood cell (WBCs) counts were counted by haemocytometer according to Stoskopf (1993). Mean Corpuscular hemoglobin concentrations were calculated according to the formula mentioned by Dacie and lewis (1975).

M.C.V. = (PCV / RBCs) x 10 as m/mm^3.

M.C.H. = (HB content gm/100ml/ RBCs) x 10 as m/mm^3.

M.C.H.C. = (HB content gm/100ml / PCV) x100 as %.

4- biochemical analysis of *O. niloticus*:

The concentration of total protein (TP) (Weichsellbaum, 1946) and albumin (A) (Doumas *et al.,* 1971) were measured by colorimetric methods, While, globulin concentrations (G) were determined by subtracting the concentration of TP from A concentration.

Electrophoretic pattern of serum protein fractions was estimated using polyacrylamide gel columns (Maurer, 1971) and gel was scanned and read according to Glick (1968).

The activity of the liver enzymes, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were determined according to (Reitman and Frankel, 1957). The activity of the kidneys creatinine were determined according to Henry (1974) and urea was determined according to Patton and Crouch (1977) kits (dp International). Alkaline phosphatase (ALP) was measured according to (Rec, 1972) by using kits reagent supplied by Diamond Diagnostic Co.

5- Immunity activities of *O. niloticus*:

a- Macrophage phagocyte indices:-

Leukocytes isolation was performed according to the method described by Faulmann *et al.* (1983) and phagocytic activities were determined according to Kawahara *et al.* (1991). Blood was collected from the caudal vessels by syringe moisten with heparin (100 IU/ml). *C. albicans* was prepared as 24 hours old culture, the number of *C. albicans* cells was counted for obtaining the required concentration $1x10^6$ yeast cells/ml. Separated peripheral blood leucocytes were adjusted to a concentration of 2.5 x 10^6 viable cells/ml, then to each I ml volume of adjusted blood leucocytes *C.albicians* suspension was added, then incubated in an incubator (CO_2 5-10%) at 37 ^{0}C for one hour. Cover slips were stained with Giemsa stain. The phagocytic assay was calculated according to the following equations:

Phagocytic activity = No. of Ingesting phagocytes / total No. of phagocytes.

Phagocytic index = No. of ingested *C. albicans* cells / No. of Ingesting phagocytes.

b- Measuring of hepatosomatic index (HSI):-

At the end of experimental period, 5 fish from each group were dissected and the viscus was examined. The liver was weighed and HSI was calculated according to (Htun-hun, 1978).

HSI = weight of the liver/fish body weight.

c-Neutrophils glass- adhesion assay

Neutrophils glass- adherent, using nitroblue tetrazolium assay, was determined according to Anderson *et al.* (1992). Briefly, one drop of heparinized blood sample was placed onto cover slip. The cover slips were incubated for 30 minutes at room temperature (25^{o}C) in humid chambers to allow the neutrophils to stick to the glass. Cover slips were gently washed with PBS (pH 7.4) and the cells were transferred to a microscope slide containing a 50 µl drop of 0.2% filtrated NBT solution (Fluka Buchs, Co. Switzerland). The positive, dark-blue stained cells were counted under the microscope.

d-Challenge test:-

A total number of 25 fish (5 fish from each treatment) were injected I/P with the pathogenic *A. hydrophila* which kindly obtained from fish diseases department, animal health research institute (0.3 ml of 10^{8}cells/ml) according to Schaperclaus *et al.* (1992), the injected fishes were kept under observation for 14 day to record the mortality rate.

Mortality rate % = No. of death in specific period/ Total population during that period x 100.

6-Statistical analysis:-

Duncan's Multiple Range Duncan (1955) was used to determine differences among means at significance level of 0.05. All statistics were run on the computer using the SPSS program (SPSS, 2004).

RESULTS AND DISCUSSION

1- Clinical and Post mortem examination of *O. niloticus:*

O. niloticus in group fed highly contaminated ration T1 suffered from loss of appetite, lethargy, loss of reflexes at the end of the experiment. El-Boshy *et al.* (2008) agreed with our findings as they stated that AFB_1intoxicated fish showed off feed, sluggish swimming, dark skin, loss of reflexes, increased mucus secretion, loss of scales and ascities .Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages.

Post examination presented in Figs (1, 2, 3and 4) revealed that *O. niloticus* in TI group which fed on high AFB_1 diet exhibit some pathological lesion as enlarged hepatopancrease, enlarged gall bladder spleenomegaly, and empty intestinal tract.

Also it was observed that the intense of clinical signs increased with high AFB_1 concentration and period of exposure. Similar pictures as spleen and the Kidneys appeared enlarged, congested and dark in color were noticed by (El-Boshy *et al.*, 2008 and Halver, 1968). Chavez *et al.* (1994) reported that *O. niloticus* fingerlings were able to tolerate the immediate effect of aflatoxin but later the fish developed external and internal abnormalities. Liver of fish were abnormal enlargement and yellowing were reported by (Roberts, 1978 and Wu, 1998). Also our results agreed with findings of Dimia *et al.* (2005) who mentioned that mycotoxins induce several disorders in fish organism; biochemical, functional, morphological and in more severe cases mortality.

2- Growth performance and feed utilization of *O. niloticus* fed different AFB1 levels:

Data presented in (Table 1) showed that group T4 (AFB_1 20µg/kg) had significantly higher growth parameters and feed utilization (ADG, TG, FW, SGR and RGR) and (FCR and FI) compared with T1 ($AFB_1$100µg /kg). SR the same trend, T4 had significantly higher recorded SR. Our findings agreed with some experimental studies as Shehata *et al.* (2009) stated that aflatoxicosis in *O. niloticus* induced by fed

on 3 mg AFB_1 significantly ($P \leq 0.05$) decreased body weight gain, relative growth rate in comparison with these of control and also they mentioned that the mortality rate significantly ($P \leq 0.05$) increased (53.33% versus 6.67% for the control) by 20µg AFB_1. Also Roberts and Sommerville (1982) mentioned that in tilapia culture aflatoxicosis was a major cause of losses. Lovell (1991) explained low survival rate as aflatoxin caused damage of liver and other body organs which led to death. These findings could be due to disturbance in metabolic processes of carbohydrate, lipid and protein (Cheeke and Shull, 1985 and Devegowda *et al*., 1998). A contrast results were obtained by (Jantrarotai and Lovell, 1990) showed that there was no significant reduction in weight gains or histopathological findings in catfish exposed to a wide range of AFB_1concentrations in diets (ranged from 100 to 2,154ppb AFB_1).

Table 1: Mean ± SE of growth performance parameters and survival rate of *O. niloticus* exposed to different levels of AFB_1.

Item	IW	FW	ADG	TG	FCR	FI	SGR	RGR	SR
T1	**23 ±0.7**	**45.6 ±1.5**	**0.27 ±0.026**	**22.7 ±2.2**	**5.57 ±0.4**	**124 ±3.4**	**0.82 ±0.077**	**99.5 ±12.6**	**80**
T4	**23.87 ±0.5**	**78.7 ±3.6**	**0.65 ±0.037**	**54.9 ±3.1**	**3.4 ±0.12**	**188 ±9.20**	**1.42 ±0.3**	**229.6 ±8.3**	**90**
Sig	**0.38**	**0.001**	**0.001**	**0.001**	**0.008**	**0.003**	**0.002**	**0.001**	**-**
F value	**0.985***	**70.72****	**70.72****	**70.72****	**23.27****	**42.31****	**53.03****	**74.32****	**-**

* Insignificant ** Significant difference at $P \leq 0.05$.

3- Haemogram picture and differential leukogram of *O. niloticus* fed different AFB_1 levels:

The application of haematological have proved to be valuable for fishery biolo01gists in assessing the health of fish and monitoring stress responses either due to fluctuations in environmental condition or due to sub lethal concentration of pollutants. Haematology concerns mainly investigations on cells present in the blood, RBCs, Hb, PCV, WBC, MCH, MCHC and MCV (Kapila, 1999). Findings presented in Table (2) showed that erthrogram of T1had significantly had lower values comparing with T4 indicating that AFB_1 at concentration 100µg /kg could cause an adverse effect on health status and *O. niloticus* may suffer from anemic condition as it obvious from results of RBCs, Hb, PCV, MCV, MCH and MCHC 1.7, 4.6, 17.7, 104.5, 27.4 and 26.4 respectively. Lowering the AFB_1 concentration to 20µg /kg (T2) for 10 days didn't significantly improve *O. niloticus* adverse condition while treatment by biological antitoxin plus lowering the AFB_1 concentration to 20µg /kg would improve significantly the health condition as RBCs, Hb, PCV, MCV, MCH and MCHC 2.32, 8.9, 32.7, 139.7, 38.3 and 27.5. The most improved health status had recorded by T5 as *O. niloticus* had fed on diet contaminated with AFB_1 20µg /kg and treated by biological antitoxin. These results could be explained by the findings of Dimia *et al.* (2005) who stated that biochemical alterations and metabolism disturbance lead to changes in nutrient resorption and primary brings to cell and organ alterations. Our findings agreed with those obtained by (Hussein *et al.,* 2000 and Rizkalla *et al.,* 1997) as they recorded anemia in *O. niloticus* fed on ration supplemented AFB_1. Neutrophilia, and lymphopenia are the main leukocytic pictures in acute stress of teleost fish (Robert 2001). Data concerning WBCs agreed with findings of Jantrarotai and Lovell (1990) reported Leukocytosis AFB_1toxicity in channel catfish *Ictularus punctatus* fed 10 mg AFB_1/Kg diet. Different findings were obtained by Hussein *et al.,* (2000) who mentioned that *Oreochromis* species exposed to 50µg crude AFB_1/Kg feed for 22 weeks, 3 mg AFB_1/ Kg diet for 90 days and 1.0

µg AFB_1/Kg BW for 10 days, respectively suffered from leukopenia these differences could be explained by the different durations. Similar findings had observed by Selim *et al.* (2013) who stated that the total erythrocyte count, hemoglobin content and total leukocyte count were significantly decreased after AFB_1exposure for 6, 8 and 10 weeks, respectively.

Table 2: Mean ± SE of complete blood picture of *O. niloticus* exposed to different levels of AFB_1.

Item	T1	T2	T3	T4	T5
RBCs x10^6	**1.7^{c}±0.1**	**1.97^{bc}±0.1**	**2.32^{ab}±0.22**	**2.58^{a}±0.15**	**2.8^{a}±0.26**
Hb g/dl	**4.6^{c}±0.14**	**6.7^{c}±0.33**	**8.9^{b}±1.16**	**10.1^{ab}±0.52**	**11.2^{a}±0.73**
PCV %	**17.7^{c}±1.2**	**25b^{c}±2.31**	**32.7^{ab}±4.7**	**37.7^{a}±1.2**	**38.3^{a}±1.8**
MCV Fl	**104.5^{b}±6.4**	**126.9^{ab}±9.1**	**139.7^{a}±8.3**	**146.7^{a}±4**	**138.3^{a}±7.21**
MCH Pg	**27.4^{c}±0.95**	**33.9^{b}±0.57**	**38.3^{a}±1.4**	**39.1^{a}±0.23**	**40.15^{a}±1.7**
MCHC%	**26.4^{a}±1.8**	**26.9^{a}±1.4**	**27.5^{a}±1.6**	**26.7^{a}±0.56**	**29.9^{a}±1.5**
WBCs 10^3	**44^{c}±2.3**	**52^{bc}±1.7**	**59^{ab}±3.5**	**61.7^{ab}±4.8**	**68.3^{a}±2.4**
Het %	**25.8^{a}±0.5**	**23.7^{b}±0.5**	**23.3^{b}±0.17**	**22.2^{b}±0.7**	**19.5^{c}±0.64**
Mono %	**5.5^{a}±0.2**	**4.6^{b}±0.16**	**4.53^{b}±0.18**	**4.3^{b}±0.22**	**3.57^{c}±0.2**
Esino %	**2.7^{a}±0.08**	**2.5^{b}±0.1**	**2.16^{c}±0.12**	**2.23^{bc}±0.1**	**2.1^{c}±0.1**
Baso %	**0.33±0.3**	**0.33±0.3**	**0.33±0.3**	**0.33±0.3**	**0.0**
Lympho %	**65.1^{d}±0.95**	**68.8^{c}±1.45**	**69.7^{bc}±1.1**	**70.5^{b}±0.5**	**74^{a}±1.2**

Group with different letter within the same column are significantly different at $P \leq 0.05$.

4- Liver enzymes, kidneys enzymes and serum total protein examination of *O. niloticus* fed different AFB_1 levels:

Serological tests are important diagnostic tools in fish diseases investigation as Kapila (1999) stated that serology deals with the constituents in the fluid part of blood such as protein, enzymes, minerals, carbohydrates pigments, hormones, immune bodies etc. Liver enzymes ALT, AST and ALP activity in serum of *O. niloticus* presented in Table (3) emphasized that *O. niloticus* fed on high AFB_1concentration 100µg /kg T1 had increased significantly comparing with T4 indicating liver damage and impairment of function as they had reached 32.3, 137.7 and 28.6 respectively. An improvement was recorded by lowering the AFB_1to20µg /kg and lowering the AFB_1to20µg /kg plus biological treatment as ALT, AST and alkaline phosphatase had decreased significantly. Creatinine and urea significantly affected by high level AFB_1indicating damages in kidneys tissue.

Table 3: Mean ± SE of liver enzymes, kidneys enzymes and serum total protein of *O. niloticus* exposed to different levels of AFB_1.

Item	T1	T2	T3	T4	T5
ALT	**32.3^{a}±1.86**	**27.3^{ab}±1.45**	**21^{c}±2.8**	**24^{bc}±1.53**	**23.3^{bc}±1.2**
AST	**137.7^{a}±2.8**	**132^{a}±1.5**	**115.3^{b}±3.18**	**115.3^{b}±2.4**	**104.7^{b}±7.1**
Urea (mg/dl)	**5.1^{a}±0.05**	**3.47^{b}±0.9**	**4.1^{ab}±0.6**	**3.77^{ab}±0.09**	**2.9^{b}±0.06**
Creatinine (mg/dl)	**1.03^{a}±0.03**	**0.89^{b}±0.02**	**0.83^{bc}±0.01**	**0.79^{c}±0.02**	**0.8^{c}±0.06**
ALP (mg/dl)	**28.6^{a}±1.7**	**26.8^{a}±1.3**	**20.1^{b}±1.25**	**20.6^{b}±1.6**	**16.9^{b}±0.5**
HSI	**4.27^{a}±0.12**	**3.27^{b}±0.12**	**2.9^{bc}±0.18**	**2.36^{c}±0.3**	**2.16^{c}±0.37**
TP (g/dl)	**2.8^{c}±0.22**	**3.4^{bc}±0.36**	**4.3^{ab}±0.5**	**4.5^{ab}±0.14**	**5.1^{a}±0.5**
A (g/dl)	**1.25±0.18**	**1.3±0.1**	**1.23±0.13**	**1.3±0.12**	**1.15±0.1**
Globulin (g/dl)	**1.6^{b}±0.12**	**2.1^{b}±0.3**	**3.1^{a}±0.3**	**3.24^{a}±0.24**	**3.9^{a}±0.4**
Alpha (g/dl)	**0.42^{c}±0.04**	**0.52^{bc}±0.16**	**0.8^{ab}±0.1**	**0.88^{a}±0.1**	**0.72^{bc}±0.04**
Beta (g/dl)	**0.34^{b}±0.05**	**0.37^{b}±0.08**	**0.9^{a}±0.14**	**0.97^{a}±0.1**	**1.12^{a}±0.14**
(g/dl)	**0.83^{c}±0.04**	**1.2^{bc}±0.14**	**1.37^{ab}±0.1**	**1.36^{ab}±0.2**	**1.75^{a}±0.13**
A/G	**78.9^{b}±1.8**	**68.1^{b}±13.9**	**41^{b}±2.17**	**41^{a}±5.5**	**29.1^{a}±1.7**

Group with different letter within the same column are significantly different at $P \leq 0.05$. A/G=Albumin Globulin ratio.

Creatinine and urea had the same trend of liver enzymes as it had improved significantly by lowering AFB_1concentration and addition of biological antitoxin. Our results could be explained by findings of Kapila (1999) who stated that AST, ALT and alkaline phosphatase enzymes are released into the circulatory system (serum) by cellular damage or destruction. Liver is rich in AST and ALT and changes in plasma levels of these enzymes may be indicative of liver dysfunction. Alkaline phosphatase is involved in membrane transport and is a good indicator of stress in biological systems (Verma *et al.* 1980). Findings of El-Boshy *et al.* (2008) had agreed with our results as they stated that the elevation of ALT and AST activities in *O. niloticus* caused as a result of supplementation diet with AFB_1 could be attributed to hepatic injury. Also Pepeljnjak *et al.* (2003) agreed with these findings as they noticed an elevation in liver enzymes of *Cyprinus carpio* fed 5.0 mg AFB_1/kg body weight for 42 days. Shehata *et al.* (2009) and Abd El-Baki *et al*. (2002) mentioned that tested blood parameters (total protein, albumin, globulin, AST and ALT) of *O. niloticus* fed on diet contaminated with AFB_1 were decreased. Our results also agreed with those obtained with Selim *et al.* (2013) who had experimented hydrated sodium calcium aluminosilicates, *Saccharomyces cerevisiae* and an esterified glucomannan, against feed contaminated with contained 200 µg/kg (ppb) AFB_1 and stated that *O. niloticus* showed reduction of the survivability, total weight gain, average daily gain and specific growth rate, evident as early as the second week of exposure, also they mentioned that prolonged administration of AFB_1led to significant increases in serum ALT, AST and creatinine activity. Determination of blood creatinine is routinely used as an index of renal function (Melby and Altman, 1974). The gills is the main organ of excretion of urea rather the kidney (Stoskoph, 1993) so the elevation of urea could indicate dysfunction of the gills of *O. niloticus* which fed on AFB_1 supplemented diet (Hussein *et al.,* 2000 and El-Boshy *et al.*, 2008). An elevation of serum creatinine might be attributed to the renal damage that had induced renal impairment. In contrast of our results El-Boshy *et al.* (2008) observed insignificant creatinine increased level in AFB_1treated group.

Serum protein concentrations can be used to monitor disease progress and general physiological status, as total protein levels tend to drop in diseased states. Sequential total protein analysis provides quantitative evidence of disease progression (Searcy *et al.,* 1964). The decrease in the serum protein level could be correlated with severe damage of hepatocytes as indicated by histopathological studies. Similar observations were observed in Nile tilapia by (Saber, 1995 and Roberts and Sommerville, 1982). Total protein, albumin, globuline, albumin / globuline ratio and globulin fraction had the same trend of liver enzymes. Total protein and albumin concentrations were decreased in fresh water fishes fed on diet contaminated with AFB_1 and this decline could be explained that mycotoxins had caused a hepatotoxic condition that resulted in impairing protein synthesis and/or liver disorder (Sahoo *et al.,* 1998, Buhler *et al.,* 2000, Pepeljnjak *et al.,* 2003 and El-Boshy *et al.*, 2008). Decrease in total protein and albumin may be attributed to aflatoxin caused hepatotoxicity that interacted with protein synthesis (Srivastava, 1984).

5- Immune status of *O. niloticus* fed different AFB_1 levels:

Immune status of examined *O. niloticus* had been tested by measuring phagocytic activities, neutrophil glass adhesion test and challenge against *A. hydrophilla*. Data presented in Table (4) indicated that *O. niloticus* fed highly contaminated diet T1 will exhibit lower immunological status and lowering AFB_1 concentration plus biological antitoxin treatment T3 could improve the immune status. The AFB1 had an immune-suppression; this reduction could be a result of the toxic

effect of aflatoxins on the hematopoietic tissues (Ghosh *et al.,* 1990). Glass-adherent NBT-positive cells which indicated the suppression of non-specific immunity level in *O. niloticus* Sahoo and Mukherjee (2001)agreed with the results of our experiment observed that decreased glass adhesion NBT-positive neutrophils of *Labeo rohita* exposed to AFB_1.The macrophage phagocytic index was significantly decreased in AFB1treated groups suppressed the macrophage phagocytic activity and macromolecular synthesis of macrophages as reported in *O. niloticus* (El-Enbaawy *et al.,* 1994 and El-Boshy *et al.*, 2008). Our results could be explained by those obtained by Rodriguez-Cervantes *et al.* (2010) as they stated that aflatoxins generate a long-term dysfunction in the specific and non-specific immune response of aquatic organisms. Also Selim *et al.* (2013) mentioned that AFB_1 reduced the serum levels of total protein, albumin and globulin and after challenge with *A. hydrophila*, AFB_1 produced a low level of agglutinating antibody titer and a scant relative level of protection. *S. cerevisiae* and esterified glucomannan effectively improved AFB_1 toxicity.

Table 4: Mean ± SE of phagocytic activities and neutrophils glass adhesion of *O. niloticus* exposed to different levels of AFB_1.

Item		T1	T2	T3	T4	T5
Phagocytic	index	**0.67^{c} ±0.33**	**0.87^{bc} ±0.44**	**1.7^{bc} ±0.38**	**1.8^{b} ±0.35**	**3.1^{a} ±0.21**
	assay	**16.7^{c} ±0.8**	**20^{bc} ±1.2**	**48.3^{ab} ±0.78**	**43.3^{b} ±3.3**	**61.7^{a} ±6**
Neutrophils glass		**6^{d} ±0.6**	**7.^{7d} ±0.3**	**10^{c} ±1.12**	**15.3^{b} ±0.6**	**17.3^{a} ±0.3**
Challenge test MR%		**100**	**100**	**60**	**40**	**20**

Group with different letter within the same raw are significantly different at P≤0.05.

CONCLUSION

AFB_1 had an adverse effect on immune status and liver function of *O. niloticus* leading to decrease feed utilization and increase susceptibility to infectious diseases. These bad effects could be lowered by decrease the level of AFB1 and use of biological antitoxin.

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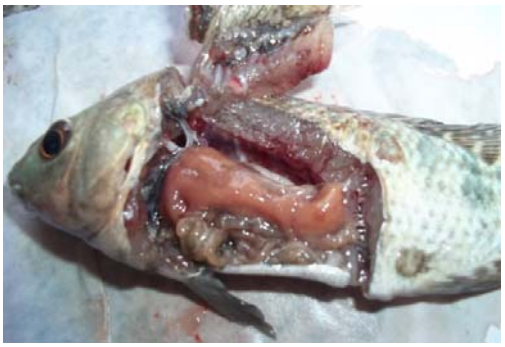

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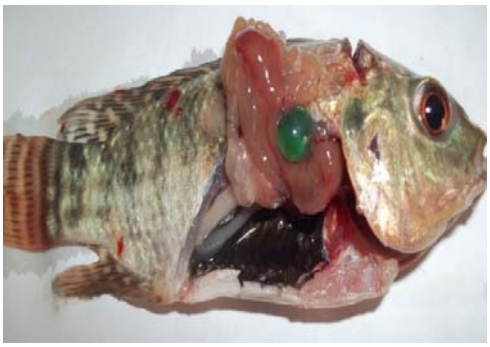

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Fig. 1: *O. niloticus* fed on diet contains $AFB_1$100µg /Kg showed hepatomegaly with pale colour.
Fig. 2: *O. niloticus* fed on diet contains $AFB_1$100 µg /Kg showed hepatomegaly, clear distended gall bladder.
Fig. 3: *O. niloticus* fed on diet contains $AFB_1$100µg /Kg showed enlarged of gall bladder and empty intestinal tract.
Fig. 4: *O. niloticus* fed on diet contains AFB_1 20µg /Kg showed spleenomegaly and normal liver.

ARABIC SUMMARY

دراسة علي تسمم أسماك البلطى النيلى بالافلاتوكسين

احمد حماد شريف[١] و شحاتة عبد المقصود عبد الحكيم[٢] و مصطفي شكري[٣]

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يهدف العمل الي دراسة تأثير الافلاتوكسين ($Aflatoxin\ B_1$)علي الحالة المناعية وصورة الدم وكفاءة الكبد. تم تغذية اسماك البلطي النيلي (وزن ٢٣,٤٢±١,٢ جرام) لمدة ٨٤ يوم علي علف مضاف اليه ١٠٠ و ٢٠ ميكرو جرام / جرام. تم معالجة الاسماك لمدة ٣ يوم بمضاد سموم بيولوجي (سنر توكس) حسب الجرعة الموصي بها من الشركة المصنعة. تم أخذ عينات الدم بعد ٧ ايام من انتهاء العلاج. وأظهرت النتائج ان تأثير السموم الفطرية يعتمد علي الجرعة ومدة الاضافة. كذلك وجد تحسن معنوي في مناعة الاسماك ووظائف الكبد عند خفض نسبة السموم المضافة للعلف مع أستعمال مضاد سموم حيوي.