THE EFFICACY OF MYCOSORB AND ZEOLITE TO ALLEVIATE T-2 TOXINS-INDUCED TOXICITY IN CULTURED *OREOCHROMIS NILOTICUS*

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ABSTRACT

The aim of this work was to find out how *Oreochromis niloticus* were fed diets containing T-2 toxins for a period of 42 days as well as study the role of efficacy of two mycotoxin adsorbents to alleviate T2 toxins -induced toxicity. The *O. niloticus* were treated with 1 g /kg from both Mycosorb (G1) and Zeolite (G2), 0.1 mg/kg of T-2 in (G3), 0.1 mg/kg of T-2 + 1 g/kg Mycosorb in (G4), 0.1 mg/kg of T-2 + 1 g/kg Zeolite in (G5) and control negative group feed on basal commercial ration (G6). T-2 toxins was dissolved in 10 ml/kg of 7% (DMSO). These doses were added to feed daily for a period of seven weeks. Some biochemical blood serum indicators of liver and kidney functions, and leucogram were monitored as well as histopathological picture of some internal organs. The results revealed an increase in the levels of liver enzymes, urea and creatinine; meanwhile, the total protein, Ca, P and Mg, were decreased. It could be concluded that both Mycosorb and Zeolite may be useful tool to minimize the toxic effects of T-2 toxicity by its potent antioxidant activity. The kidney plays an important physiological. Fish treated with both Mycosorb and Zeolite showed an amelioration of the adverse effects of T-2 toxicity and significant improvement in serum electrolytes (calcium, inorganic phosphate, and magnesium) and creatinine level compared with the positive control group (P ≤ 0.05). Mycosorb and Zeolite have the ability to improve the kidney function after T-2 intoxication of *O. niloticus*.

INTRODUCTION

The use of plant-based protein sources in feeds designed for aquaculture has increased in the last decade. This is due to their lower costs compared with fishmeal (Spring and Fegan 2005, Santos *et al.*, 2010 and Encarnação 2011), and its production is believed to be declining (Josupeit 2010).

Production of carnivorous species still requires considerable input of fishmeal (Naylor *et al.,* 2000) but it has been replaced successfully in diets of some omnivorous and herbivorous species (Spring and Fegan 2005). However, this trend is not a panacea because plant-based aquafeed formulations are commonly contaminated with mycotoxins (Santos *et al.,* 2010 and Encarnação 2011).

Mycotoxin contamination of feedstuffs is a serious threat to animal welfare and production, and can result in dramatic economic losses (Iheshiulor *et al.,* 2011). It has been estimated that 25% of the world's feed crops are affected by mycotoxins (Hooft *et al.,* 2010 and Iheshiulor *et al.,* 2011), and that feed is contaminated with one or more mycotoxins (Santos *et al.,* 2010).

Mycotoxins can be mutagenic (Anonymous 2002), hepatotoxic (Manning 2001, Santos *et al.,* 2010), carcinogenic (Spring and Fegan 2005), teratogenic (D´Mello *et al.,* 1999) and often impair immune and reproductive function leading to poor growth rates and increases mortality in animals (Santacroce *et al.,* 2008 and Santos *et al.,* 2010).

Mycotoxins are of increasing concern in aquaculture because mycotoxin contamination is persistent in fish

flesh and residues have been found in marketed fish products beyond acceptable. *Fusarium* fungi are among the three most widespread genera (*Aspergillums*, *Penicillium* and *Fusarium*) in crops. These three genera of fungi produce more than 400 different mycotoxins that are known as the aflatoxins, ochratoxins, fumonisins, zearalenones and trichothecenes (Santos *et al.*, 2010).

From the trichothecenes group, T-2 toxin reduces feed intake, growth, hematocrit and hemoglobin levels in fish. Moreover, mortality is increased via increased susceptibility to bacterial disease (Santos *et al.*, 2010).

Careful selection and adequate processing of raw materials are the best way to prevent mycotoxin contamination, yet it is difficult to guarantee the complete absence of these toxins. Mycotoxin remediation and detoxification procedures are under research and development. Adsorbents can be used to bind mycotoxins in the digestive tract, thereby reducing toxin absorption. The most common substances used for adsorption are clays, bentonites, zeolites, silicas and aluminum silicates, which are effective against aflatoxins only (Encarnação, 2011). Enzymatic deactivation has been used to reduce negative influence of trichothecenes, ochratoxin A and zearalenone (Santos *et al.*, 2010). Few studies have investigated the effects of T-2 toxin on fish despite their potential impacts on aquaculture productivity and human health. *Oreochromis niloticus* is one of the most farmed species worldwide and surprisingly, little is known about the possible responses of *O.niloticus* to T-2 toxin. The aim of this study was to evaluate the effects of T-2 toxin on the some immune parameters, enzymatic activities of liver and kidney as well as the minerals levels of *O.niloticus* and evaluation the effect of two mycotoxin adsorbents to alleviate T-2 toxins.

MATERIALS and METHODS

Experimental
A Total of 180, apparently healthy *Oreochromis niloticus* were obtained from a commercial private fish farm at El-Behera governorate. The fish were acclimatized for 14 days to allow for physiological adjustment, before which data collection started. *O. niloticus* consumed the food *ad libitum*. The basal diets were tested for possible residual of T-2 before feeding (Hansen, 1993) and there were no detectable levels present (detection limit 1 g T-2 and kg-1 in food; recovery of the extraction method 95 per cent). When the *O. niloticus* reached to 6 weeks of breeding, the feeding trial was terminated and 9

O. niloticus from each treatment were selected at random and blood samples were collected into heparinized test tubes from caudal blood vessels vein to determine biochemical and haematological parameters. Red Blood Cell (RBC), White Blood Cell (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), haematocrit values, Total and differential leucocytic count, were determined by a haemocytometer method using Natt-Herrick solution; haematocrit values were measured.

The hematological parameters were determined according to the methods described by Jain (1986). The leukogram was read from blood smears prepared during the animal exsanguination and stained with Giemsa stain. Blood samples were taken without anticoagulant for separation of serum for determination of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Reitman and Frankel (1957), Urea Patton and Crouch (1997), Creatinine, Henry (1974), Calcium, Gitelman (1967), Inorganic phosphorus and Magnesium Burits and Ashwood (2001) and Total proteins Peters (1968).

Serum biochemical indices investigated were total protein, globulin, albumin, and albumin: globulin ratio, creatinine, glucose, uric acid, and cholesterol, alanine amino transferase (ALT) and aspartate amino transferase (AST). The material for the pathohistological examination of the liver, lung, kidney, spleen, pancreas, jejunum, thymus and testicles was fixed in 4% formaldehyde for 2 hours.

Chemicals:
1. Toxin: T-2 toxin, 99% purity (Sigma company in Alexandria branch and obtained from Prof. Dr. Riad Hassan Khalil Faculty of veterinary medicine, Alexandria University MYCOLAB Co., Chesterfield, Missouri 63017, USA). All mycotoxins and chemicals used were supplied by Sigma – Aldrich, Ltd.

2. Dimethylsulfoxid (DMSO) was obtained from El-gomoheria Company, Alexandria, Egypt.

3. Mycosorb was provided from alltech, K.Y., USA.

4. Zeolite was provided by Incal Biotechnology and mining ltd., Izmir, Turkey; and the chemical formula was $KNa_2ca_2 (Si_{29} Al_7) O72, 32H_2O$

Experimental Design:
This study was carried out for seven successive weeks. The *Oreochromis niloticus* were classified into six groups of as follow:

Table 1: Showing the experimental design of study the efficacy of two mycotoxins adsorbents to alleviate T2 toxins -induced toxicity in *Oreochromis niloticus*:

Groups	Treatment	Replicates
T 1	*O. niloticus* were treated with 1 g/kg of Mycosorb.	(3 replicates/ 10 fish each)
T 2	*O. niloticus* were treated with 1 g /kg of Zeolite.	(3 replicates/ 10 fish each)
T 3	*O. niloticus* were treated with 1.0 mg/kg of T-2	(3 replicates/ 10 fish each)
T 4	*O. niloticus* were treated with 1 g /kg of Mycosorb + 1.0 mg/kg of T-2	(3 replicates/ 10 fish each)
T 5	*O. niloticus* were treated with 1 g /kg of Zeolite + 1.0 mg/kg of T-2	(3 replicates/ 10 fish each)
T 6	*O. niloticus* were feed on basal diet	(3 replicates/ 10 fish each)

Hematological studies:

At week 6, two fish per replicate were bled using hypodermic syringe. Blood was drained into two different carefully labelled bottles for haematological and serum biochemistry investigation.

The blood samples for haematological parameters were collected into the bottle pre-treated with anticoagulant (EDTA). Blood samples for biochemical indices were collected into another bottle without anticoagulant. These samples were spurned in the centrifuge at 3,000 rpm and the clearer portion decanted into small sample tubes stored in a freezer. The haematological indices examined include (RBC), (WBC), (PCV), haemoglobin concentration (Hb) and Leucocytes differential count (monocyte, lymphocyte, eosinophil etc.).

PCV was determined by spinning about 75 μl of each blood samples in heparinised capillary tube in a haematocrit centrifuge for about 5 min and read on haematocrit reader (Benson *et al.,* 1989).

Erythrocyte and Leucocytes counts were determined using Neubaur chamber method as described by Lamb (1981). The blood sample collected in each treatment was diluted at a ratio of 1: 200 for RBC counts using red cell diluting fluid while a dilution ratio of 1: 20 (blood: white cell diluting fluid) was used for WBC counts. Samples of RBC and WBC counts were obtained using the relationship:

RBC/μl = Numbers of red blood cells counted × 5 × 10 × 200
WBC/μ = Numbers of white blood cells counted × 0.25 × 10 × 20.

Haemoglobin was estimated using cyanomethaemoglobin method. 0.02 ml of blood was expelled into 4 ml drabkin solution. The mixture was allowed to stand for 5 min for full colour development (Jain, 1986).

Haemoglobin (g per 100 ml) MCH = × 10 RBC count million per cu.mm or

The biochemical examination of blood serum was performed with the automatic spectrophotometer the following parameters were monitored: urea nitrogen, creatinine, Triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol and glucose.

The serum total protein was determined by the Biuret method (Reinhold, 1953) using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method (Doumas and Biggs, 1971). The globulin and albumin/globulin ratio were determined according to the method of Coles (1986).

The serum creatinine and urea nitrogen were estimated by deproteinisation and Urease Berhelot colorimetric methods, using a commercial kit (Randox Laboratories Ltd, U.K). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods, respectively using commercial kit (Quimica Clinica Applicada, S.A), while the serum enzymes; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

However, measurement of Ca, P and Mg. were carried out as the technique described in the references (Brown *et al.,* 1986 and Brown and Taylor, 1995).

Histopathological studies:

From the recent dead cases, tissue specimens were collected directly from liver, spleen and kidneys for histopathological examination. They were kept in 10% neutral buffered formalin for at least 24 hours, routinely processed by the standard paraffin

embedding technique and stained with Hematoxyline and Eosin (Bancroft *et al.,* 1994).

Statistical Analysis:
All data generated on performance, haematology and serum biochemistry of the experimental *O. niloticus* were subjected to statistical analysis of variance procedures of SAS Institute Inc. (SAS, 2006). The treatment means were compared using the Duncan's procedures of the same software.

RESULTS

1- Results of clinical signs of T-2 toxicity in *Oreochromis niloticus*:
O. niloticus experimentally exposed to T-2 toxin (group III) showed severe darkness colouration of the dorsal area, caudal peduncle, and eye cataract throughout the experimental period compared with other groups, Fig. 1, 2 and 3.
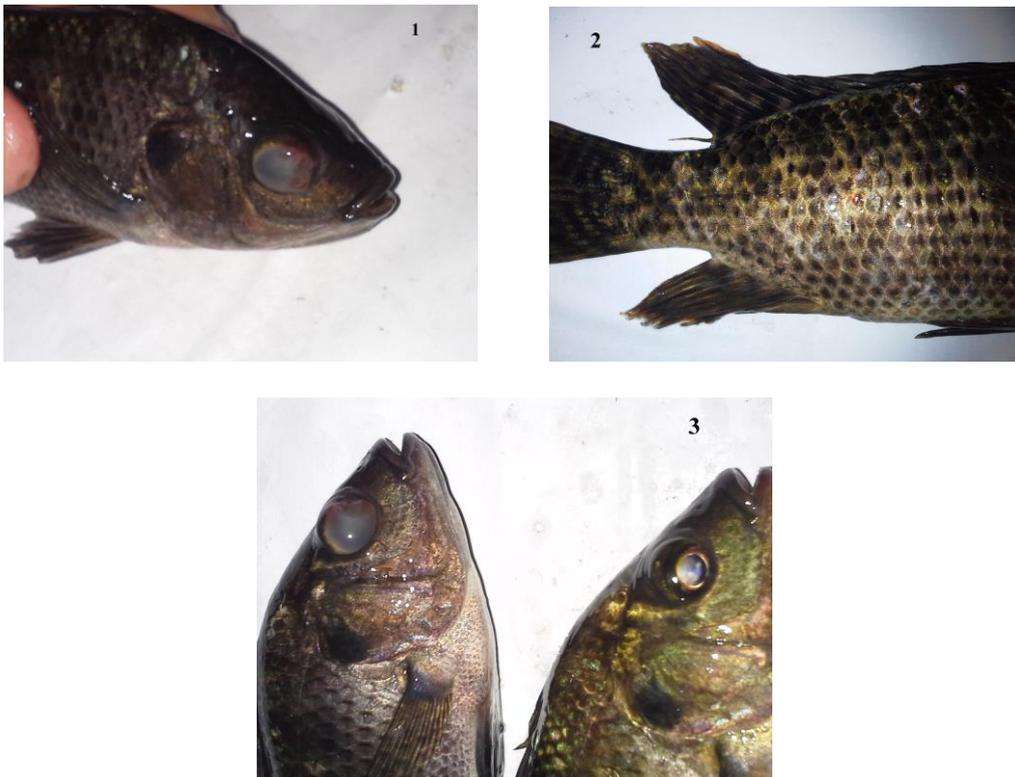

Fig. 1, 2 and 3: Showed severe darkness colouration of the dorsal area, caudal peduncle, and eye cataract.

2- Results of postmortem lesions of T-2 toxicity in *Oreochromis niloticus*:
The postmortem lesion of *O. niloticus* experimentally exposed to T-2 toxin (G3) showed yellowish discolorations of the liver and numerous nodules appeared on the surface of the liver as well as whitish and sever necrosis of the kidney Fig. 4 and 5.
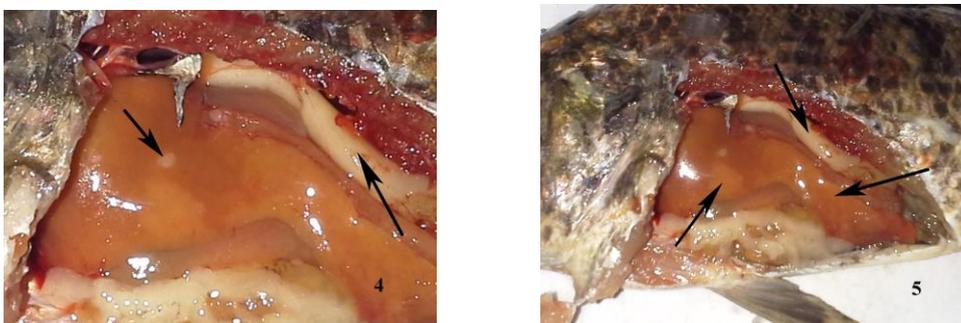

Fig. 4 and 5: Showed yellowish discolorations of the liver and numerous nodules appeared on the surface of the liver as well as whitish and sever necrosis of the kidney.

3- Results of hematological parameters of T-2 toxicity in *Oreochromis niloticus*:

According to Table, 2 T-2 toxin effects on hematological indicators were reflected rather in changes of the red blood picture, packed cell volume hemoglobin and haematocrit value, but changes of the white blood picture were repeatedly observed as well as a decrease in the leucocyte. In view of the state that T-2 toxin induces distinct decrease in leucocyte count corresponding to a neutrophile decrease after a single application of T-2 toxin to fish.

Table 2: Changes in erythrocyte counts (RBCs), Leucocyte count (WBCs), packed cell volume (PCV), hemoglobin content (HB) and haematocrit value (Hct) in the blood of *Oreochromis niloticus* fed diet containing T-2 toxins and two mycotoxins adsorbents to alleviate T2 toxins -induced toxicity in *Oreochromis niloticus* after the end of experiment (42 days).

Groups	G1	G2	G3	G4	G5	T6
Erythrocyte count 10^6/mm^3	1.03 [a] ± 0.03	1.02 [a] ± 0.03	0.721 [c] ± 0.02	0.98 [b] ± 0.03	0.96 [b] ± 0.03	1.02 [a] ± 0.03
Leucocyte count 10^4/mm^3	26.42 [a] ± 2.23	26.33 [a] ± 2.20	18.35 [c] ± 1.37	22.36 [b] ± 1.13	22.29 [b] ± 1.12	25.54 [a] ± 1.36
Packed cell volume (PCV %)	31.54 [a] ± 2.01	34.21 [a] ± 2.14	23.37 [a] ± 1.74	26.94 [a] ± 2.02	29.08 [a] ± 2.08	32.56 [a] ± 2.42
Hemoglobin (g %)	10.87 [a] ± 1.09	11.37 [a] ± 1.13	7.79 [c] ± 0.38	9.53 [b] ± 0.77	9.39 [b] ± 0.83	10.92 [a] ± 1.08
Haematocrit value (%)	17.0 [a] ± 0.81	19.0 [a] ± 0.86	9.75 [c] ± 0.478	14.36 [b] ± 0.59	13.41 [b] ± 0.53	18.75 [a] ± 0.87

abc, mean with difference superscript are significantly different ($P<0.05$).

4- Results of differential leucocytic count of T-2 toxicity in *Oreochromis niloticus*:

According to Table 3, T-2 toxin effects on differential leucocytic count were reflected rather in changes of the decrease neutrophile numbers and a decline in the lymphocyte and monocyte numbers and/or a decrease of the lymphocytes/neutrophiles ratio. In view of the data concerning the general leucocyte count, it is state that T-2 toxin induces lymphopenia. Similarly, neutrophile decrease after a single application of T-2 toxin to fish.

Table 3: Changes in differential leucocytic count in the blood of *Oreochromis niloticus* fed diet containing T-2 toxins and two mycotoxins adsorbents to alleviate T2 toxins -induced toxicity in *Oreochromis niloticus* after the end of experiment (42 days).

Groups	G1	G2	G3	G4	G5	T6
Neutrophils (%)	55.62 [a] ± 2.43	56.96 [a] ± 2.42	41.30 [c] ± 1.23	48.54 [b] ± 2.31	47.04 [b] ± 2.25	54.04 [a] ± 2.41
Eosinophils (%)	9.80 [a] ± 0.94	9.87 [a] ± 0.95	8.85 [b] ± 0.86	8.94 [b] ± 0.89	9.49 [a] ± 0.91	9.72 [a] ± 0.94
Basophils (%)	3.66 [a] ± 0.37	3.36 [a] ± 0.25	3.43 [a] ± 0.26	3.28 [a] ± 0.24	3.88 [a] ± 0.32	3.59 [a] ± 0.35
Lymphocytes (%)	29.34 [d] ± 1.78	28.11 [d] ± 1.74	45.85 [a] ± 2.88	38.67 [b] ± 2.89	37.70 [b] ± 2.83	31.73 [c] ± 2.88
Monocytes (%)	1.64 [b] ± 0.06	1.54 [b] ± 0.06	2.10 [a] ± 0.05	1.29 [b] ± 0.04	1.41 [b] ± 0.05	1.28 [b] ± 0.05

abc, mean with difference superscript are significantly different ($P<0.05$).

5- Results of hepatic and kidney marker enzymes as well as some immune parameters of T-2 toxicity in *Oreochromis niloticus*:

5.1. Evaluation of hepatic marker enzymes and total protein:

The effects of T-2 toxin intoxication as well as the preventive effects of Mycosorb and Zeolite on hepatic marker enzymes and total protein are shown in (table 4). Significant increases (P<0.05) in serum liver function marker enzymes (AST, ALT) were recorded in T-2 toxin intoxicated fish as compared to negative control group. Significant decrease (P<0.05) in serum total protein was also detected in the positive control group. Meanwhile, Mycosorb and Zeolite treated groups showed significant improvement (P<0.05) in AST, ALT and total protein comparing with positive control. The results indicated that the Mycosorb and Zeolite treated groups were more effectively improves the ALT level compared with the other groups. The effect of feeding T-2 toxin on serum chemistry showed numerical reduction in plasma triglyceride and increase in glucose levels.

5.2. Evaluation of kidney markers

Similarly, serum urea and creatinine levels showed significant increases (P<0.05) in T-2 toxin intoxicated fish compared with the negative control and other treated groups. Significant improvement (P<0.05) in the serum urea and creatinine levels was recorded in Mycosorb and Zeolite treated groups. Renal function tests data are shown in table 4. Results indicated that the improvement in the serum urea and creatinine levels were close in all treated groups when compared with the positive and negative control groups.

Table 4: Changes in glucose levels, total proteins, Albumin, Globulin, aspartate aminotransferase (AST), alanine amino transferase (ALT), lactated dehydrogenase (LDH) activities, Urea, Creatinine, Cholesterol and Triglyceride in plasma of *Oreochromis niloticus* fed diet containing T-2 toxins and two mycotoxins adsorbents to alleviate T2 toxins -induced toxicity in *Oreochromis niloticus* after the end of experiment (42 days).

Groups	G1	G2	G3	G4	G5	T6
Glucose (mg/L)	91.73 [c]± 5.59	93.8 [c]± 7.10	121.12 [a]± 4.45	104.47 [b]± 5.59	109.84 [b]± 7.10	85.12 [c]± 4.45
Total protein (g/dl)	5.28 [a]± 0.29	5.66 [a]± 0.34	2.75 [d]± 0.17	3.74 [c]± 0.20	3.39 [c]± 0.20	4.87 [b]± 0.28
Albumin (g/dl)	2.31 [a]± 0.17	2.39 [a]± 0.17	2.13 [b]± 0.17	2.11 [b]± 0.17	2.05 [b]± 0.17	2.09 [b]± 0.17
Globulin (mg/dl)	2.97 [b]± 0.15	3.27 [a]± 0.17	0.62 [d]± 0.04	1.63 [c]± 0.09	1.34 [c]± 0.06	2.78 [b]± 0.17
AST(IU/L)	119.75 [c]± 3.15	112.55 [c]± 3.05	145.65 [a]± 5.15	127.38 [b]± 3.58	130.18 [b]± 4.22	112.55 [c]± 3.75
ALT (IU/L)	13.96 [c]± 1.05	14.79 [c]± 1.11	21.55 [a]± 2.38	16.33 [b]± 1.67	16.29 [b]± 1.68	13.84 [c]± 1.12
LDH (IU/L)	125.1 [c]± 6.66	129.2 [c]± 6.36	189.5 [a]± 8.38	155.56 [b]± 4.78	151.32 [b]± 4.57	127.5 [c]± 6.32
Urea (mg/dl)	0.21 [c]± 0.02	0.19 [c]± 0.02	0.72 [a]± 0.09	0.43 [b]± 0.04	0.47 [b]± 0.04	0.23 [c]± 0.02
Creatinine (mg/dl)	0.12 [c]± 0.01	0.13 [c]± 0.02	0.92 [a]± 0.11	0.71 [b]± 0.09	0.69 [b]± 0.09	0.11 [c]± 0.01
Cholesterol (mg/dl)	93.64 [c]± 3.21	88.02 [c]± 3.09	122.89 [a]± 4.16	108.67 [b]±3.68	109.43 [b]±3.66	94.87 [c]±3.23
Triglyceride (mg/dl)	121.57 [c]± 3.16	127.38 [c]± 3.27	89.35 [c]± 2.27	112.19 [c]±3.23	108.25 [c]±3.21	117.66 [c]±3.28

abc, mean with difference superscript are significantly different (P<0.05).

6- Results of evaluation of serum electrolytes of T-2 toxicity in *Oreochromis niloticus*:

Changes in the serum calcium (Ca), phosphorus (P) and magnesium (Mg) were recorded in table 5. T-2 toxin intoxicated fish showed significant decrease (P<0.05) in serum Ca, P and Mg levels compared with the negative control. Mycosorb and Zeolite treated groups ameliorates the T-2 intoxication changes in the studied serum parameters. Significant improvement (P<0.05) in the serum Ca, P and Mg level was observed. The results indicate that the Mycosorb and Zeolite treatment group effectively improves the electrolyte level close to the normal level compared with the other treated groups and the negative control group.

Table 5: Changes in Ca, P and Mg levels in plasma of *Oreochromis niloticus* fed diet containing T-2 toxins and two mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in *Oreochromis niloticus* after the end of experiment (42 days).

Groups	G1	G2	G3	G4	G5	T6
Calcium mg%	13.39 [a]± 0.25	13.35 [a]± 0.24	7.28 [c]± 0.14	9.33 [b]± 0.17	9.12 [b]± 0.17	12.57 [a]± 0.23
Phosphorus mg %	9.61 [a]± 0.18	9.74 [a]± 0.18	4.98 [c]± 0.08	6.58 [b] ± 0.06	6.74 [b]± 0.06	8.98 [a]± 0.006
Magnesium ug/g	349.43[a]± 20.74	351.72 [a] ± 20.86	277.96[c]± 16.56	311.56 [b]± 18.14	305.72[b]± 18.36	349.54[a]± 20.56

abc, mean with difference superscript are significantly different (P<0.05).

9- Results of histopathological changes of T-2 toxicity in *Oreochromis niloticus*:

The histopathological changes of liver of *O. niloticus* fed on diet incorporated with T-2 showed moderate fatty change and congestion in central vein and moderate hydropic degeneration and activation of kupffer's cells sinusoidal congestion. The kidney of *O. niloticus* fed on diet incorporated with T-2 showed hemorrhage, necrosis in lining epithelium and hypercellularity in glomeruli, vaculation of proximal convoluted tubules, dilatation of Bowman's space due to glomerular atrophy and interstitial edema and vaculation in tubular epithelium with glomerular atrophy and dilatation of Bowman's space.
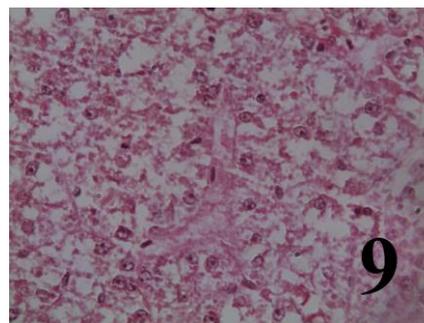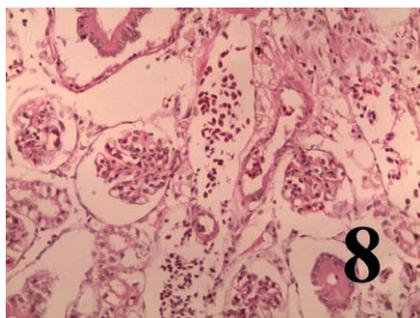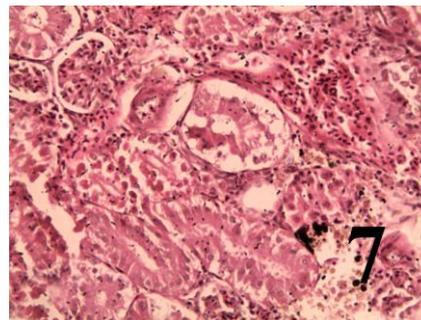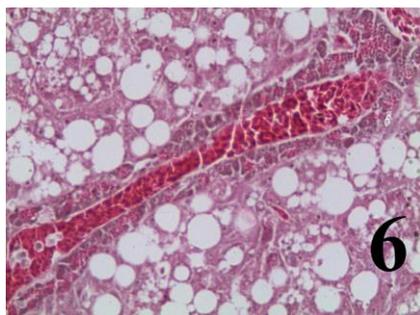

Figure 6: Liver of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 after 6th week showed moderate fatty change and congestion in central vein with higher magnification. H&E ×20.

Figure 7: Kidney of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 after 6 weeks showed hemorrhage, necrosis in lining epithelium and hypercellularity in glomeruli. H&E ×20.

Figure 8: Kidney of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 feed after 6 weeks showed vaculation of proximal convoluted tubules, dilatation of Bowman's space due to glomerular atrophy and interstitial edema. H&E ×20.

Figure 9: Liver of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 feed after 6 weeks showed moderate hydropic degeneration and activation of kupffer's cells. H&E ×40.
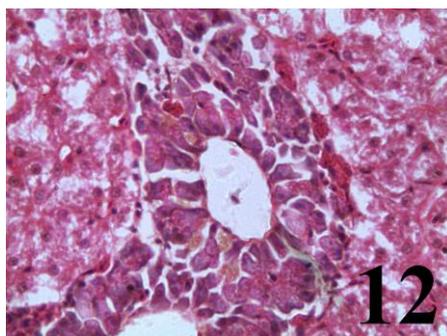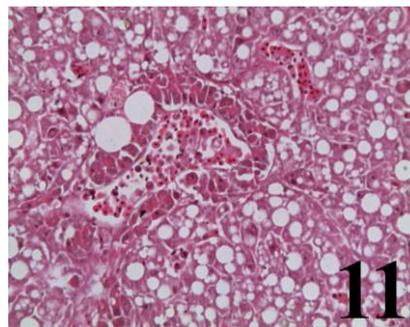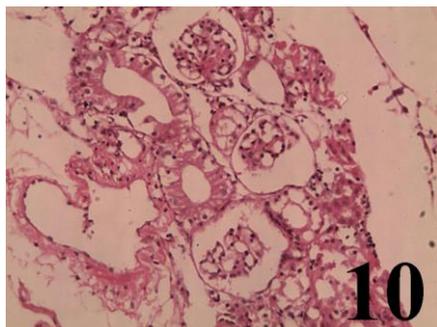
Figure 10: Kidney of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 feed after 6 weeks showed vaculation in tubular epithelium with glomerular atrophy and dilatation of Bowman's space. H&E ×20.

Figure 11: Liver of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 showed sinusoidal congestion, moderate fatty change and congestion in central vein. H&E ×20.

Figure 12: Liver of *O. niloticus* fed on diet incorporated with 1.0 mg/kg of T-2 feed after 2nd week showed moderate hydropic degeneration with mild congestion H&E × 40.

DISCUSSION

The T-2 toxin is a trichothecene mycotoxin produced by certain molds of the genus *Fusarium* that infect the grains, wheat by-products, and oilseed meals used in the production of animal feeds. An aquarium study was conducted with juvenile channel catfish *Ictalurus punctatus* Bruce *et al.,* 2003.

The effects of fusarium toxins in human and animals ranged from carcinogenic, nephrotoxic and immunosuppressive health effects (Morriss, 1997). Although the main route of human exposure to mycotoxins has been identified as the direct ingestion of contaminated cereals and grains (Morriss, 1997), while, there are many studies about whether the ingestion of meat, milk, and eggs originating from mycotoxin-exposed food-production animals is a significant exposure pathway for mycotoxins among humans (Hassan *et al.,* 1997; Wafia and Hassan, 2000 and Hassan *et al.,* 2004 and 2009). The search focused to recover the clinical signs and immunosuppressive health effects as well as the histopathological effects of the T-2 toxin in cultured *Oreochromis niloticus* were examined.

Concerned to clinical signs of T-2 toxin in cultured *Oreochromis niloticus* revealed that yellowish discolorations of the liver and numerous nodules appeared on the surface of the liver as well as whitish and sever necrosis of the kidney. These results may be due to destructive effects of T-2 toxin on fish. Smalley (1973) mentioned that T-2 in trout induced severe oedema and fluid accumulation in the body cavity and behind the eyes are produced in addition to the loss of the intestinal mucosa, also, Kravchenko *et al.* (1989) in carp.

After 6 weeks red blood picture, packed cell volume, hemoglobin and haematocrit values were adversely affected by the inclusion of T-2 toxin compared to treated groups with antimycotoxins.

As seen in Tables 2, the addition of Mycosorb and Zeolite to a T-2-containing diet significantly ameliorated the adverse effects of T-2 on blood parameters (RBCs, WBCs, PCV, HB, haematocrit value (Hct) and leucocytic count) in *Oreochromis niloticus* (P ≤0.05). These results indicated the depressing effect of T-2 toxin on hemopoietic tissue

and immune responses as reported by others (Kubena *et al.,* 1998; Keçeci *et al.,* 1998 and Huff *et al.,* 1988b). In addition, *Fusarium* toxin has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction have been reported by Rocha *et al.* (2005) including disruption of normal cell function by inhibiting RNA, DNA, and protein synthesis; inhibition of cell division; stimulation of ribotoxic stress response; and activation of mitogen-activated protein kinases.

The mean percentages of monocyte, lymphocyte and heterophil counts are presented in Table 3. The diet containing of T-2 toxin caused a significant decrease on the lymphocyte counts over 42 days. A significant increase of heterophil counts obtained during the last week of the experiment. As to the T-2 toxin effects, Corrier and Ziprin (1987) state that T-2 toxin induces lymphopenia and lowers neutrophil chemotactic migration. Niyo *et al.* (1988) observed decreased mean corpuscular volume, leucocyte count, and – just below the significance level – decreased neutrophile count in rabbits after a 3-week administration of 0.75 mg/kg/day of T-2 toxin. Similarly, Gentry *et al.* (1984) found a distinct decrease in leucocyte count corresponding to a neutrophile decrease after a single application of T-2 toxin to calves. On the contrary, Pang *et al.* (1987) found marked neutrophilia after a single T-2 toxin administration. Hayes *et al.* (1980) observed anemia, lymphopenia and eosinopenia in mice after 3 weeks. However, granulocytopoesis and erythropoiesis regeneration occurred later. It is probable that due to the lack of data on leucocyte number, the changes observed in our experiments were based on the neutrophile and/or lymphocyte decrease rather than on the real increased number of monocytes. Evaluation of hepatic marker enzymes and total protein, the effects of T-2 toxin intoxication as well as the preventive effects of Mycosorb and Zeolite on hepatic marker enzymes and total protein are shown in Table, 4. Significant increases (P<0.05) in serum liver function marker enzymes (AST, ALT) were recorded in T-2 toxin intoxicated fish as compared to negative control group. Significant decrease (P<0.05) in serum total protein was also detected in the positive control group. It is reported that the significant effect of fusarium toxins are the alteration in serum concentration of kidney and liver enzymes, total protein, albumin, minerals and lipid profiles (Kubena *et al.,* 1997 and Mogeda *et al.,* 2002). *Fusarium* toxin inducing significantly decreased values in serum total protein, alpha globulin, beta globulin and while slightly increase in gamma globulin, these results agree with (Rotter *et al.,* 1994).

The globulin component Table 3, showed drop in globulin in all the experiment while decrease globulin. This may be attributed to that *Fusarium*

fungi cause's hepatotoxic, nephrosis, hemorrhages (liver and kidneys) (Tietz, 1996) *Fusarium* mycotoxins might affect the synthesis of globulins of hepatic origin as well as globulins of lymphoid origin. Rotter *et al.* (1994) suggested that *Fusarium* mycotoxins can directly affect -globulin synthesis in the liver.

The results of this study are in agreement with those of Chowdhury and Smith (2004), who observed that excessive serum concentrations of uric acid in laying hens were a result of feeding feedborne *Fusarium* mycotoxins. Moreover, in a subsequent study with laying hens, they found that feeding contaminated grains led to reduced hepatic fractional protein synthesis rates (Chowdhury and Smith, 2005). Dänicke *et al.* (2006) also observed a reduction in fractional protein synthesis rates in the kidneys, spleen, and ileum of pigs exposed to DON.

Mitigating T-2 toxin negative effect was achieved by addition of both Mycosorb and Zeolite, by reduction of AST and ALT, and an increase of glucose compared with T-2 toxin fed group. The effect of feeding T-2 toxin on blood chemistry showed numerical reduction in plasma triglyceride and increase in glucose levels which may be affected by the effect of T-2 toxin on liver (Trinder, 1969). The effect of feeding T-2 toxin on blood chemistry showed numerical reduction in plasma triglyceride and increase in glucose levels which may be affected by the effect of T-2 toxin on liver (Trinder, 1969). Decontamination procedures have focused on degrading, destroying, inactivating or removing T-2 by physical, chemical and biological methods. Recently, researchers have directed efforts toward finding effective means of the biological degradation of T-2 (Aziz, 2005 and Omar, 1996). These compounds must not be adsorbed from the gastrointestinal tract and must have the ability to bind physically with chemical substances, precluding their adsorption (Miazzo *et al.,* 2000 and Santurio *et al.,* 1999). The addition of Zeolite and Mycosorb to the 1 ppm T-2 toxin -containing diet provided a partial improvement in the adverse effects of T-2 toxin on total billirubin and direct billirubin values. The AS and LDH enzyme activities increased compared with control, while the addition of Zeolite to the T-2 toxin -containing diet caused significant increase on the AST activities compared with control. This was in agreement with results obtained by Celik *et al.* (2000). Our results agreed with those reported by Kubena *et al.* (1993) who found significant decreases in the biochemical parameters at exposure level of T-2 toxin ranging from 2.5 to 5 ppm. The elevation of AST may be due to disruption of hepatic cell as a result of necrosis or a consequence of altered membrane permeability Coles (1974). The depressing effect of T-2 toxin on hemopoietic tissue and immune

responses as reported by others (Kubena *et al.,* 1998; Keçeci *et al.,* 1998 and Huff *et al.,* 1988a).

Many studies have been recently made to determine whether Zeolite in the diets of chickens have a beneficial effect on immune response Ramos *et al.* (1996). Our results seem to differ from those observed by Kececi *et al.* (1998). Others concluded that AST and ALT are the serum enzymes which are sensitive specific indicator of liver damage. The increase in LDH activity due to mycotoxicosis was found to be significant ($p < 0.05$) as Benjamin (1978) stated that LDH may be elevated in many disease processes in which there is cell necrosis.

The depression in serum albumin concentration resulting from feeding T-2 was clear indication of impairment in protein synthesis by inactivation of initiation and termination, possibly through its binding to ribosomes (Uneo, 1977). The finding of lower serum albumin values in broilers receiving T-2 were also reported by Kubena *et al.* (1998) and Bailey *et al.* (1998).

The inconsistency of serum enzymes during T-2 toxicosis was also reported by Chi and Mirocha (1978); Kubena *et al.* (1998); Raju and Devegowda, (2002) and Denli and Okan, (2006). These findings were in agreement with the findings of Kubena *et al.* (1998) who reported a similar reduction in uric acid and albumin values of broilers fed T-2. The results were contrary to the findings of Huff *et al.* (1988a) and Bailey *et al.* (1998) who reported no significant effects at 8mg/kg of T-2 on uric acid levels in broilers. Bailey *et al.* (1998) and Garcia *et al.* (2003) reported similar reduction in uric acid and albumin values in broilers receiving diets containing 5ppm T-2. Reduction in serum uric acid levels due to T-2 was also reported by Huff *et al.* (1988a).

At the same time concentrations of serum calcium and serum phosphorus were decreased due to feeding *Fusarium* mycotoxin-contaminated diets this results were agree with Diaz and Smith (2006).

CONCLUSION

The current study confirms that T-2 toxin alternate serum biochemical parameters. It is recommended to focus on supplementation of fish and aquaculture farms with antioxidants such as Zeolite or Mycosorb to alleviate pollutant stress and improve the fish health and productivity especially in contaminated areas.

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تأثير الميكوزورب والزوليت لمنع سميه ت۲ فى اسماك البلطى النيلى المستزرع

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تهدف هذه الدراسه إلى تقييم قدره إثنين من المواد الماصه للسموم الفطريه على التخلص من الاثار السميه لــ T-2 Toxins في أعلاف أسماك البلطي النيلي المستزرع. تم تقسيم ۱۸۰ سمكه بلطي نيلي الى سته مجموعات، تم معالجه الاسماك بإضافه ۱جم/كجم علف من كل من ميكوزورب والزوليت في الجموعه (۱ – ۲) كل على حده، وإضافه ۱مجم/كجم علف من T-2 Toxins في المجموعه (۳) وإضافه ۱مجم/كجم علف من T-2 Toxins مع ۱جم/كجم علف من كل من ميكوزورب في المجموعه (٤) وإضافه ۱مجم/كجم علف من T-2 Toxins مع ۱جم/كجم علف من كل من الزوليت في المجموعه (٥) وتركت المجموعه (٦) كمجموعه ضابطه بدون معاملات. تم إضافه هذه الجرعات إلى الاسماك يوميا لمده سبعه أسابيع. تم اجراء بعض التحاليل البيوكيميائيه والدراسات الهستوباثولوجيه لتقييم حاله الكلى والكبد للاسماك في المجموعات المختلفه. اوضحت النتائج ارتفاع ملحوظ في وظائف الكبد والكلى (الكرياتينين – اليوريا) بينما انخفضت نسبه البروتين الكلي والكالسيوم والفوسفور والماغنسيوم في المجموعه (۳) مقارنه بتحسن ملحوظ في هذه النتائج في المجموعات المعالجه بـ الميكوزورب والزوليت. نخلص من هذه النتائج إلى أن إضافه الميكوزورب والزوليت إلى أعلاف الأسماك يقلل من الأثر السمي الناتج عن T-2 Toxins.