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Comparative ameliorative effect of Hydrated sodium calcium aluminosilicate and *Saccharomyces cerevisiae* (Brewer's yeast) against toxic impact of aflatoxin B_1 in *Oreochromis niloticus* (Nile tilapia)

Abd El-Alim F. Abd El-Alim[1], Azza A. A. Galal[1*], Shahira H. Mahmoud[2] and Walaa A. El-Sayed[2]

[1]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt
[2]Animal Health Research Institute, Zagazig Branch, Zagazig 44516, Sharkia, Egypt

Abstract

Different ways have been used in an attempt to decrease the risk of aflatoxicosis in fish. This study was undertaken to compare the possible alleviative effects of hydrated sodium calcium aluminosilicate (HSCAS) and *Saccharomyces cerevisiae* against the toxic impact of aflatoxin B_1 (AFB_1) on *Oreochromis niloticu*s (*O. niloticus*). Therefore, 180 normal cultured monosex *O. niloticus* were randomly allocated into 6 equal groups. Group 1, was received the basal ration only. Group 2, was fed a basal ration supplemented with 0.5% HASCAS. Group 3, was fed a basal diet enriched with 0.25% *Saccharomyces cerevisiae*. Group 4, was received a diet intoxicated with 2.5 ppm aflatoxin B_1. Group 5, was fed a diet intoxicated with 2.5 ppm AFB_1 with 0.5% HSCAS. Group 6, was fed a diet intoxicated with 2.5 ppm AFB_1 with 0.25% *S. cerevisae*. AFB_1 intoxication induced mortality 16.67 %, leucopenia, lymphopenia, neutrophilia with a significant decrease in phagocytic % and index. Furthermore, significant increases in serum creatinine, ALT and ALP as well as a significant decrease in total protein, albumin and globulin were recorded. Moreover, accumulation of aflatoxin residues in *O. niloticus* flesh (5 ppb) and liver (15 ppb). While, supplementation of AFB_1 intoxicated diet either with *S. cerevisiae* or HSCAS ameliorated the drastic effects of aflatoxin on *O. niloticus* and *S. cerevisiae* appear to be more effective in the protection of fish from aflatoxicosis than HSCAS.

Keywords: Aflatoxin B_1, Residues, Hematology, Phagocytosis, HSCAS, *Saccharomyces cerevisiae*

Introduction

Mycotoxins are unavoidable contaminants in foods and feed stuffs and are a major problem throughout the world [1]. Aflatoxins (AFs) are a group of structurally related mycotoxins produced as food-borne metabolites by toxigenic strains of *Aspergillus parasiticus*, *Aspergillus flavus* and to lesser extent *Aspergillus nominus* [2]. Aflatoxins have a serious impact on the cultured fish, inducing disease with elevated death-rate and a steady decrease fish quality, in this manner revealing a critical issue in aquacultures [3]**.** Aflatoxin B_1 is the most common and toxic aflatoxins for human, land animals and aquatic organisms, due to its strong carcinogenic, immunosuppressive and mutagenic effects [4].

In spite of good screening programs, election of high quality feed ingredients and raw materials and good storage it is extremely hard to ensure the nonappearance of mycotoxins in aquaculture feeds. Subsequently, it is insistent to find appropriate ways to face the problem via an effective handling of the hazards caused by mycotoxins contamination [5]. Hydrated sodium calcium aluminosilicate (HSCAS) clay considered an easy, inexpensive and effectual way of aflatoxicosis as it firmly and specifically captures aflatoxins in GIT, lowering their bioavailability and accompanied problems [6,7].

Brewer's yeast (*Saccharomyces cerevisiae*) was found to have an important antagonistic role toward aflatoxicosis and has immuno-stimulant in chickens and quails [8,9]. The yeast preparations appear to be effective on a broad range of mycotoxins [10], due to the ability of glucomannans from *S. cerevisiae* to adsorb mycotoxins [11]. *S. cerevisiae* able to degrade aflatoxin and other mycotoxins as T-2 toxin and zearalenone [12,10]. Therefore, the present experiment was carried out to compare the preventive impacts of HSCAS and

***Corresponding author email: (azzapharma@gmail.com), Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.**

Saccharomyces cerevisiae against the drastic effects of AFB_1 on *Oreochromis niloticus*.

Material and Methods

Aflatoxin B_1 ($C_{17}H_{12}O_6$), generated by toxigenic *Aspergillus flavus* utilizing polished raw rice as a growth substrate [13] with minor modifications [14].

HSCAS: Trade name, Condition feed, is commercial product made in India and imported by Pharma chemical international company and is composed of Hydrated sodium calcium aluminosilicate 100%.

Saccharomyces cerevisiae: Trade name, Diamond v original xp, it contains dried yeast (*Saccharomyces cerevisiae*) fermented product 100%, made in U.S.A.

Experimental Fish

One hundred and eighty apparently normal cultured monosex *Oreochromis niloticus*, with average body weight (35±5 g), were obtained from Abbassa Fish hatchery, Sharkia Governorate, Egypt. Fish were transported in polyethylene bags filled with one third dechlorinated water enriched by air (2/3) to the laboratory of Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University. Fish were acclimated for 2 weeks and kept in glass aquaria filled with chlorine free tap water under laboratory conditions (natural photoperiod 12 h and temp (25.5±2 °C). Persistent aeration was kept in each aquarium by an electric air pump and temp was maintained by heaters. The water parameters (dissolved oxygen, pH and electric conductivity of the tap water) used in this study were measured using Hack Method (Sigma Laboratory) following WHO, (2001).

Diets used for experimental fish

The experimental fish were fed on basal diet (contained 30.38% crude protein and 3000 kcal/kg metabolizable energy which composed of fish meal, poultry by product, soybean, vegetable oils, wheat flour, yellow corn, minerals and vitamins mixture. The basal ration was formulated from commercial constituents and was compressed (1mm size pellets) at Fish Research Unit, Faculty of Veterinary Medicine, Zagazig University. Fish were fed twice daily (8 am and 2 pm) at the rate of 3% of their biomass.

Experimental protocol

After adaptation period, the healthy fish were haphazardly assigned into six equal groups (3 replicates/group), each replicate contains 10 fish kept in well prepared and persistently aerated aquarium (80x40x30cm) containing dechlorinated tap water, experiment had lasted for 42 days.

Group 1 received a basal ration only. Group 2 (HSCAS group): Fish were received a basal diet supplemented with 0.5% HSCAS [15]. Group 3 (*S. cerevisae* group): Fish were received a basal ration enriched with 0.25% *Saccharomyces cerevisiae* [15]. Group 4 (AFB_1 group): Fish were received a diet intoxicated with 2.5 ppm aflatoxin B_1 [16]. Group 5 (AFB_1+HSCAS group): Fish were fed a diet intoxicated with 2.5 ppm AFB_1 and supplemented with 0.5% HSCAS. Group 6 (AFB_1+*S. cerevisiae* group): Fish were fed a diet intoxicated with 2.5 ppm AFB_1 and supplemented with 0.25% *S. cerevisae*.

Hematological, immunological and biochemical analysis

Blood samples were collected on 1st, 2nd, 4th and 6th weeks of the experiment from the caudal blood vessels. Three blood samples were collected from each group. The 1st sample was gathered in clean sterilized tubes containing heparin as anticoagulant for estimation of phagocytic activity according to method previously illustrated [17,18]. The 2nd blood sample was collected in clean sterilized tubes containing EDTA for hematological examination. The 3rd blood sample was collected in plain centrifuge tubes without anticoagulant then centrifuged at 3000 rpm for 15 minutes for serum separation. Hepato-renal injury biomarkers were assessed in the separated serum. Serum alanine aminotransferase (ALT) was measured [19] and alkaline phosphatase (ALP) [20]. Total protein, albumin and creatinine were detected [21-23] respectively.

Measurement of AFB$_1$ residues

Muscle and liver samples from five fish of each group on 1st, 2nd, 4th and 6th weeks of the experiment, were pooled and thoroughly homogenized in a mortem. AFB$_1$ was extracted, filtrated and quantitatively measured by HPLC [24].

Statistical analysis

All data were analyzed using the SPSS program using one-way ANOVA**.** Duncan's Multiple Range Test (DMRT) was used to determine differences among means at significance level of 0.05.

Results

Effects on survival rate

Fish fed AFB$_1$ toxicated diet showed 83.33% survival rate. Adding of HSCAS or *S. cerevisiae* to AFB$_1$ toxicated diet increased the survival rate to 96.67%.

Effects on some hematological parameters

Inclusion of AFB$_1$ to the *O. niloticus* ration evoked a significant reduction in the total RBCs, Hb concentration and PCV% in descending manner at different experimental periods comparing with those fed a basal diet only (Fig 1 A, B &C). Group 5 and 6 revealed a significant elevation in RBCs count, Hb and PCV% compared to fish received AFB$_1$ intoxicated ration only (Group 4). Addition of *S. cerevisiae* to AFB$_1$ intoxicated diet (Group 6) induced a significantly higher RBCs count, Hb content and PCV% when compared with fish group treated with aflatoxin B$_1$ and HSCAS.

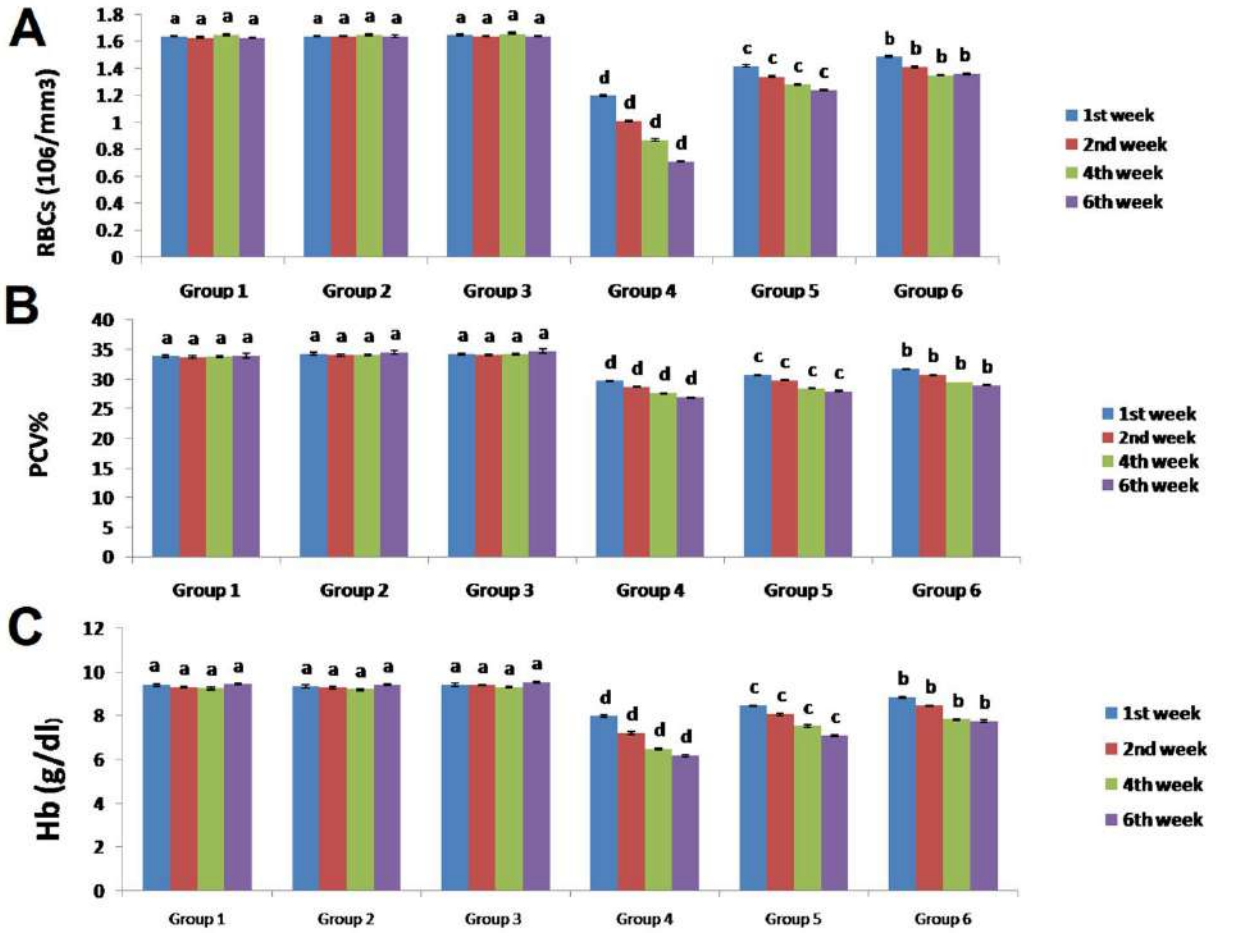

Figure 1: Effect of aflatoxin B$_1$ and different antimycotoxins on serum level of RBCs (A), PCV% (B) and Hb concentration (C) of *O. niloticus* during different experimental periods

Fish fed diet enriched with *S. cerevisiae* (Group 3) showed a marked elevation in total leukocytic and lymphocytic counts comparing with the control group, but fish in HSCAS group revealed non-significant change in comparison with the control group (Table 1). The total leukocytes count significantly decreased ($P \leq 0.05$) in descending manner at different experimental periods in AFB_1 group. Leucopenia, lymphopenia and neutrophilia are the main picture of the leukogram of AFB_1 group. Fish of group 5 and 6 showed a marked increase in WBCs counts compared to fish in group 4 throughout the experimental periods. Addition of *S. cerevisiae* to AFB_1 intoxicated diet induced a significant increase in WBCs count and lymphocytic counts when compared with AFB_1+HSCAS group.

Table 1: Effect of aflatoxin B_1 and different antimycotoxins on total WBCs, lymphocytes and neutrophils of *O. niloticus* at different experimental periods

Parameters \ Groups	Period	G1	G2	G3	G4	G5	G6
WBCs (10^3/ mm^3)	1st week	25.70 ± 0.05^b	25.69 ± 0.02^b	26.51 ±0.05^a	22.46 ± 0.05^e	23.71 ± 0.04^d	25.02 ± 0.06^c
	2nd week	25.60 ± 0.04^b	25.62 ± 0.02^b	26.60 ± 0.03^a	20.75 ± 0.06^e	23.02 ± 0.05^d	24.15 ± 0.05^c
	4th week	25.58 ± 0.03^b	25.56 ± 0.03^b	26.70 ± 0.01^a	18.74 ± 0.07^e	21.76 ±0.07^d	23.19 ± 0.03^c
	6th week	25.67 ± 0.02^b	25.70 ± 0.02^b	27.25 ± 0.38^a	16.64 ± 0.03^e	20.48 ± 0.03^d	22.99 ± 0.04^c
Neut.%	1st week	46.00 ± 0.32^d	45.40 ± 0.24^d	44.00 ± 0.32^e	49.60 ± 0.24^a	48.40 ± 0.51^b	47.20 ± 0.37^c
	2nd week	45.80 ± 0.37^d	46.00 ± 0.32^d	44.60 ± 0.24^e	55.00 ± 0.32^a	49.40 ± 0.24^b	48.00 ± 0.32^c
	4th week	46.20± 0.20^c	46.60± 0.24^c	44.60± 0.37^d	55.60± 0.24^a	47.40 ± 0.24^b	46.40 ± 0.24^c
	6th week	45.80 ± .37^{bc}	46.40 ± 0.24^b	44.20 ± 0.20^d	56.60 ± 0.24^a	46.40 ± 0.24^b	45.40 ± 0.24^c
Lymph.%	1st week	38.00 ± 0.32^b	37.60 ± 0.24^b	39.80 ± 0.37^a	36.00 ±0.32^c	37.60 ± 0.24^b	39.00 ± 0.32^a
	2nd week	38.20 ± 0.37^b	38.80 ± 0.37^b	40.80 ± 0.37^a	29.60 ± 0.24^d	35.40 ± 0.24^c	38.40 ± 0.24^b
	4th week	38.40 ± 0.24^c	39.60 ± 0.24^b	40.60 ± 0.37^a	28.60 ±0.24^e	36.80 ± 0.20^d	39.20 ± 0.20^b
	6th week	38.40 ±0.24^b	38.60 ± 0.32^b	41.00 ± 0.37^a	27.60 ±0.24^c	38.8 0± 0.20^b	39.60 ± 0.24^{ab}

Values are expressed as mean ± standard error, n=5. Means within the same raw carrying different superscripts are significant at ($P \leq 0.05$).

Effects on immune status

The addition of biological antimycotoxin *S. cerevisiae* to the basal diet (Group 3) significantly increase both phagocytic % and index when compared with control group, while adding of HSCAS to the basal diet (Group 2) evoked non-significant changes in phagocytic % or index comparing with control (Fig 2 A & B). There was a significant decrease ($P \leq 0.05$) in phagocytic % and index in descending manner at different experimental periods as indicator for nonspecific immunity in AFB_1 group. The addition either of HSCAS or *S. cerevisiae* to aflatoxicated diet significantly increased ($P \leq 0.05$) both phagocytic % and index compared to AFB_1 group all over the experimental periods. AFB_{1+} *S. cerevisiae* group showed a significant improvement in the phagocytosis when compared to HSCAS+AFB_1 group.

Effects on some biochemical parameters

Fish fed diet intoxicated with 2.5 ppm AFB_1 displayed a marked elevation in ALT, ALP and creatinine (Figs 3 A, B & C). Those fish also displayed a marked reduction in

serum total protein, albumin and globulin (Table 2). Addition of HSCAS or *S. cerevisiae* could ameliorate the alterations of these parameters compared with AFB_1 group. Group 6 showed significant decrease in ALT, ALP and creatinine as well as significant increase in serum total protein, albumin and globulin compared to group 5 throughout the experimental period.

Effects on aflatoxin residues

Exposure of fish to AFB_1 intoxicated diet for 42 day resulted in accumulation of aflatoxin residues in *O. niloticus* flesh and liver in ascending manner at different experimental periods till reach high levels (5 ppb) in muscle and (15 ppb) in liver at the end of experiment. Addition of chemical antimycotoxin HSCAS or biological one *S. cerevisiae* to AFB_1 treated diet significantly reduced ($P \leq 0.05$) aflatoxin residues in both liver and muscle of *O. niloticus* all over the experimental periods. Fish in group 6showed a significant reduction in aflatoxin residues followed fish in group 5 (data not shown).

Discussion

The mortality rate was increased in fish fed aflatoxin B_1 intoxicated diet (16.7%) in comparison with control group (0%). Fish death may be due to the organs dysfunction, anemia and impaired immunity caused by aflatoxicosis as recorded in our study. In similar way, Santacroce *et al*., [3] stated that the mortality rate increased in fish fed diets contaminated with aflatoxin. Addition either of HSCAS or *S. cerevisiae* to aflatoxin toxicated diet, improved the survival rate (96%) when compared with aflatoxicated diet only. This could be attributed to the ability of both antimycotoxins to bind aflatoxin in the gastrointestinal tract decreasing its uptake and bioavailability [25]. Our results were reinforced by Pooramini *et al*., [26].

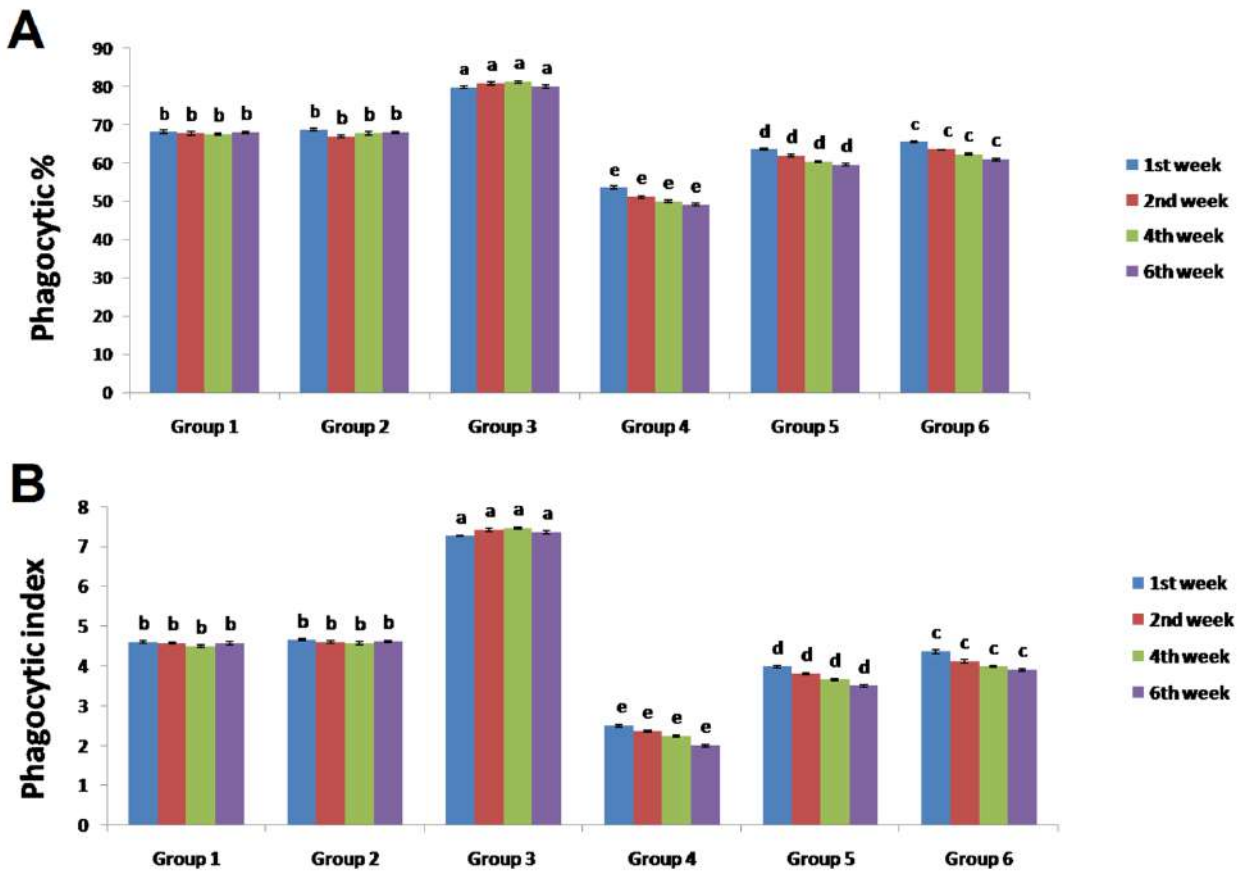

Figure 2: Effect of aflatoxin B_1 and different antimycotoxins on serum level of phagocytic % (A), and phagocytic index (B) of *O. niloticus* at different experimental periods.

AFB_1 had adverse impacts on fish hematological parameters, as it significantly decreased RBCs, PCV%, Hb concentration, total WBCs and lymphocytes. Meanwhile, neutrophils significantly increased. Lowering of RBCs, PCV %, Hb concentration indicated anemia, possibly due to the hemopiotic organs damage mainly anterior kidney [27] or an increase of RBCs destruction in hematopoietic tissues [28]. While, the reduction in leukocytic count may be due to the release of epinephrine during stress, which is capable of causing the spleen contraction and a decrease of leucocytes count, which accordingly results in the weakening of the immune system [29], renders the fish vulnerable to infection. Besides, the release of neutrophils into the blood occurs as a non-specific response to a variety of stress stimuli in mammals and fishes [30]. Our findings were supported by those reported for fish aflatoxicosis in *O. niloticus* [16,15].

Marked elevation in WBCs, RBCs, PCV % and Hb in groups 5 and 6 compared with fish in group 4. This can be explained by the ability of *S. cerevisiae* to degrade mycotoxins and prevent their toxic effects [31] and the ability of HSCAS to bind AF strongly and prevent its absorption across the gastrointestinal tract [32]. Our results were reinforced by Osman *et al.*, [33].

In the present study, AFB_1 significantly reduced both phagocytic % and index, which proved the immunosuppressive effect of aflatoxin. Our results were supported by Sahoo and Mukherjee, [34] who reported that AFB_1 cause suppression of neutrophil function, macrophage phagocytic activity, humeral immune response and globulin levels in rohu (*Labeo rohita*). Furthermore, Rodríguez-Cervantes *et al.*, [35] stated that aflatoxins induced chronic alterations in the immune system of aquatic organisms.

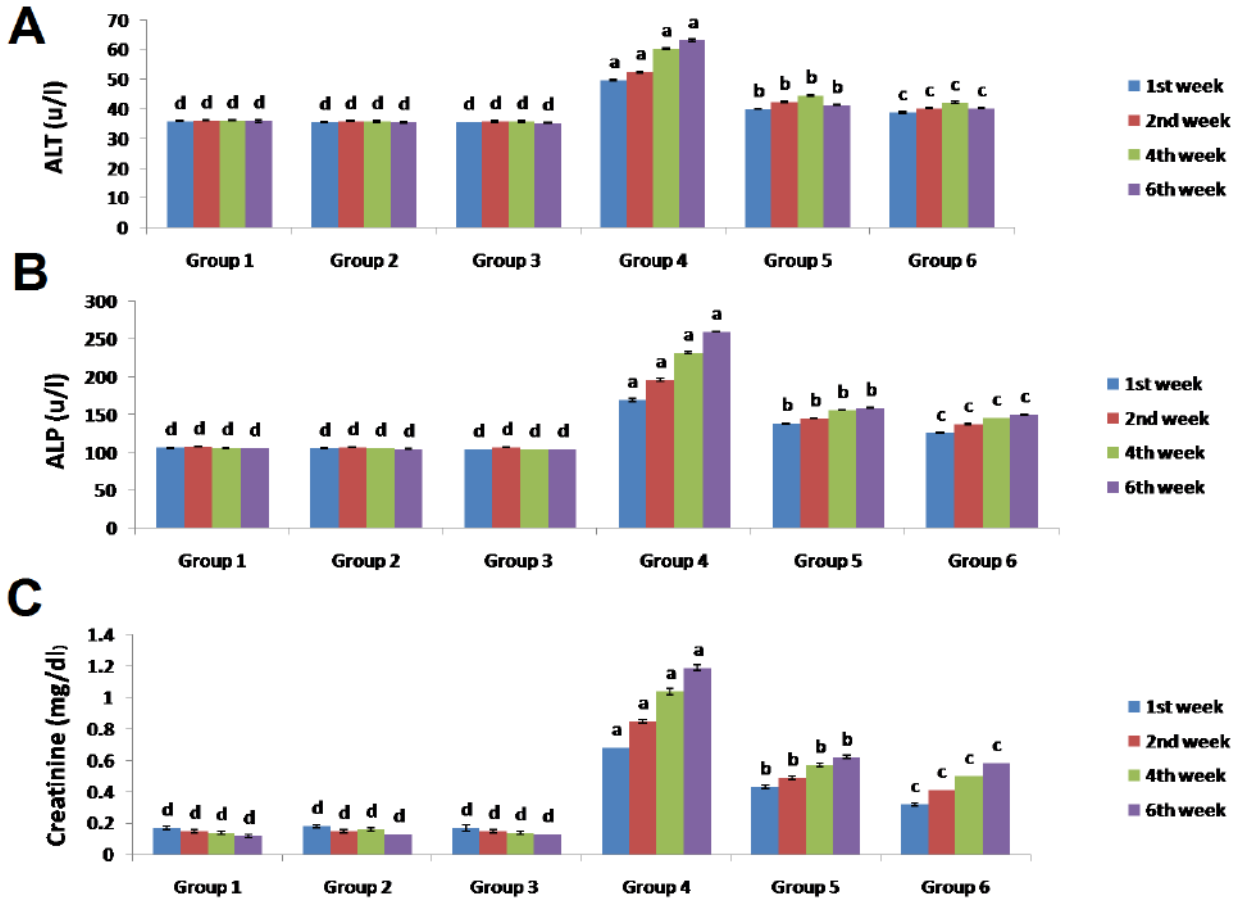

Figure 3: Effect of aflatoxin B_1 and different antimycotoxins on serum level of ALT (A), ALP (B) and creatinine (C) of *O. niloticus* during experimental periods.

Adding either HSCAS or *S. cerevisiae* to aflatoxicated diet significantly improved phagocytic % and index comparing to AFB_1 intoxicated group. These results may be attributed to the ability of HSCAS to bind AFB_1 in the gastrointestinal tract, reducing bioavailability to the blood stream [36]. Furthermore *S. cerevisiae* contains various immunostimulating compounds [37] beside its ability to capture the mycotoxin molecule changing it into nontoxic substance [10]. Our results are nearly agreed with Wang *et al.*, [38] who reported that β-glucan enhance phagocytic index in *O. niloticus* fed on aflatoxin treated diet.

Concerning liver and kidney function, fish fed AFB_1 treated diet showed a significant increase in ALT, ALP and creatinine as well as significant decrease in total protein, albumin and globulin indicating the stressful effects of AFB_1 on the hepatic and renal tissues and impairment of their function. The reduction in total protein and albumin could be attributed partly to the damaging effects of AFB_1 on hepatic cells [34], which was detected in this study, as evidenced by the increase in serum ALT and ALP activities, whereas the reduced globulin levels in AFB_1-treated fish may be due the result of lymphocytolysis [39]. Our results were strengthened by those reported for aflatoxicosis in *O. niloticus* [40].

Supplementation of AFB_1 toxicated diet either with HSCAS or *S. cerevisiae* could alleviate the alterations of biochemical parameters compared to AFB_1 group. This could explain by the ability of *S. cerevisiae* and HSCAS to bind with mycotoxins and limit their bioavailability in the digestive tract and protect animals against its adverse effects. Our results were reinforced by those previously recorded [41,15,42].

Table 2: Effect of aflatoxin B_1 and different antimycotoxins on serum total protein, albumin and globulin of *O. niloticus* during experimental periods

Parameter \ Groups	Period	G1	G2	G3	G4	G5	G6
Total protein (g/dl)	1st week	7.03± 0.09^{a}	7.02± 0.07^{a}	7.07± 0.07^{a}	4.94± 0.07^{d}	5.99± 0.03^{c}	6.21± 0.06^{b}
	2nd week	7.19± 0.12^{a}	7.07± 0.11^{a}	7.13± 0.10^{a}	4.19± 0.04^{d}	5.36± 0.05^{c}	5.92± 0.03^{b}
	4th week	7.03± 0.07^{a}	7.04± 0.06^{a}	7.08± 0.05^{a}	3.45± 0.07^{d}	5.15± 0.05^{c}	5.52± 0.04^{b}
	6th week	6.94± 0.07^{a}	6.94± 0.06^{a}	7.01± 0.05^{a}	2.87± 0.09^{d}	5.01± 0.03^{c}	5.25± 0.02^{b}
Albumin (g/dl)	1st week	4.20± 0.02^{a}	4.20± 0.02^{a}	4.22± 0.02^{a}	3.78± 0.02^{c}	3.99± 0.04^{b}	4.05± 0.06^{b}
	2nd week	4.21± 0.02^{a}	4.21± 0.03^{a}	4.21± 0.03^{a}	3.20± 0.02^{d}	3.76± 0.03^{c}	3.93± 0.02^{b}
	4th week	4.16± 0.01^{a}	4.18± 0.01^{a}	4.19± 0.02^{a}	2.99± 0.02^{d}	3.38± 0.01^{c}	3.47± 0.01^{b}
	6th week	4.16± 0.01^{a}	4.17± 0.01^{a}	4.19± 0.02^{a}	2.42± 0.02^{d}	3.29± 0.01^{c}	3.36± 0.01^{b}
Globulin (g/dl)	1st week	2.83± 0.07^{a}	2.82± 0.05^{a}	2.85± 0.05^{a}	1.40± 0.01^{d}	1.92± 0.02^{c}	2.13± 0.02^{b}
	2nd week	2.98± 0.10^{a}	2.86± 0.08^{a}	2.92± 0.08^{a}	1.08± 0.02^{d}	1.62± 0.02^{c}	1.98± 0.01^{b}
	4th week	2.96± 0.03^{a}	2.86± 0.05^{b}	2.89± 0.03^{ab}	0.57± 0.01^{e}	1.77± 0.03^{d}	2.05± 0.03^{c}
	6th week	2.78± 0.06^{a}	2.77± 0.06^{a}	2.82± 0.04^{a}	0.44± 0.07^{d}	1.73± 0.02^{c}	1.89± 0.02^{b}

Values are expressed as mean ± standard error, n=5. Means within the same raw carrying different superscripts are significant at ($P \leq 0.05$).

Maintainable mycotoxin residues in fish are a food safety problem [43]. In the current work, exposure to 2.5 mg AFB_1/kg diet for 42 day resulted in accumulation of aflatoxin residues in Nile tilapia flesh and liver in ascending increase manner at different experimental periods till reach high levels (5 ppb) in muscle and (15 ppb) in liver at the end of experiment. Most of AFB_1 residues were recorded mainly in the liver. These findings indicate that liver has an essential role in AFB_1 metabolism, toxic metabolites activation or detoxification [44,45]. Our results strengthened by Deng et al., [46] who detected AFB_1 residues in Nile tilapia liver when received smaller doses of toxin (less than 2 mg/kg). Moreover, Rajeev Raghavan *et al.*, [47] concluded that residual AFB_1 was detected at high levels (5 ppb) in fish musculature after prolonged feeding of sea bass with low levels of AFB_1.

Supplementation of AFB_1 toxicated diet either with HSCAS or *S. cerevisiae* reduced residues of aflatoxin significantly in liver and muscle of *O. niloticus* indicating the protection of fish liver and musculature against AFB_1 residues by HSCAS and *S. cerevisiae* through their ability to bind aflatoxin and formation of adduct which is not affected by the gastrointestinal tract enzymes consequently reduce the toxin bioavailability.

Conclusion

From the previously mentioned outcomes, it could be concluded that the supplementation of AFB_1 intoxicated diet either with 0.25% *S. cerevisiae* or 0.5% HSCAS succeed in mitigation of the drastic effects of aflatoxin on survival rate, hematology, phagocytosis and biochemical parameters as well as its residues in *O. niloticus*. Furthermore, *S. cerevisiae* appear to be more effective in protection of fish from aflatoxicosis than HSCAS.

Conflict of interest

The authors declare that they have no conflict of interest.

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الملخص العربى

التأثير التحفيزي المقارن لكالسيوم ألومينوسيليكات الصوديوم وسكروميسز سيريفيسياي (خميرة بروير) ضد التأثير السام للأفلاتوكسين B1 في أسماك البلطي النيلي

عبد العليم فؤاد عبد العليم[١]، عزة جلال[١]، شهيرة محمود[٢] و ولاء السيد[٢]

[١]قسم الفارماكولوجي، كلية الطب البيطرى، جامعة الزقازيق، الزقازيق ٤٤٥١١، الشرقية، مصر

[٢]معهد بحوث صحه الحيوان – معمل فرعى الزقازيق – مصر

تعتبر الثروة السمكيه من أهم مصادر البروتين الحيوانى. ووجود السموم الفطريه فى الأعلاف من أهم المشكلات التى تواجه الاستزراع السمكى. تم اجراء هذه الدراسه لاختبار تأثير سم الأفلاتوكسن ومضادات السموم الفطريه على صحة ومناعة وبقايا سموم الأفلاتوكسن فى أسماك البلطى النيلى. فى هذه الدراسه تم استخدام ١٨٠ سمكه بلطى يتراوح أوزانهم بين ٣٥-٤٠ جرام تم تقسيمهم الى ستة مجموعات (كل مجموعه تشمل ٣٠ سمكه) كل مجموعه مقسمه على ثلاثة أحواض, والمجموعات كالآتى: المجموعه الأولى: هى مجموعه ضابطه تتغذى على عليقه أساسيه بدون أى اضافات. المجموعه الثانيه: هى مجموعه تتغذى على عليقه أساسيه مع اضافه ٠,٥% سيليكات. المجموعه الثالثه: هى مجموعه تتغذى على عليقه أساسيه مع اضافه ٠,٢٥% خميره. المجموعه الرابعه: هى مجموعه تتغذى على عليقه أساسيه تحتوى على ٢,٥مجم أفلاتوكسن ب١ على كجم عليقه. المجموعه الخامسه: هى مجموعه تتغذى على عليقه تحتوى على ٢,٥ مجم أفلاتوكسن ب١/كجم عليقة مع اضافه ٠,٥% سيليكات. المجموعه السادسه: هى مجموعه تتغذى على عليقه تحتوى على ٢,٥ مجم أفلاتوكسن ب١ /كجم عليقة مع اضافه ٠,٢٥% خميره. وأوضحت النتائج أن اضافة الأفلاتوكسن الي عليقة الأسماك أدت الي معدلات نفوق ١٦%. ومع اضافة السيليكات والخميره الي العليقه أدت الى نقص معدل النفوق الي ٣%. وجد أن الأسماك التي تم تغذيتها علي ٢.٥ جزء في المليون أفلاتوكسن أسفرت عن انخفاض معنوي في خلايا الدم الحمراء والهيموجلوبين والهيماتوكريت وخلايا الدم البيضاء ومع اضافة كلا من السيليكات والخميره أدى الى تعديل هذا الانخفاض. الأسماك التي تم تغذيتها علي عليقه مسممه بالأفلاتوكسن أظهرت انخفاض معنوي في نسبة البلعمه وتم زيادة هذا الانخفاض مع اضافة مضادات السموم البيولوجي والكيميائي. المجموعه التي تم تغذيتها علي عليقه تحتوي علي الأفلاتوكسن أسفرت عن ارتفاع معنوي في انزيمات الكبد والكلي ومع اضافة كلا من مضادى السموم أدي الي انخفاض معنوي لهذا الارتفاع في الانزيمات. أيضا اضافة الأفلاتوكسن الي عليقة الأسماك أدي الي انخفاض معنوي في نسبة البروتين والألبيومين والجلوبيولين وتحسنت هذه النتائج باضافة كلا من السيليكات والخميره. الأسماك التي تم تغذيتها علي عليقه ملوثه بالأفلاتوكسن أدت الي ارتفاع معنوي في نسبة بقايا سموم الأفلاتوكسن في العضلات والكبد وهذه الزياده تصاعديه علي مدار التجربه واضافة مضادات السموم أدى الي انخفاض معنوى في نسبة بقايا السموم في كلا من العضلات والكبد. والجدير بالذكر ان اعطاء مضاد السموم البيولوجي (الخميره)أدي الي نتائج أفضل نسبيا من مضاد السموم الكيميائي (السيليكات).