

## Diminution of Aflatoxicosis in *Tilapia nilotica* Fish by Dietary Supplementation with Fix in Toxin and *Nigella Sativa* Oil

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**Abstract:** Mycotoxins are toxic metabolites of fungal origin, they are produced by certain strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Under favorable conditions of temperature and humidity, these fungi grow on certain foods, resulting in the production of aflatoxins, which can enter into the human food chain directly through foods of plant origin (cereal grains), indirectly through foods of animal origin (kidney, liver, milk, eggs); however their continuous intake even in microdoses can result in their accumulation. Aflatoxins are hepatotoxic, hepatocarcinogenic and immunotoxic and cause growth retardation in animals and exposed human populations. Fix in Toxin is a kind of pentonite clay, consisting of sodium calcium aluminosilicate, a non toxic agent and absorbent for a wide variety of toxic agents. It acts as an enterosorbant that rapidly binds aflatoxins in the gastrointestinal tract resulting in decreased aflatoxin uptake and bioavailability. *Nigella sativa* is a spicy potent oil belonging to ranunculacea seeds oil showed antibacterial as well as antifungal effects. This study was conducted to evaluate the ability of Fix in Toxin 0.2 % and *Nigella sativa* oil 1% to diminish the clinical signs of aflatoxicosis in *Tilapia nilotica* fish and based on this evidence, it's hypothesized that clay based enterosorption of aflatoxin may be a useful strategy for prevention of aflatoxicosis in human population. *Tilapia nilotica* fish were divided into three groups, 20 fish for each group: Group 1 served as control and will be fed on commercial fish diet. Group 2 were supplied by aflatoxin contaminated ration with corn 80 ug toxin /kg ration. Group 3 were supplied by aflatoxin contaminated ration with corn 80 ug toxin/kg ration and treated with 0.2 % Fix in Toxin and 1 % *Nigella sativa* oil injected daily I/P. Analysis of hematological parameters and clinical chemistry revealed significant differences between the control groups and the aflatoxin groups. Administration of Fix in Toxin 0.2% and *Nigella sativa* oil injection 1% of body weight reduced the aflatoxicosis in liver and kidney by improving all liver and kidney enzymes. The dietary Fix in Toxin clay remedy is novel, inexpensive and easily disseminated, proving efficacy in diminishing the clinical signs of aflatoxicosis in fish, where it acts as an aflatoxin enterosorbant that tightly and selectively binds the poison in the gastrointestinal tract of the fish, decreasing their bioavailability and associated toxicities. In addition the *Nigella sativa* oil has a synergistic effect with Fix in Toxin in diminishing aflatoxicosis in fish. These findings support their use for dietary intervention studies in human populations at high risks for aflatoxicosis, specially in Egypt, where studies have shown that concurrent infection with the hepatitis B virus (HBV) during aflatoxin exposure increases the risk of hepatocellular carcinoma (HCC).

**Key words:** Aflatoxicosis · *Tilapia nilotica* fish · Fix in Toxin effect · *Nigella sativa* oil effect · Hematological parameters · Clinical chemistry

### INTRODUCTION

When certain types of fungus grow on food, they produce minute amounts of toxins called *mycotoxins*. Most fungi-produced mycotoxins are harmless and even

helpful. For example, the antibiotic penicillin came from a fungus and it is a mycotoxin [1].

The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Under

favorable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins, which can enter into the human food chain directly through foods of plant origin (cereal grains), indirectly through foods of animal origin (kidney, liver, milk, eggs) [2].

The most pronounced contamination has been encountered in tree nuts, peanuts and other oilseeds, including corn and cottonseed. The major aflatoxins of concern are designated B1, B2, G1 and G2. These toxins are usually found together in various foods and feeds in various proportions [3]; however, aflatoxin B1 is usually predominant and is the most toxic. Aflatoxin M a major metabolic product of aflatoxin B1 in animals and is usually excreted in the milk and urine of dairy cattle and other mammalian species that have consumed aflatoxin-contaminated food [4].

These poisons are completely heat stable, so neither cooking nor freezing destroys the toxin. They remain on the food indefinitely. aflatoxins grow on grains and legumes mostly during storage, so the grains and legumes must be stored correctly to limit this problem [5].

Aflatoxins produce acute necrosis, cirrhosis and carcinoma of the liver in a number of animal species; no animal species is resistant to the acute toxic effects of aflatoxins; hence it is logical to assume that humans may be similarly affected. Aflatoxin B1 is a very potent carcinogen in many species, including nonhuman primates, birds, fish and rodents. In each species, the liver is the primary target organ of acute injury [6-8].

*Nigella sativa* is a spicy potent oil belonging to ranunculacea seeds oil showed antibacterial fungicidal effects [9]. *Nigella sativa* inhibited chemical carcinogenesis, some investigators reported that its antioxidants effect inhibited chemical carcinogenesis. Ascorbic acid and *Nigella sativa* could reduce aflatoxin induced liver cancer [10].

Fix in Toxin is a kind of pentonite (clay) consisting of sodium calcium aluminosilicate, a non toxic agent and absorbent for a wide variety of toxic agents [11]. It acts as an enterosorbant that rapidly and preferentially binds aflatoxins in the gastrointestinal tract resulting in decreased aflatoxin uptake and bioavailability [12].

**Aim of the present work:** This study was conducted to evaluate the ability of Fix in toxin 0.2 % and *Nigella sativa* oil 1% to diminish the clinical signs of Aflatoxicosis in *Tilapia nilotica* and based on this evidence, it's hypothesized that clay based entorsorption of aflatoxin may be a useful strategy for prevention of aflatoxicosis in human population.

## MATERIALS AND METHODS

**Experimental design:** 60 *Tilapia nilotica* fish (50-100g each) were obtained from Abbassa and were acclimatized to laboratory conditions. They were kept in glass aquaria supplied with dechlorinated tap water at a rate of one liter for each cm of fish body.

The 60 *Tilapia nilotica* fish were divided into three groups, 20 fish for each group:

- Group 1 served as control and will be fed on commercial fish diet.
- Group 2 were supplied by Aflatoxin contaminated ration with corn 80 ug toxin/kg ration.
- Group 3 were supplied by Aflatoxin contaminated ration with corn 80 ug toxin/kg ration and treated with 0.2 % Fix in Toxin and 1 % *Nigella sativa* oil injected daily I/P.

The fish were fed by hand twice daily and feed consumption in all groups was recorded daily, also mortality and body weight due to Aflatoxin were recorded.

**Samples analysis:** serum was collected 3 times at 3 months interval and sera were frozen at -20°C.

**Biochemical and hormonal studies:** The activities of aspartic aminotransferase (AST) and alanine aminotransferase (ALT) as well as cholesterol, urea and creatinine levels were determined according to the method of Varley *et al.*, [13] by using commercial kits (Bio Merieux, France), total lipids were estimated according to the method of Siesta [14]. Total serum protein was estimated according to Drupt [15].

**Haematological studies:** Blood hemoglobin was assessed and hematocrit value was carried out by using microhematocrit capillary tubes centrifuged at 2000 P.M. for 5 min according to the method of Drabkin [16].

**Statistical analysis:** Data are collected, tabulated for Statistical analysis according to the method of Gad and Weil [17].

## RESULTS

Table 1 showed that aflatoxicosis produce a significant decrease in body weight if compared with the control groups.

Tables 2, 3 & 4 showed that there is a significant decrease in PCV and Hemoglobin (P <0.01). There is a

Table 1: Effect of aflatoxin on body weight of fish during the course of the experiment

Groups	1 month	2 months	3 months
Group 1	58±0.21p	69±0.16*	100±0.72*
Group 2	50±0.10	60±0.2*	75±0.13*
Group 3	55.5±0.06	65±0.73*	85±0.64*

(\*P <0.01)

Table 2: Effect of aflatoxin after one month on biochemical and hematological parameters in fish and after treatment with Fix in Toxin 0.2 % + *Nigella sativa* 1 %

Parameters	Group 3		
	Group 1 Control N=20	Group 2 Aflatoxin N=20	Aflatoxin + Fix in Toxin 0.2 % <i>Nigella sativa</i> 1% N=20
Total protein mg/l	5.6±0.18	3.4±0.73**	4.5±0.71
Alkaline phosphatase mg/dl	18.9±0.38	28.7±0.34**	22±0.14
AST U/l	82±0.24	112±0.05**	104±0.04
ALT U/l	18±0.68	28±0.73**	23±0.75
Urea mg/dl	2.98±0.28	4.5±0.63**	5.3±0.28*
Creatinine mg/dl	0.82±5.5	0.99±0.63**	0.89±0.35
Total lipids mg/dl	98±0.98	142±0.24**	105±0.28*
Cholesterol mg/l	188±0.78	210±2.3**	198±0.34*
Hemoglobin mg/dl	8.2±0.24	5.3±0.75**	7.0±1.61
P.C.V%	39.0±64	34±0.04*	34±0.08

(\*\*P <0.01; \*p < 0.05)

Table 3: Effect of Aflatoxin after two months on biochemical and hematological parameters in fish and after treatment with Fix in Toxin 0.2 % + *Nigella sativa* 1 %

Parameters	Group 3		
	Group 1 Control N=20	Group 2 Aflatoxin N=20	Aflatoxin + Fix in Toxin 0.2 % <i>Nigella sativa</i> 1% N=20
Total protein mg/l	5.8±0.22	3.2±0.14**	4.8±0.27
Alkaline phosphatase U/L	18.9±0.18	34.9±0.28**	20±0.13
AST U/L	85±1.27	120±2.3**	95.6±0.09
ALT U/L	19±0.72	30±0.89**	20±0.16
Urea mg/dl	2.9±0.74	5.2±913**	3.2±0.21
Creatinine mg/dl	0.84±0.26	1.4±0.51**	0.84±0.27
Total lipids mg/dl	99±0.14	185±13**	100±0.77
Cholesterol mg/dl	187±0.64	240±3.6**	190±2.3
Hemoglobin %	8.7±0.29	4.9±0.73**	7.2±1.14
P.C.V%	41±0.71	29±0.03**	38±0.28

(\*\*P <0.01)

Table 4: Effect of Aflatoxin after three months on biochemical and hematological parameters in fish and after treatment with Fix in Toxin 0.2 % + *Nigella sativa* 1 %

Parameters	Group 3		
	Group 1 Control N=20	Group 2 Aflatoxin N=20	Aflatoxin + Fix in Toxin 0.2 % <i>Nigella sativa</i> 1% N=20
Total protein mg/l	5.8±0.23	3.0±0.44**	5.6±0.76
Alkaline phosphatase U/L	18.7±0.28	36.2±0.92**	18.4±0.33
AST U/L	82±0.18	134±6.3**	83±0.28
ALT U/L	19.2±0.23	26±0.38**	19.4±0.08
Urea mg/dl	2.78±0.23	5.2±0.19**	2.79±0.36
Creatinine mg/dl	0.80±0.47	1.6±0.53**	0.83±0.33
Total lipids mg/dl	97±0.74	190±1.4**	96±0.83
Cholesterol mg/dl	185±0.95	255±2.4**	184±0.74
Hemoglobin %	8.6±0.43	4.8±0.72**	8.7±0.38
P.C.V%	39±0.22	27±0.16**	40±034

(\*\*P <0.01)

significant decrease in mean of total protein and a significant increase in AST, ALT. There is a significant increase in urea, creatinine, total lipid, cholesterol and alkaline phosphatase (P <0.01).

Post treatment with Fix in Toxin 0.2% and *Nigella sativa* oil injection 1% for 3 months. All this parameters return to normal level gradually as shown in Tables 1, 2, 3 and 4 if compared with control groups.

## DISCUSSION

Aflatoxicosis is poisoning that results from ingestion of aflatoxins in contaminated food, so humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to be avoided because fungal growth in foods is not easy to prevent [18]. Aflatoxins produce acute necrosis, cirrhosis and carcinoma of the liver and also impair immunity which ultimately led to increased susceptibility to disease in a number of animal species; no animal species is resistant to the acute toxic effects of aflatoxins [19-21]; hence it is logical to assume that humans may be similarly affected.

Evidence of acute aflatoxicosis in humans has been reported from many parts of the world [6,8,22,23] and there is a positive association between dietary aflatoxins and hepatocellular carcinoma (HCC), especially aflatoxin B1 which is a potent carcinogen in some animals, In 1988, the IARC placed aflatoxin B1 on the list of human carcinogens.

Studies have shown that concurrent infection with the Hepatitis B virus (HBV) during aflatoxin exposure increases the risk of hepatocellular carcinoma (HCC). As HBV interferes with the ability of hepatocytes to metabolize aflatoxins, This effect is synergistic with the resulting damage far greater than just the sum of aflatoxin or HBV individually [22].

The biochemical results detected in Tables 2, 3 & 4, showed significant increase in AST, ALT, while there was significant decrease in total protein level in group 2. These findings agreed with those found by by Jassar and Balwant [24], Rasmassen *et al.* [25], Sisk *et al.* [26] due to liver injury induced by aflatoxicosis. The elevation of ALP activity comes in consistence with that mentioned by Jassar and Balwant [24], Svobodava *et al.* [27] in chicken due to degenerative changes in the liver, causing leakage of enzymes into serum and cause the highest concentration of alkaline phosphatase.

The biochemical results, detected in Tables 2, 3 & 4 showed significant increase in urea and creatinine, which

are indicative of abnormal kidney functions group 2, Similar finding were reported by Newperne [10]. These changes due to necrosis of kidneys reported by Pier [19], Jindal and Mahipal [28] and Mansfeld and Kautna [29]. The lipid metabolism was altered during aflatoxicosis as judged by increase of total lipid content. In the present experiment, there is a highly elevation of total lipid and cholesterol in serum which agree with Sisk *et al.* [26] and Sippel *et al.* [30].

It is obvious that administration of Fix in Toxin 0.2% and *Nigella sativa* oil injection 1% of body weight reduced the aflatoxicosis in liver and kidney, group 3. These findings agreed with those found by Sniezko [31], Harvey *et al.* [32], Phillips [33, 34], Wang *et al.* [35], Afriyie Gyawu [36] and Afriyie Gyawu [37] in relation to the dietary Fix in Toxin clay. In addition the *Nigella sativa* oil has a synergistic effect with Fix in Toxin in diminishing aflatoxicosis in fish due to its antibacterial as well as antifungal and antioxidants effects [11].

The present study showed a significant decrease in PCV, HB concentration in the affected fish that was proportionally correlated with the severity of aflatoxicosis. This result is in accordance with El-Bouhy *et al.* [11] and Robert [38]. They found similar results in broilers chickens common carp. Fish and this indicates that the toxin causes a deleterious effect on the hemopoietic system.

In conclusion, based on the present research, the dietary HSCAS clay remedy is novel, inexpensive and easily disseminated and proves its efficacy in diminishing the clinical signs of aflatoxicosis in fish, where it acts as an aflatoxin enterosorbant that tightly and selectively binds the poison in the gastrointestinal tract of the fish, decreasing their bioavailability and associated toxicities. In addition that, *Nigella sativa* oil has a synergistic effect with Fix in Toxin in diminishing this aflatoxicosis in fish. These findings support their use for dietary intervention studies in human populations at high risks for aflatoxicosis, specially in Egypt, where studies have shown that concurrent infection with the hepatitis B virus (HBV) during aflatoxin exposure increases the risk of hepatocellular carcinoma (HCC).

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