Diminution Of Aflatoxicosis In Tilipia Zilli 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 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); 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) consists 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 belonging to ranunculacea seeds oil showed antibacterial, fungicidal 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 Zilli* fish, and based on this evidence, it's hypothesized that clay based entersorption of Aflatoxin may be a useful strategy for prevention of Aflatoxicosis in human population.60 *Tilapia Zilli* 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 be supplied by Aflatoxin contaminated ration with corn 80 ug toxin /kg ration. Group 3 were be 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, clinical chemistry revealed significant differences between the control groups and the aflatoxicotic 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 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 alfatoxin 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). [Nature and Science 2010;8(2):43-49]. (ISSN: 1545-0740).

Key words: Aflatoxicosis, *Tilapia Zilli* fish, Fix in Toxin effect, *Nigella sativa oil* effect, Hematological parameters, Clinical chemistry dynamic simulation; model; composting; domestic solid waste

1. 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 [Magan N 2005] [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 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) Rojas-Duran T 2006 [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 [Takatori K 2006] [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 [Martins HM 2007] [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 [Kabak S 2006] [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 [Gong Y 2004], [Egal S 2005], [Wagacha JM 2008] [6],[7] and [8].*Nigella sativa* is a spicy potent belonging to ranunculacea seeds oil showed antibacterial fungicidal effects (Akguil, 1989) [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 (Newperne *et al.*1999) [10].Fix in toxin is a kind of pentonite (clay) consists of (sodium calcium aluminosilicate) a non toxic agent and absorbent for a wide variety of toxic agents (El-Bouhy *et al.*, 1993) [11]. It acts as an enterosorbant that rapidly and preferentially binds aflatoxins in the gastrointestinal tract resulting in decreased aflatoxin uptake and bioavailability [Phillips TD 2002] [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 Zilli*, and based on this evidence, it's hypothesized that clay based entersorption of Aflatoxin may be a useful strategy for prevention of Aflatoxicosis in human population.

Material and Methods:

Experimental Design: 60 *Tilapia Zilli* 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 Zilli* 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^{o}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 Merieus, France), total lipids were estimated according to the method of Siesta (1981) [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 (1964) [16].

Statistical analysis: Data are collected, summarized then tabulated for st Statistical analysis according to the method of Gad and Weil (1986) [17].

Results:

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

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

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	57 ± 0.21p	68 ± 0.16*	101 ± 0.72*
Group 2	51 ± 0.10	61 ± 0.2*	74 ± 0.13*
Group 3	54.5 ± 0.06	64 ± 0.73*	84 ± 0.64*

(*P <0.01)TABLE

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 1 Control N =20	Group 2 Aflatoxin N =20	Group 3 Aflatoxin + Fix in Toxin 0.2 % *Nigella sativa* 1% N=20
Total protein mg/L	5.5 ± 0.18	3.13 ± 0.73**	4.4 ± 0.71
AST U/L	81 ± 0.24	112 ± 0.05**	103 ± 0.04
ALTU/L	17 ± 0.68	27 ± 0.73**	24 ± 0.75
Urea mg/dl	2.88 ± 0.28	4.4 ± 0.63**	5.2 ± 0.28*
Creatinine mg/dl	0.72 ± 5.5	0.98 ± 0.63**	0.88 ± 0.35
Total lipids cholesterol mg/dl	98 ± 0.98	143 ± 0.24**	104 ± 0.28*
Cholesterol mg/l	188.0.78	210 ± 2.3**	198 ± 0.34*
Alkaline phosphatase mg/dl	18.9 ± 0.38	28.7 ± 0.34**	22 ± 0.14
Hemoglobin mg/dl	8.3 ± 0.24	5.4 ± 0.75**	7.1 ± 1.61
P.C.V%	39.1 ± 64	34.1 ± 0.04*	34.1 ± 0.08

****p < 0.01**

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 1 Control N =20	Group 2 Aflatoxin N =20	Group 3 Aflatoxin + Fix in Toxin 0.2 % *Nigella sativa* 1% N=20
AST U/L	84 ± 1.27	121 ± 2.3**	94.6 ± 0.09
ALT U/L	18 ± 0.72	31 ± 0.89**	19 ± 0.16
Urea mg/dl	2.8 ± 0.74	5.1 ± 913**	3.1 ± 0.21
Creatinine mg/dl	0.83 ± 0.26	1.3 ± 0.51**	0.83 ± 0.27
Total protein mg/l	5.7 ± 0.22	3.1 ± 0.14**	4.7 ± 0.27
Total lipids mg/dl	98 ± 0.14	184 ± 13**	99 ± 0.77
Cholesterol mg/dl	186 ± 0.64	239 ± 3.6**	189 ± 2.3
Alkaline phosphates U/L	18.8 ± 0.18	33.9 ± 0.28**	201 ± 0.13
Hemoglobin %	8.6 ± 0.29	4.8 ± 0.73**	7.1 ± 114
P.C.V%	42 ± 0.71	28 ± 0.03**	37 ± 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 1 Control N =20	Group 2 Aflatoxin N =20	Group 3 Aflatoxin + Fix in Toxin 0.2 % *Nigella sativa* 1% N=20
Total protein mg/l	5.7 ± 0.23	3.1 ± 0.44**	5.5 ± 0.76
AST U/L	81 ± 0.18	133 ± 6.3**	82 ± 0.28
ALT U/L	19.1 ± 0.23	25 ± 0.38**	19.3 ± 0.08
Urea mg/dl	2.77 ± 0.23	5.1 ± 0.19**	2.78 ± 0.36
Creatinine mg/dl	0.79 ± 0.47	1.5 ± 0.53**	0.82 ± 0.33
Total lipids mg/dl	96 ± 0.74	189 ± 1.4**	95 ± 0.83
Cholesterol mg/dl	184 ± 0.95	254 ± 2.4**	183 ± 0.74
Alkaline phosphatase U/L	18.6 ± 0.28	35.2 ± 0.92**	18.3 ± 0.33
Hemoglobin %	8.5 ± 0.43	4.7 ± 0.72**	8.6 ± 0.38
P.C.V%	38 ± 0.22	26 ± 0.16**	39 ± 034

(P <0.01)**

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 avoid because fungal growth in foods is not easy to prevent [Bennett JE 2005] [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 [Pier AC 1987, Pier AC 1999, Wildi Dis J 2004] [19, 20, 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 [Williams JH 2004, Kovacs M 2004, Gong Y 2004, Wagacha JM 2008] [22, 23,6, 8] and there is a positive association between dietary aflatoxins and hepatocellular carcinoma (HCC). especially aflatoxin B1, is potent carcinogens 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 (Williams, 2004) [22].

The biochemical results detected in Table 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 (1993), Rasmassen *et al*. (1986), Sisk *et al*. (1988), due to liver injury induced by Aflatoxicosis [24, 25, 26]. The elevation of ALP activity comes in consisitence with that mentioned by Jassar and Balwant (1993), Svobodava *et al*. (1999) in chicken due to degenerative changes in the liver causing leakage of enzymes into serum and cause the highest concentration of alkaline phosphatase [24, 27].

The biochemical results, detected in table 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 (1999) [12]. These changes due to necrosis of kidneys reported by Jindal and Mahipal (1994) Mansfeld (1989), Pier (1987) [28, 29, 19]. 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 Sippel, *et al*. (1983), Sisk *et al*. (1988) [30, 31].

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 Harvey RB 1991, Phillips TD 1999, 2002, 2008, Wang JS 2005, Afriyie Gyawu E 2005, 2008 in relation to the dieatry HSCAS clay [32, 33, 12, 34, 35, 36, 37]. In addition the *Nigella sativa oil* has a synergistic effect with Fix in Toxin in diminishing aflatoxicosis in fish due to its antibacterial fungicidal antioxidants effects [El-Bouhy *et al*. (1993)] [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 Robert (1989), El-Bouhy *et al*. (1993) [38,11]. They found similar results in broilers chinckens common carp. Fish and this indicates that the toxin causes a deleterious effect on the hemopoeitic 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 alfatoxin 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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