ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pharmaceutical Effect Of Aflatoxin on Grey Mullet.

Mona S Zaki[1]*, MH Osfor[2], and AH Hammam[3].

[1]Hydrobiology Department, National Research Centre, Dokki, Giza, Egypt.
[2]Nutritional Department, National Research Centre, Dokki, Giza, Egypt.
[3]Animal Reproductive, National Research Centre, Dokki, Giza, Egypt.

ABSTRACT

The effect of fix in tox 0.5% and *Nigella sativa* oil 3% on aflatoxin induced liver toxicity was investigated, Aflatoxicosis causes significant increase in liver enzyme SGOT and SGPT, Alkaline phosphastose activity and an increase in the level of cholesterol total lipids, decrease the level of total protein and hemoglobin and P.C.V. Moreover the liver exhibited some clinicopathological changes and decreased body weight. Both oil of Nigella sativa 3% and Fix in Tox 0.5% reduced the development of hepatotoxicity by Aflatoxin. *Nigella sativa* showed more improvement of all enzymes of kidney and liver, and also total lipid and cholesterol were reduced and body weight increased.

Keywords: Aflatoxin toxicity Nigella sativa oil effect Fix in Tox effect clarion lazara catfish

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Aflatoxin a number of biological active mycotoxins group is produced by a strain of *Aspergillus flavus*. Aflatoxin cause damages in the liver, kidneys producing a variety effects including disruption of lipids CHO and protein metabolism. It decreases growth rate and lowered productivity. (Jantrarotoi et al. 1990)

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) (Berezi, 1989).

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 (Chen, 1980); 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.

Aflatoxicosis is a disease that can affect many species of fish, and results when feed contaminated with aflatoxins is eaten by the fish (Ashley, 1970). Aflatoxins are chemicals produced by some species of naturally occurring fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) commonly known as molds. Aflatoxins are common contaminants of oilseed crops such as cottonseed, peanut meal, and corn. Wheat, sunflower, soybean, fish meal, and nutritionally complete feeds can also be contaminated with aflatoxins. Four major aflatoxins (AFB1, AFB2, AFG1 and AFG2) are direct contaminants of grains and finished feeds. Factors that increase the production of aflatoxins in feeds include environmental temperatures above 27°C, humidity levels greater than 62%, and moisture levels in the feed above 14%. The extent of contamination will vary with geographic location, feed storage practices and processing methods. Improper storage is one of the most important factors favoring the growth of aflatoxin-producing molds, and it is a major element that the fish producer needed to control (Sotolu**,***et al.,* 2014).

The liver, spleen and gills of fishes treated with concurrent administration of biological antidotes and aflatoxins were within the normal limits. Liver showed normal hepatocytes with normal vacuolation consistent with glycogen deposition. Spleen also showed marked lymphocytes within the white pulp and normally scattered melanomacrophages centers. The gill lamellae were obviously separated unless the lining epithelial cells hyperplasia of the gill lamellae (Hegazi et al.,2013).

Aflatoxin treated fishes showed marked yellowish icteric coloration, pale scales and marked protrusion of the eye ball with marked eye opacity. Fin and gill rot were also noticed. Body organs examination revealed liver jaundice associated with marked gall bladder enlargement and whitish grayish nodules on the liver surface (Hegazi et al.,2013).

Aflatoxins were first isolated in turkeys and of cancer in rainbow trout fed on rations formulated from peanut and cottonseed meals. The toxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus*fungi when the temperatures are between 24 and 35 °C, and they will form within many commodities whenever the moisture content exceeds 7% (10% with ventilation). Other factors may also influence aflatoxin production: substrate composition, water activity, pH, atmosphere (concentration of oxygen and carbon dioxide), microbial competition, mechanical damage to the seeds, mold lineage, strain specificity and variation, instability of toxigenic properties, plant stress, insect infestation, and use of fungicides or fertilizers It is important to remember that aflatoxin contamination is cumulative, and the moment of harvesting and drying, and storage conditions may also play an important role in aflatoxin production.

Effect of aflatoxin on fishes and other animals have been reported by many workers. Nunez *et al*. (1991) reported hepatocellular adenoma and hepatocellular carcinoma in Rainbow trout when exposed to aflaroxin B1.Caguan*et al*. (2004) reported loss of appetite, low survival percent and decreased mean total biomass in

tilapia when fed with aflatoxin contaminated feed. Faisal *et al*. (2008) reported spermatotoxic effect of aflatoxin in male wister rat.

MATERIAL AND METHODS

Experimental conditions: 60 catfish clariouslazera (100-150g 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. They were fed commercial fish diet were supplied by Aflatoxin contaminated ration with corn 80 ug toxin/kg ration, as shown in Table 1. A total number of 60 cutfish were used in this experiment: 20 Fish each group, 20 cutfish control, 20 fed Aflatoxin and 20 treated with Faxatation Nigella sativa.

The third group Aflatoxin contaminated ration + 0.5 Fix in Tox + 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

Serum was collected 3 times at 3 months interval and sera were frozen at -20. Tested kits supplied from biomerieux, France were used for detetmination of the activity of serum glutamic pyurvictransaminse and glutamic oxalocetictransaminase as described by Reitman and Frankel (1956), serum creatinine was determined according to Henery, (1968). Enzymatic determination of urea was done according to King (1965). Blood hemoglobin was assessed by cyame hemoglobin method Hematocrit value was carried out by using microhematacrit capillary tubesrentrifuged at 2000 P.M. for 5 min according to the method of Drabkin (1946) serum cholesterol according to the method Flegg (1973), total lipids according to the method of Siesta (1981), and statistical analysis according to the method of Gad and Weil (1986).

Table 1. ingredients and proximate chemical composition of diets used in the experiments.

Fngredient	Control		Proximate chemical composition
Fish meal	30	Crude protein CPg%	35.87
Meat meal	8	M.E/kg	2297.21
Bone meal	1	Ether extract g%	2.78
Soya bean	5	Crude liber g%	3.91
Skimmed milk	3	Ash g%	8.735
Wheat bran	20	Calcium mg%	2.069
Wheat flour	20	Lysine mg%	2.105
Yeast	10	Mehtionine mg%	0.562
Cod liver oil	1		
Mineral and premix	2		

Mineral and vit. premix per/kg of pellet food

Vit. A 8000 1U, vit. D 900 1U, vit. E 2 1U, vit. K4 mg, B_2 3.6mg, niacin 20mg, choline chloride 160mg, pantothenic acid 7mg, pyridoxine 0.2 mg, vit. B_{12} 5ug, Mn 70mg, Zn 60mg, Fe 20mg, Cu 2mg, Co 0.2mg.
N.B.: we added 80Ug polluted corn with Aflatoxin B_1 ,in this ration.

RESULTS

Aflatoxicosis produced a significant decrease in body weight if compared with control group as shown in Table 2. Statistical analysis revealed effect of Aflatoxin B_1 on erythrogram. There is a significant decrease in P.C.V. Hemoglobin ($P < 0.01$) as shown in Table 2. There is a significant decrease in mean of total protein and a significant increase in SGOT, SGPT, urea, creatinine, total lipid, cholesterol and alkaline phosphatose ($P < 0.01$). Post treatment with Fix in Tox 0.5 % and Nigella sativa oil injection 3% of body weight for 3 months. All this parameters return to normal level as shown in Tables 3 and 4 if compared with control group.

ISSN: 0975-8585

DISCUSSION

Aflatoxins are hepatotoxins (Pier, 1987, 1999) and also impair immunity which ultimately led to increased susceptibility to disease (Zaki, 1999). The present work demonstrated a severe necrosis in liver of catfish. The liver is the primary site of metabolism of ingested Aflatoxin. (Butler and Clifford, a985; Ali et al, 1994). The pathological changes of liver observed in the present investigations may be due to primary site of metabolism of ingested Aflatoxins as well as the primary lacratian of residues and lesions. Similar finding reported by Newperne (1999). The increase of enzyme Urea, creatinine. These changes due to necrosis of kidneys reported by Jindal and Mahipal (1994), Mansfeld (1989), Pier (1987). 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). It is obvious that administration of Fix in Tox 0.5 % and Nigella sativa oil injection 3% of body weight reduced the Aflatoxin in liver, kidney, of infected fish and may protect liver from free radical reactions due to Aflatocin, also total lipid, cholesterol return to normal level.

The present study showed a significant decrease in P.C.V., 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). They found similar results in broilers chickens common crap. Fish and this indicates that the toxin causes a deleterious effect on the hemopoeitic system.

Regarding the biochemical serum analysis, the noticed decreased in T.P. may be attributed to the improved protein synthesis as a result of liver function due to Aflatoxiocsis. (Ali et al., 1994, A kguil 1989, Edds, 1993). The increase in ALT and AST activities recorded by Jassar and Balwant (1993), Rasmassen et al. (1986), Sisk et al. (1988), due to liver affection in case of Aflatoxicosis the elevation of ALP activity comes in consistence with 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. The great increase of alkaline phosphatase activity due to damage of liver degeneration. Similar results were described by Kubena et al, (1990), who used hydrated sodium calcium alumino silicate (Fix in Tox) for preventing the absorption of Aflatoxins from gastrointestinal tract.

Table2(Effect of Aflatoxin after 1-2 months) on Clinicopathological changes in catfish after treatment with fix in Tox 0.5 % and Nigella sativa 3%.

Parameters	Control w(19)	Aflatoxin 1 month w(19)	Aflatoxin + Fix in Tox 0.5% Nigella sativa 3%	Control	2 months Aflatoxin group	Aflatoxin + Fix in Tox 0.5% Nigella sativa 3%
AST U/L	82±0.23	111±0.05**	103±0.04	84±1.26	119±2.3**	94.6±0.08
ALT U/L	17±0.66	27±0.71**	22±0.73	18±0.71	29±088**	19±0.17
Urea mg/dl	1.98±0.26	3.5±0.63**	4.3±0.26*	1.9±0.73	4.2±911**	2.2±0.19
Creatinine mg/dl	0.81±4.4	0.98±0.22**	0.88±0.33	0.83±0.25	1.3±0.49**	0.83±0.27
Total protein mg/dl	4.6±0.16	2.4±0.71**	3.5±0.69	4.8±0.21	2.2±0.13**	99±0.73
Total lipids cholesterol mg/dl	97±0.98	141±0.22**	104±0.26*	98±0.13	184±1.1**	99±0.73
Cholesterol	187±0.78	209±1.8**	197±0.38*	186±0.63	239±2.6**	189±1.1
Alkaline phosphates mg/dl	17.9±0.36	27.7±0.32**	21±0.17	17.9±0.17	33.9±0.26**	19±0.11
Hemoglobin mg/dl	7.2±0.22	4.3±0.73**	6.0±1.50	7.7±0.28	3.9±0.71**	6.2±1174
P.C.V %	38±0.62	33±0.04	33±0.08	40±0.70	28±0.01	37±0.27

P<0.01

ISSN: 0975-8585

Table3 Effect of Aflatoxin after 3 months on clinicopathological changes in catfish and treated by Fix in Tox 0.5% and Nigella sativa 3%

Parameters	Control 3 Months	Aflatoxin 3 Months	Aflatoxin plus Faxatoxin + Nigella sativa 3 months
AST U/L	81±0.13	133±5.1**	82±0.26
ALT U/L	18.1±0.19	25±0.36**	18.3±0.06
Urea mg/dl	1.77±0.21	4.1±0.17**	1.78±0.38
Creatinine mg/dl	0.79±0.45	0.5±0.53**	0.82±0.31
Total protein mg/dl	4.7±0.23	2.0±0.44**	4.5±0.73
Total lipid mg/dl	96±0.93	254±1.2**	183±0.72
Cholesterol mg/dl	184±0.93	254±1.2**	183±0.72
Alkaline phosphates U/L	17.6±0.26	35.1±0.90**	17.3±0.72
Hemoglobin %	7.5±0.43	3.7±0.71**	7.7±0.36
P.C.V %	38±0.19	23±0.14**	39±0.24

P<0.01

Table 4 Effect of Aflatoxin on body weight of catfish during the course of experiment

Group	1 month	2 months	3 months
Control 20 fish	57±0.20p	98±0.15*	119±071
Aflatoxin group (20 fish)	89±0.9	79±0.1*	74±012
Aflatoxin + Fix in Tox 0.5% + Nigella sative (20 fish)	84.4±0.05	104±0.72*	134±063

(*P<0.01)

In conclusion, the metabolism of Aflatoxin result in the alteration of various metabolic processes within hepatocytes which leads to severe serum biochemical alterations and serious pathological changes which affect fish production but treatment with Fix in Tox and Neigella sativa give an excellent of results.

REFERENCES

[1] Akguil, A. (1989) Antimicronial activity of black seed (Nigella sativa of Aflatoxin) essential oil. Caso Univ. Eczacilik Fax. Derg., 6, 63.

[2] Ali, M. V., Mohi-Eddin, S. M. and Eedy, V. M., (1994) Effect of dietary Aflatoxin on cell mediated immunity and serum protein in broiler chicken. Indian Vet. J., 78, (8) 760.

[3] Ashley, L.M (1970). Pathology of fish feed Aflatoxins and other anti-metabolites. In: A symposium on diseases of Fishes and shell fishes. American Fisheries Society Special Publication 5, 366-379.

[4] Berezi, I. (1989): Immunoregulation by neuroendocrine factors Dev, comp Immunol 13:329-341.

[5] Bullerman, L. B. (1979) Significance of mycotoxins to food Protection. 42, 65.

[6] Butler, W. H. and Clifford, J. I. Extraction of Aflatoxin from rat liver. Nature, 206 (5), 1045.

[7] Caguan, A.G; Tayaban,R.H; J.R. Somga; Bartolome,R.M. Effect of Aflatoxin Contaminated feed in Nile tilapia (*Oreochromis niloticus*L.). In Proceeding of the 6th International symposium on tilapia in aquaculture (R.B. Remedios, G.C. Imir and K. Fitzsimons. eds.):172-178 (2004).

[8] Canton, J. H., Kroes, R., Van Loglen, M. J. and Van Schathorst, M., (1998) The carcinogenicity of aflatoxin B_1 in rainbow trout. Fd. Consmet. Toxicol, 19, 564.

[9] Chen, Y. (1980): Effect of thyroxine on the immune response of mice invivo and vitro Immunol org 9, 269-276.

[10] Drabkin, D. J. (1946) Clinc. Chem. 164, 703.

[11] Edds, G. T. (1973) Acute Aflatoxicosis: A Review. V. M. A. J. A. 162, 4, 304.

[12] El-Bouhy, Z. M., Ali A. A. and Helmy, M. S. (1993) Preliminary studies on Aflatoxicosis in Nile cat fish and trials for detoxification of contaminated food. Zagazig Vet. J. 21, (4), 607.
[13] Faisal. K; perisamy, V. S.; Sahabuddin,S; Radha, A; Anandhi,R; Akbarsha, M. A. Spermatotoxic effect of aflatoxin B1 in rat: extrusion of outer dense fibers and associated axonemalmicrotubule doublets of sperm flagellum. *J. Soci. Repro. Fert.* 135: 303 - 310, (2008).
[14] Flegg, (1973) Clin. Chem, 29, 1075.
[15] Gad, S. C. and Weil, C. S. (1986) Statistics for toxicologists, In; Hayes, A. W. (2nd ed). Principles and Methods of Toxiology: Raven Press, Mew York, pp. 273-320.
[16] Hegazi, S.M., El-Sabagh, M. R., El-Keeidy, A. and I. Zein El-Dein. A. (2013): Aflatoxin in feed and its effect on fish health. Kafrelsheikh Vet. Med. J. 11(2) 317:329.
[17] Jantrarotai, W., Lovell, R. T. and Gizzle T. M. (1990) A cute toxicity of aflatoxin B_1 Channel catfish. J. Aquatic Animal Health 2, 238.
[18] Jassar, B. S. and Balwant, S. (1993) Biochemical changes in experimental Aflatoxicosis in Broiler chicken. Indian J. Animal Science 63 (8), 784.
[19] Jindal, IV. And Mhaipal S. K. (1994) Toxicity of aflatoxin B_1 in broiler chicks and its reduction by activated charcoal. Research in Vet. Science 56, 37.
[20] King, J. (1965) Practical Clinical Enzymology. Van Nostrand Co. Ltd. Rpge 132.
[21] Kubena, F. L., Harvey, B.R., Huff, E., W. and Corrier, E.D. (1990) Ffficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T. 2 Toxin. Poultry Science 69, 1078.
[22] Manfeld, R., Grunert, E. and Kautna, J. (1989) Mycotoxicosis-a problem of dairy cowherds. Monatsheft fur veterinary medizin 44 (12), 409.
[23] Newperne, P.M. (1999) Chronic aflatoxicosis in animals and Poultry. J. Am. Vet. Med. Assoc., 263, 1269.
[24] Nunez. J. D.H; Duimishra, J. R. Ultra structure of hepato cellular neoplasms in aflatoxin B1 (AFB1) initiated rainbow trout (*Oncorhychus mykiss*). *ToxicolPathol*. 19 (i) 11-21, (1991).
[25] Pier, A.C. (1987) Aflatoxicosis and immuno suppression in mammalian animals. In M.S. Zuber, GB. Lillehoj and B.I. Rsnfor (Ed). Aflatoxin in Maize. pp. 65. Cimmyt, Mexico.
[26] Pier, A.C. (1999) Major biological consequences of Aflatoxicosis in animal production. J. Anim. Sci, 70, 3964.
[27] Rasmassen, B.H., Larsen, K., Hald, B., Maller, O.B. and Elling, F. (1986) Outbreak of liver cell carcinoma among salt water reared rainbow trout salmogairdneri in Denmark. Diseases of Aquatic Organisms 1, 191.
[28] Reitman, S. and Frankel, S. (1946) Am. J. Clin. Pathol. 28, 26.
[29] Robert, R. A. (1989) Fish Pathology. Second Edition, Baillier Tindall, Lodon. Philadelphia, Sydney, Tokyo, Toronto.
[30] Siesta, D. (1981) Am. Clin. Biochem., 6,24.
[31] Sippel, W.I., Burnside, J.C. and Atwood, M.B. (1983) A disease of swine and cattle caused by eating mouldy corn. Proc. 6th Ann. Meet. Am. Vet Med. Assoc. pp. 174-181.
[32] Sisk, D.B., Carlton, V.U. and Curtin, T.M. (1988) Experimental Aflatoxicosis in young swine. Am J. Vet. Res., 39, 1591.
[33] Sotolu, A.O, Sule, S.O, Oshinowo, J.A. and Ogara I. M., (2014):Implication of Aflatoxin In Fish Feeds and Management Strategies For Sustainable Aquaculture. *PAT*. 10 (1):38-52.
[34] Svobodava, Z., Piskac, A., Havlikova, J. and Groch, L. (1999) The influence of feed with different contents of Aflatoxin B_1 on the carp health condition, ZivocisnaVyroa 27, (11), 811.
[35] Zaki, Mona S. (1999) Effect of Aflatoxins on clinicopathological changes in Tilapodaniloticus, Banisuef Journal of Vet Science, July.