

## Reduction of Aflatoxin in Clarius lazara Catfish By Ginseng Extract and Nigella sativa Oil

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**Abstract:** Aflatoxins are the major toxic metabolites of fungi which are able to induce chronic liver damages. The antioxidant and hepatoprotective effects of Ginseng extract and Nigella sativa Oil 1% on Aflatoxin were investigated. Aflatoxicosis causes significant increase in liver enzyme SGOT and SGPT, Alkaline phosphatase activity and an increase in the level of cholesterol total lipid, decrease the level of total protein and hemoglobin and P.C.V. Moreover the liver exhibited some clinicopathological changes and decreased body weight. Both Ginseng extract and Nigella sativa Oil 1% 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.

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### Introduction:

Aflatoxin is a toxic compound produced by *Aspergillus flavus* and *A. parasiticus*. The molds can grow in improperly stored feeds and feeds with inferior quality of ingredients. Aflatoxins represent a serious source of contamination in foods and feeds in many parts of the world. These toxins have been incriminated as the cause of high mortality in livestock and in some cases of death in human beings (Murjani, 2003).

Aflatoxin is a potent hepatocarcinogen, strong mutagen and a potential teratogen. (Canton, et al. 1998; Bulter and Clifford, 1985). There are four main Aflatoxins: B1, B2, C1, C2. Aflatoxin B1 is known to be the most significant form that causes serious risk to animals and human health. The carcinogenic effect of aflatoxin B1 has been studied in fishes such as salmonid, rainbow trout, channel catfish, tilapia, guppy and Indian major carps (Jantrarotai and Lovell, 1990; Lovell, 1992; Tacon, 1992; Wu, , 1998; Chavez et al., 1994; Murjani, 2003). Aflatoxins inhibited RNA synthesis and DNA in liver. (Jindal et al. 1994).

*Nigella sativa* is a spicy potent belonging to ranunculacea seeds oil showed antibacterial fungicidal (Akguil, 1989). *Nigella sativa* inhibited chemical carcinogenesis. Some investigators reported that its antioxidant effect inhibited chemical carcinogenesis. Ascorbic acid and *Nigella sativa* could reduce Aflatoxin induced liver cancer (Newperne et al., 1999).

*Panax ginseng* C.A. Meyer is an herbal root that has been used for more than 2000 years throughout Far Eastern countries including China, Japan and Korea. Its beneficial effects have been analyzed by extensive preclinical and epidemiological studies (Yun, 2003). Recently, 20-O-( $\beta$ -D-glucopyranosyl)-20(S)-protopanaxadiol (IH-901), a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2 was isolated and purified after giving ginseng extract p.o. to humans and animals (Hasegawa et al., 1996). IH-901 has been shown to enhance the efficacy of anticancer drugs in cancer cell lines previously resistant to several anticancer drugs (Lee et al. 1999).

### Aim of present work

This study was conducted to evaluate the effect of Aflatoxin and ginseng with *Nigella sativa* oil 1% on some nutritional status and clinicopathological changes in Catfish toxicated with Aflatoxin and treated with ginseng and *Nigella Sativa* oil 1%.

### Material and Methods

**Experimental conditions:** 60 catfish *Clarius lazara* 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 80ug toxin/kg ration, as shown in Table (1). A total number of 60 cutfish were used in this experiment: 20 Fish each group, 20 cotfish control, 20 fed Aflatoxin and 20 treated with Faxatation Nigella Sativa.

The third group Aflatoxin contaminated ration + 0.2 ginseng + Niegella sativa oil injected daily 1/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 were collected 3 times at 3 months interval and sera were frozen at -20. Tested kits

supplied from biomerieux, France were used for determination of the activity of serum glutamic pyruvic transaminase and glutamic oxalocetic transaminase 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 tubes centri futed at 2000P.M. for 5min 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.**

Ingredient	Control		Proximate chemical composition
Hah meal	30	Crude protein Pg%	35.87
Meas meal	8	M.E/kg	2297.21
Bone meal	1	Ether extract g%	2.78
Soya bean	5	Crude fiber 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	Methionine mg%	0.562
Codliver oil	1		
Mineral and premix	2		

#### Mineral and vit. Premix per/kg of pellet food

Vit. A 8000 IU, vit. D 900 IU, vit. E 2 IU, vit. K4mg, B2 3.6mg, niacin 20mg, choline chloride 160mg, pantothenic acid 7mg, pyridoxine 0.2mg, vit. B12, 5ug, Mn 70mg, Zn 60mg, Fe 20mg, Cu 2mg, Co 0.2mg.

**N.B.:** we added 80Ug polluted corn with Aflatoxin B1, in this ration.

#### Results

Aflatoxicosis produced a significant decrease in body weight if compared with control group as shown in Table2. statistical analysis revealed effect of Aflatoxin, B1 on erythrogram. There is a significant decrease in P.C.V. Hemoglobin ( $P < 0.01$ ) as shown in Table2. there is a significant decrease in mean of total protein and a significant increase in SGOT, SGOT, Urea, creatinine, total lipid, cholesterol and alkaline phosphatase ( $P < 0.01$ ).

Post treatment with ginseng and Nigella sativa oil injection 1% of body weight for 3 months. All this parameters return to normal level as shown in Table3 and 4 if compared with control group.

**Table(2). (Effect of Aflatoxin after 1-2 months on clinicopathological changes in catfish after treatment with ginseng and Nigella sativa 1%)**

Parameters	Control N=(20)	Aflatoxin 1month N=(20)	Alfatoxint + ginseng +Nigella sativa 1% N=20	Control N=20	2months group N=20	Aflatoxint + ginseng +Nigella sativa 1% N=20
AST U/L	82±0.23	133±0.06**	103±0.05	84±1.27	121±2.4**	946±0.09
ALT U/L	17±0.67	27±0.72**	22±0.74	18±0.72	31±0.89**	21±0.18
Urea mg/dl	2.87±0.27	4.6±0.64**	5.2±0.27*	2.7±0.74	5.3±912**	3.3±0.20
Creatininemg/dl	0.72±5.4	0.8±0.23**	0.88±0.34	0.83±0.26	1.3±0.50**	0.83±0.28
Total protein mg/dl	46±0.17	3.5±0.72**	4.4±0.70	5.7±0.22	3.3±0.14**	4.4±0.60
Total lipidscholesterol mg/dl	88±0.99	143±0.23**	104±0.27*	97±0.14	184±1.2**	101±0.74
Cholesterol	178±0.79	212±2.8**	197±0.39*	188±0.64	244±3.6**	191±2.1
Alkaline phosphates mg/dl	16.9±0.37	28.8±0.33**	23±0.18	18.7±0.18	34.8±0.27**	21±0.12
Hemoglobin mg/dl	7.2±0.23	5.4±0.74**	7.1±1.60	8.6±0.29	4.8±0.72**	7.3±11.75
P.C.V%	38±0.63	33±0.05	33±0.05	42±0.71	28±0.02**	37±0.27

P<0.01

**Table(3). Effect of Aflatoxin after 3 months on clinicopathological changes in catfish after treatment with ginseng and Nigella sativa 1%**

Parameters	Control 3 months	Aflatoxin 3 months	Aflatoxin plus ginseng + Nigella sativa 1% 3 months
AST U/L	81±0.14	133±6.2**	82±0.27
ALT U/L	182±0.20	25±0.37**	18.3±0.07
Urea mg/dl	2.88±0.22	5.3±0.18**	2.64±0.39
Creatinine mg/dl	0.81±0.46	1.5±0.54**	0.81±03.2
Total protein mg/dl	5.7±0.24	3.1±0.45**	5.4±0.74
Total lipid mg/dl	98±0.78	191±1.4**	94±0.82

Cholesterol mg/dl	184±0.94	254±2.3**	182±0.73
Alkaline phosphatase U/L	18.8±0.27	36.4±0.91**	18.2±0.32
Hemoglobin %	8.7±0.44	4.6±0.72**	8.6±0.37
P.C.V. %	37±0.21	23±0.15**	40.3±0.24

P<0.01

**Table(4). Effect of Aflatoxin on body weight of catfish during the course of experiment**

Group	1month	2months	3months
Control 20 fish	68±0.21p	98±0.16*	121±0.72
Aflatoxin group (20fish)	92±0.10	81±0.2*	74±0.13
Aflatoxint + gensing + Nigella sativa (20fish)	86±0.06	104±0.73*	134±0.64

\*P<0.01

**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, 1985; Ali et al., 1994). The pathological changes of liver observed in the present investigations may be due to primary site of metabolism o ingested Aflatoxins as well as the primary laceratian laceratian 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, here is a highly elevation of total lipid and cholesterol in serum which agree with Sipple, et al. (1983),Sisk et al.(1988). It is obvious that administration of ginseng and Nigella sativa oil injection 1% of body weight reduced the Aflatoxin in liver, kidney, of infected fish and may protect liver from free radical reactions due to Aflatoxin, also total lipid, cholesterol return to normal level Mona,et al.(2002).

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 carp 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 Aflatoxicosis. (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 phosphates. The great increase of alkaline phosphates activity due to damage of liver. The detection of Aflatoxin in the liver tissues explain the liver degeneration. Similar results were described by Kubena et al., (1990), who used ginseng for preventing the absorption of Aflatoxins from gastrointestinal tract.

In conclusion, the metabolism of Aflatoxin result in the alteration of various metabolic process within hepatocytes which leads to severe serum biochemical alterations and serious pathological changes which affect fish production but treatment

with ginseng and *Nigella sativa* give an excellent of results.

## References

1. AH, 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.
2. Akgpı!, A, (1989) Antimicrobial activity of black seed (*Nigella saliva* of Aflatoxin) essential oil. *Cast Univ. Eczacılık Fax. Derg.*, 6, 63
3. Butler, W.H. and Clifford, J, L (1985) Extraction of Aflatoxin from rat liver. *Nature*, 206 (5), 1045.
4. Canton, J.H., Kroes, R., Van Logkn, MJL and Van Schathorst, M.s (1998) The careiriogenicity of aflatOxih D1 in rainbow trout, *Fd. Consmet. Toxicol* 19, 564,
5. Chavez, S., P. Martinez, M. Osorio, C. A. M. Palacios, and I. O. Mareno. 1994. Pathological effects of feeding young *Oreochromis niloticus* diets supplemented with different levels of aflatoxin. *Aquaculture* 127 (1): 49-61
6. Drabkin, DJ. (.1946) *Clinc. Chem.* 164, 703,
7. Edds, G.T, (1973) Acute Aflatoxicosis: A Review. *V.M.AJ.A.* 162,4, 304.
8. Fiegg, (1973) *Cl«. Chem.*, 29, 1075.
9. Gad, S.C. and Weil, C.S. (1986) Statistics for lexicologists, In; Hayes, A. W, (2nd ed.). *Principles and Methods of Tautology* : Raveo Press, New York, pp. 273 - 320.
10. Hasegawa, H., Sung, J.H., Matsumiya, S. and Uchiyama, M. (1996). Main ginseng saponin metabolites formed by intestinal bacteria. *Planta Med.* 62, 453-457.
11. Jantrarotai, W. and R. T. Lovell. 1990. Subchronic Toxicity of Dietary Aflatoxin B1 to Channel Catfish. *J. of Aquatic Animal Health*, 2: 248 – 254.
12. Jassar, B.S. and Balwant, S. (1993) Biochemical changes in experimental Aflatoxicosis in Broiler chicken. *Indian J. Animal Science* 63 (8), 784.
13. King, J, (1965) *Practical Clinical Eazymology.* Van Nostrand Co, Ltd. Rpg ) 132.
14. Kubena, F,L,» Harvey, B.R., Huff, £., W, and Corrier, E.D. (1990) Ffficacy of a hydrated sodium calcium aluminosilicate 10 reduce the toxicity of aflatOxiii and T. 2 Toxin, *Poultry Science* 69, 1078.
15. Lee, S.J., Sung, J.H., Lee, S.J., Moon, C.K. and Lee B.H. (1999). Antitumor activity of a novel ginseng saponin metabolite in human pulmonary adenocarcinoma cells resistant to cisplatin. *Cancer Lett.* 144, 339-343.
16. Lovell, R.T. 1992. Mycotoxins: hazardous to farmed fish. *Feed International*, 13(3): 24–28.
17. Marisfeld, R,? Graoert, E. and Kautna, J. (1989) Mycotoxieosis-a problem of dairy Cowherds. *Monatsheft fur veterinar medizin* 44 (12), 409.
18. Mona, S. Zaki., M.H. Osfor., A. Tohamy and Iman M. Zytuun (2002). Some Clinicopathological and Nutritional Studies on Reduction of Aflatoxin Induced Hepatotoxicity in Clarius lazara Catfish By Fax-Atoxin and *Nigella sativa* Oil. *Egypt. J. Microbiol.* 37(2), 185-195.
19. Newperne, P.M. (1999) Chronic aflatoxicosis In animals and Poultry, *J. Am. Vet. Med. Assoc.*, 263,1269.
20. Pier, A.C. (1987) Aflatoxicosis and immuno suppression in mammalian animals. In M.S. Zuber, GB. Liilehoj and B,L. Rssifor (Ed.). *Aflatoxin in Mam*, pp. 65. Cimmyt, Mexico.
21. Pier, A.C, (1999) Major biological consequences of Aflatoxicosis in animal production, *J, Artwi. Set*, 70, 3964.
22. Rasmassen, B.H., Larscn, K., Hald, B., Mailer, O.B. and Ellllng, F. (1986) Outbreak of liver cell carcinoma among salt water reared rainbow trout saimo gairdoert in Denmark. *Diseases of Aquatic Organisms* 1,191.
23. Reitman, S, and Frankel, S, (1946) *Am. J. Clin. PuihoL* 28, 26.
24. Robert, RA. (1989) *Fish Pailiolo.gy.* Second Edition, Baillier Tindall, London. Philadelphia., Sydney» Tokyo, Toronto.
25. Siesta, D. (1981) *Am. Clin. Biochem.*, 6, 24.
26. Slppeı, W.I., Burnside, J.C. and Atwood, MB, (1983) A disease of swine and cattle caused by eating mouldy corn. *Proc. 6' Ann. Meet. Am. Vei Med. Assoc.* pp. 174-181.
27. Sisk, D.B., Ciarlton, V.U. and Curtin, T,M, (1988) Experimental Aflatoxicosis in young swine. *Am J. Vet. Res.*, 39,1591.
28. Tacon, A.G. J. 1992. Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmed fish. *FAO Fish Technical Paper No. 330.* Rome. 75 p.

29. Wu, F.C. 1998. Retention of diet-related mycotoxins in tissues of channel catfish. (<http://www.egsz.or/BiologicalCurrentContent/Zoology?Comparative%2Physiology/TOXICOLOGY.html>).
30. Tun, T.K. (2003). Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. Mutat. Res. 523-524, 63-74.

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