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Research Article

Toxicology For Safe Therapy

Reduction of Effects of Dietary Aflatoxin B_1 on Biochemical Parameters of the Fish *Clarias Batrachus* by Vitamin C

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Abstract: Aflatoxins are produced mainly by molds *Aspergillus flavus* and *Aspergillus parasiticus*. They cause toxicity called aflatoxicosis in animals and human beings which is of great concern in aquaculture also. Aflatoxin B_1 is the most potent of all the known aflatoxins which mediates its effects by producing reactive oxygen species (ROS) and by irreversible damage to DNA and proteins leading to genotoxicity and cytotoxicity. Vitamin C is a potent antioxidant and reduces reactivity of ROS by donating electrons to them. Previous investigations suggest its role in alleviating aflatoxicosis in Nile tilapia. Present studies were conducted to determine the reduction in the negative effects of aflatoxin B_1 on blood biochemical parameters and liver glycogen of fish *Clarias batrachus* by dietary supplementation of vitamin C. In the present investigation 60 fish were divided into four groups. Each group comprised fifteen fish. Four types of feed were prepared on the basis of presence of aflatoxin B_1 and vitamin C. Feed I consists of a basal diet given to the first group of fish or control. Feed II consisted of aflatoxin B_1 contaminated feed given to second group of fish. In feed III and IV 300 mg/kg and 600 mg/kg vitamin C was added to aflatoxin B_1 contaminated feed The objective of the present investigation was to explore the role of Vitamin C in reduction of adverse effects of aflatoxin on plasma biochemical parameters and liver glycogen of the fish *Clarias batrachus.* The group of fish that was given the feed II showed a significant increase in serum level of ALT, AST, bilirubin, blood urea, glucose and albumin globulin ratio but significant decrease was observed in total serum proteins and liver glycogen as a result of dietary aflatoxin .Supplementation of Vitamin C in the feed significantly decreased the adverse effects of aflatoxin and the parameters showed significant improvement. The group of fish fed with 600 mg/kg vitamin C showed best results and were nearly similar to those of the control. The present investigation revealed that feed containing 600 mg/kg vitamin C diminishes the effect of aflatoxin B_1 in the fish reducing the health risk in humans consuming the fish as aflatoxins has the potential to accumulate and stay in the tissues of animals for long period of time.

Keywords: Aflatoxin, Vitamin C, Total serum protein, Albumin, Globulin, Blood glucose.

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1. INTRODUCTION

Fisheries and aquaculture is one of the fastest growing industries in the world [1] and provides livelihood for more than 10 % of world population [2] Fish flesh is considered as a rich source of protein, essential amino acids, omega 3 fatty acids and vitamins[3].The national per capita consumption of fish is about 11 kg which makes fish an important component of domestic food security in India [4] .To meet the requirement aquaculture production in India has been increasing over the years contributing significantly in national economy and employment opportunity particularly in the economically less developed rural area [5].However fish farming is also associated with the risk of infectious diseases, contamination and decrease of food quality that can adversely affects the fish health[6].One of the risks associated with aquaculture is contamination of aflatoxin in the feed.[7] Aflatoxins are mainly produced by two molds *Aspergillus flavus* and *Aspergillus paratisiticus* [8] which can grow on a variety of improperly stored food[9-12] and produce mainly four types of aflatoxins, B_1,B_2,G_1 and G_2 [13,14] . Among them, aflatoxin B_1 is the most fatal and found in maximum quantity in culture [15] . It shows resistance to both heating and freezing which enables it to remain in the food chain for an indefinite period of time [16] **.** The toxic effects of aflatoxin depends upon the species, dose of the aflatoxin as well as the time of exposure [17-19]. The principal target organ is liver and long time exposure to aflatoxin retards growth and increase mortality due to immunosuppression, kidney and liver dysfunctions [20-23,] .Long time exposure to aflatoxin B1 causes hepatocellular sarcoma and hepatocellular carcinoma in rainbow trout (*Oncorhynchus mykiss*) [24] . Dietary administration of aflatoxin B1 decreased growth performance and haematological parameters but increased serum ALT,AST and creatinine in *Oreochromis niloticus* [25] . In catfish *Rhamdia quelen,* low level of aflatoxin B_1 decreased Total Leucocyte Count but TLC increased when the fish is exposed to a higher level of the toxin [26] .Aflatoxin B1 residues were detected in the tissues after exposure to the toxin in seabass *Dissentracus labrax* [27] and *Oreochromis niloticus* [28] . This may leads to health risk in human also due to consumption of contaminated fish.[29,30] .Maximum accumulation of aflatoxin B_1 was reported in tissues of African catfish (*Clarias gariepinus*) when compared with Carp (*Cyprinus carpio*), Tilapia(*Oreochromis niloticus*) and Canga (*Heterotis niloticus*)[31] . Previous investigations suggested that aflatoxins are mutagenic, hepatotoxic and hepato carcinogenic in both animals and humans even small amount of the toxin cause oxidative stress leads to dysfunction of cellular components such as enzymes, nucleic acid, proteins and membranes [32,33] . Vitamin C is considered as an important component of the diet for growth and other biological functions. It improves the immunity and increases protein synthesis[34,.]Dietary administration of vitamin C improved the growth performance in Asian catfish (*Clarias batrachus*)[35]. Positive effects of vitamin C on growth performance, plasma proteins and haematological parameters have been reported in *Catla catla* [36] , Japanese sea bass *Lateolabrax japonicus* [37] and starry flounder *Platichthyes stellatus* [38] .Vitamin C Improved immunity through enhanced phagocytosis, lysozyme activities, increased antibody level and respiratory burst activity of phagocytes in Indian major carp *Labeo rohita* exposed to aflatoxin B_1 [39,40].Dietary supplementation of vitamin C improved the growth performance, improved blood level of liver function enzymes and total serum protein in Nile tilapia (*Oreochromis niloticus*) exposed to aflatoxin B_1 [41] . Vitamins C also inhibits *Aspergillus* growth and aflatoxin gene expression[42].Asian catfish of genus *Clarias batrachus* popularly known as Mangur is found throughout Asia and Africa. It is considered as an important fish due to its taste and excellent nutritional profile[43]. In many part of India, it is frequently prescribed to lactating pregnant , anaemic and malnutritional individuals[44]. However the fish showed a drastic decline from their natural habitat in India during the last few years. The objective of the present investigation was to explore the role of Vitamin C in reduction of adverse effects of aflatoxin on plasma biochemical parameters and liver glycogen of the fish *Clarias batrachus.*

2. MATERIALS AND MENTHODS

2.1 Experimental Design

A total of 60 apparently healthy *Clarias batrachus* were obtained from a private fish farm at Dholpur. The length of the fish was about 10 to 20 cm and the weight was about 40 to 50 grams. The fishes were kept in twelve aquariums measuring $2^1X\ 1^1\ X1^1$. Five fishes were kept in each aquarium. Three aquaria were kept as control and nine aquaria were divided into three sets. Each set consisted of three aquaria and kept as experimental sets.

2.2 Preparation of feed

Four types of feeds were prepared for the fishes on the basis of presence of aflatoxin or Vitamin C in the feed and they were distinguished as Feed I, Feed II ,Feed III and Feed IV. Feed I or good feed contained 100 percent good feed and no mouldy feed or Vitamin C. Feed I was given to control or fishes of the first set of aquaria comprising IA IB and IC. Feed II consisted of 100 percent mouldy feed without vitamin C. Feed II was given to fishes of the second set of Aquaria comprising 2A 2B and 2C.Feed III. consisted of 100 percent mouldy feed mixed with vitamin C. The concentration was 300mg vitamin C per Kg of feed. Feed III were given to fishes of third set of aquaria comprising 3A 3B and 3C. Feed IV consisted of 100 percent mouldy feed mixed with vitamin C. The concentration was 600mg vitamin C per Kg of feed. Feed IV was given to fishes of fourth sets of aquaria comprising 4A 4B and 4C.Mouldy feed was prepared in the laboratory. The commercial fish feed procured from market was first sprinkled with a small amount of water to make the feed moist and then mixed with cultured *Aspergillus flavus* procured from ICAR New Delhi. The inoculation was made in a transfer chamber to avoid contamination. The mixed feed was then covered with a plastic sac. For the preparation of feed III and feed IV 300 mg and 600mg of Vitamin C per kg feed was added to feed III and feed IV respectively. The infected feed was kept in a condition which is favourable for growth of the mould. The feeding started from the second day two times a day at a feeding rate of 4% of the body weight. The quantitative analysis of serum protein was made by the method of Kingsley (1942) followed by Mehl (1945) and Weichselbaum (1946).[45-47] The quantitative estimation of blood urea was carried out by the phenol hypochlorite method using Berthelot reaction (Fawcett & Scott 1960; Chaney & Marbach 1962). [48] Estimation of SGOT was carried out by the method of Karmen (1955).[49] Estimation of SGPT was carried out by the method of Wroblewski and La Due (1956) [50]Blood glucose was analysed quantitatively by the O- Toluidine method of Cooper and Mc Danile (1970). [51] Estimation of serum bilirubin was carried out by the method of Evelyne and Malloy (1937)[52]The quantitative estimation of

glycogen from liver was done according to a modification of the method of Kemp and Andrien (1954)[53]

3. STATISTICAL ANALYSIS

Statistical analysis was carried out by the method of one way analysis of variance (ANOVA) of Guenther (1964)[54] . The data were presented as mean ± standard deviation (SD). Probability value (P) of less than 0.05 was considered statistically significant. Besides graphs were drawn on the basis of data obtained as mean ± SEM.

Table-1. Showing effects of dietary aflatoxin and vitamin C on blood parameters of the fish.

feed	I	II	III	IV
Total Serum protein (g/100ml)	4.83 ± 0.14	3.28 ± 0.04	3.71 ± 0.06	4.27 ± 0.04
Serum albumen (g/100ml)	3.44 ± 0.13	2.68 ± 0.05	2.84 ± 0.06	3.14± 0.33
Serum globulin (g/100ml)	1.38 ± 0.14	0.60 ± 0.03	0.87 ± 0.07	1.12 ± 0.37
Blood Urea (mg/100ml)	4.10 ± 0.06	5.15 ± 0.04	4.92 ± 0.05	4.35 ± 0.05
Blood Glucose (mg/100ml)	74.95 ± 1.40	106.9 ± 1.53	97.06 ± 0.79	87.56 ± 1.39
Liver Glycogen (mg/g)	31.61 ± 0.48	21.33 ± 0.60	25.35 ± 0.45	29.00 ± 0.50

Values are Mean ± SD (n=8). Mean Values are Significant at p < 0.05

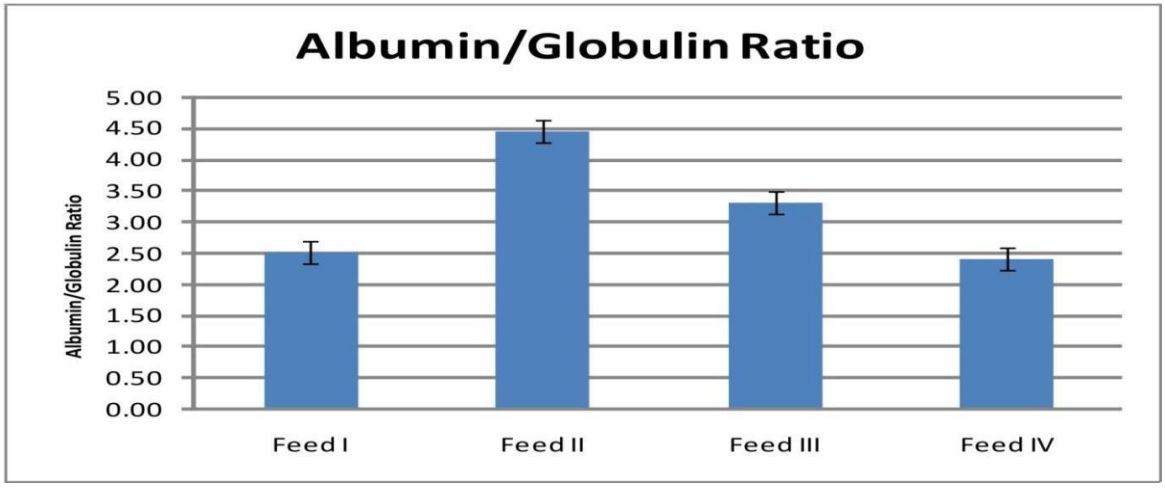

Fig-1 Effects of vitamin c and dietary aflatoxin on albumin /Globulin ratio in *C. batrachus*. Bars indicate mean ± SEM.

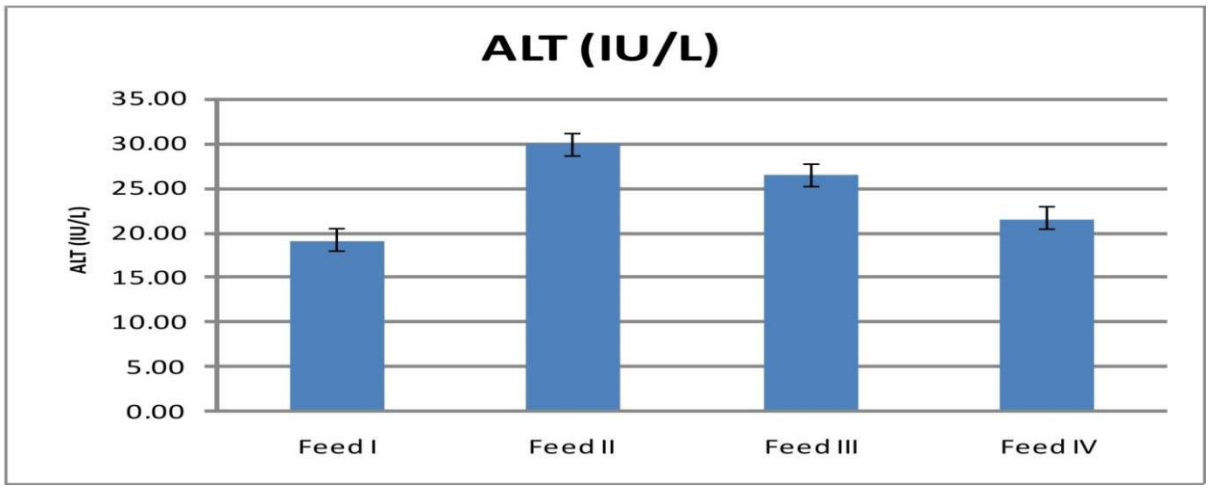

Fig-2 Showing effects of vitamin c and dietary aflatoxin on Serum ALT level *in C. batrachus* .Bars indicate mean ± SEM.

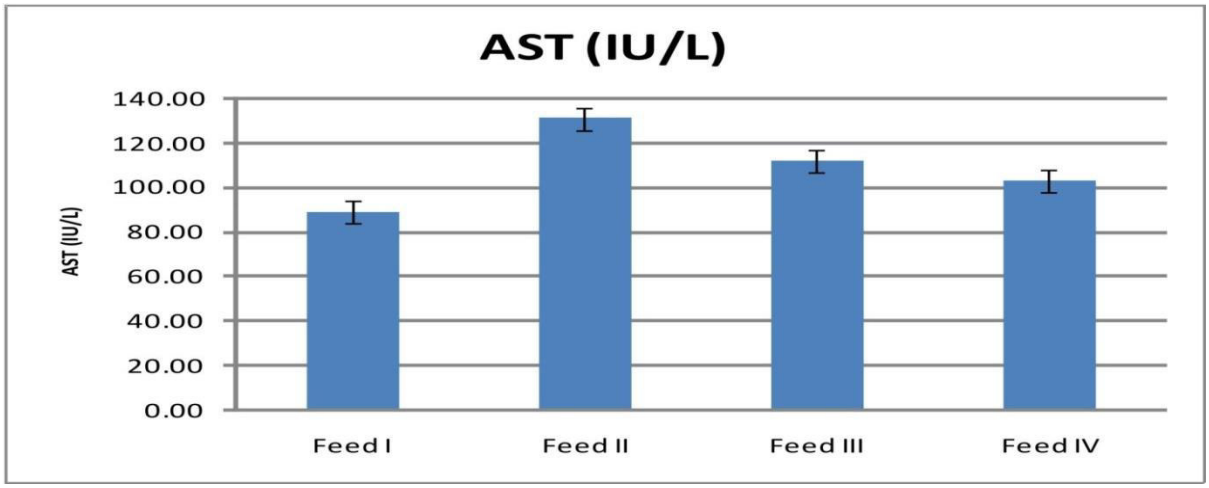

Fig -3 Showing effects of vitamin c and dietary aflatoxin on Serum AST level *C. batrachus*. Bars indicate mean ± SEM.

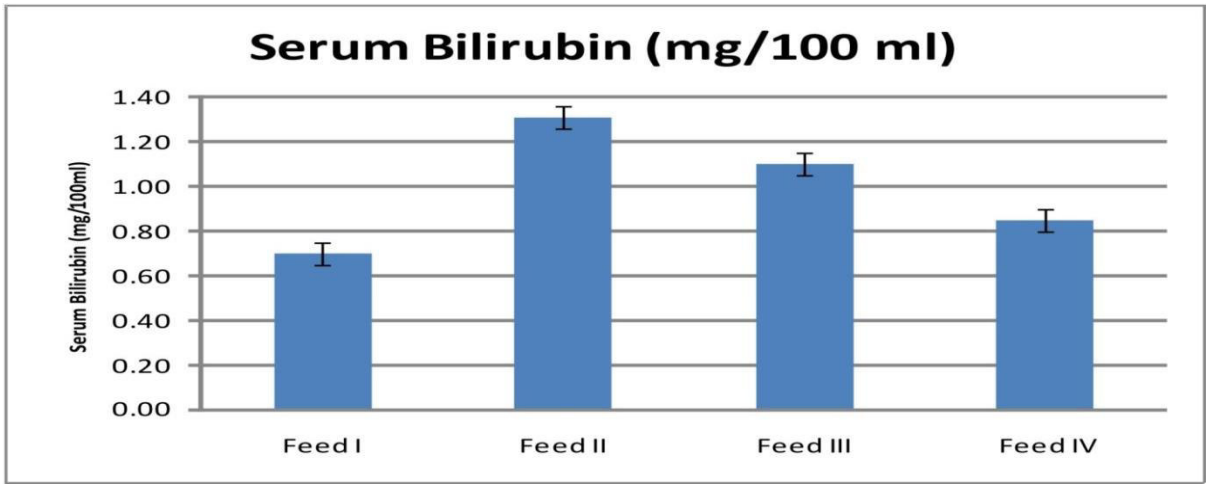

Fig- 4 Showing effects of vitamin c and dietary aflatoxin on serum Bilirubin level in *C. batrachus* .Bars indicate mean ± SEM.

4. RESULT AND DISCUSSION

4.1 Serum Protein and Urea

There was a significant ($p < 0.05$) decrease in the total serum protein albumin and globulin in fish fed with aflatoxin contaminated feed as compared to control(Table-1). The present investigation is in accordance with those of earlier investigations [26,41,55]. Aflatoxin decreases protein synthesis at both transcription and translation level [56,57, 58].There was a significant ($p < 0.05$) increase in blood urea level in fish fed with aflatoxin contaminated feed as compared to control. Fishes have very little amount of carbohydrate [59] and may utilize protein as an alternative source of energy to meet the increased energy demand under the condition of stress [60]. Thus in the present studies, decrease in total serum protein may be due to decrease in its synthesis as well as increased utilization for energy formation through gluconeogenic pathways in the condition of toxic stress as a result of aflatoxin. Presence of vitamin C in the feed significantly decreased the adverse effects of aflatoxin and thereby serum level of protein was increased. Present finding agrees with those of Nayek *et. al.*,[39]: Shehata *et. al.*,[41] There was a significant ($p < 0.05$) increase in Albumin globulin ratio in fish exposed to aflatoxin contaminated feed which suggests under production of immunoglobulin and suppression of immune system as a result of aflatoxin [61]. There was a significant ($p < 0.05$) increase in blood urea level in fish fed with aflatoxin contaminated feed as compared to control which indicates abnormal kidney function due to necrosis. Increase in urea level in blood indicates abnormal kidney function due to necrosis[62-64], The supplementation of Vitamin C significantly ($p < 0.05$) reduced the level of urea in fish. The present findings are in agreement with those of Zaki *et.al.*,[65] Soheir [66].

4.2 Serum ALT,AST

There was a significant ($p < 0.05$) increase in serum alanine transaminase (ALT) and aspartate transaminase (AST) in fish fed with aflatoxin contaminated feed as compared to control (Fig-1-4). The present findings are in agreement with those of Kheir-Eldin *et. al.*,[67], Mahfouz *et.al.*,[23] Selim *et.al.*,[25]. Previous findings suggested hepatocyte necrosis ,[68,69] and hepatocellular lipid deposition [70] in fishes exposed to aflatoxin. Any increase in these enzymes in serum is an indicator of cellular damage and consequently its increased permeability due to increased oxidative stress[26,71-73]. Aflatoxin B_1 significantly alters the stability of lysosomal membrane and thereby increases the permeability of hepatocytes resulting in high levels of ALT and AST in the serum [74]. Thus in the present findings, the increase in serum

ALT and AST in experimental fishes are probably due to hepatocyte degeneration, altered hepatic permeability and decreased lysosomal stability as a result of exposure to aflatoxin.

4.3 Serum bilirubin

Serum bilirubin also showed a significant ($p < 0.05$) rise in fish fed with B_1 containing feed as compared to control(Table-1). The present findings are in accordance with those of Rizvi *et.al*., [75] in broiler chicken. The rise in the level of serum bilirubin is an indicator of abnormal liver function. Sepahdari *et.al*.[21], reported hepatocyte degeneration in Beluga (*Huso huso*) when exposed to aflatoxin**.** Caguan *et.al.,*[20] reported yellowing of the body of aflatoxin treated Nile tilapia indicating an increased serum bilirubin level. Administration of aflatoxin increases the size of RBC and thus enhances its breakdown during passage through reticuloendothelial cells [76]. Thus the increase in serum bilirubin may also be due to enhanced breakdown of RBC as a result of aflatoxin.

4.4 Blood Glucose and Liver Glycogen

There was a significant ($p < 0.05$) rise in the level of blood glucose but decrease in liver glycogen in fish fed with aflatoxin contaminated feed(Table-1).The result agrees with the findings of EL-Boshy *et.al.,* [77]**.** Hyperglycemia in fishes are a part of stress response [78,79]. Glucose is the immediate source of energy and increases under the condition of stress to meet the increased energy demand as a result of hypoxia induced due to stress [80,81,82]. Zaki *et.al*.[22] reported increased cortisol and decreased insulin levels in *Clarias lazera* when exposed to aflatoxin. Fatmi *et.al.* reported depletion of liver glycogen in *Labeo calbasu* when treated with aflatoxin contaminated feed [83]. Thus in the present investigation, increase in blood glucose level and decrease in liver glycogen may be attributed to increased glycogenolysis, gluconeogenesis and decreased gluconeogenesis as a result of alteration in the levels of insulin and cortisol as well as the increased demand of energy formation under the condition of stress induced by aflatoxin. Addition of Vitamin C significantly ($p < 0.05$) improved the blood parameters and liver glycogen content of the fish in the present investigation. In fish that were given the feed III and IV, the level of parameters investigated gradually shifted near to those of the control group. These results agree with those of Shehata *et. al.,*[41]. Sahoo and Mukherjee [40] reported that vitamin C improved the immunity in *Labeo rohita* exposed to aflatoxin . Nayek *et, al*.[39], reported increased serum protein in vitamin c treated *Labeo rohita* .Young *et.al*.,[38] reported decrease in serum ALT and AST in fish when supplemented with vitamin C during the condition of oxidative stress[21]. The adverse effect of aflatoxin on the cell is mediated through production of free radicals and reactive oxygen species [84,85] and induces oxidative stress in animals and humans even when present in small amounts.[32,33] Vitamin C is a strong reducing agent and by donating electrons to free radicals impedes their reactivity which subsequently reduces the damaging effects of free radicals on cells.[59,86-88] . Vitamin C decreases hepatotoxicity by preventing glutathione depletion during oxidative stress which wok as intracellular free radical scavenger decreasing lipid peroxidation. [89] Moreover vitamin C indirectly reduces oxidative stress by its ability to regenerate antioxidant such as vitamin E and I glutathione in its reduced form.[90] Increased oxidative stress increases serum ALT and AST levels, but the effects were reduced when vitamin C was supplemented [38,53,91]. Aflatoxin B_1 in liver is metabolised by Cytochrome P450 enzymes to a highly reactive aflatoxin-8,9 epoxide (AFBO) which then binds with DNA to form aflatoxin- DNA adduct causing irreversible damage to DNA [92].Soheir *et.al* [66] reported decrease in aflatoxin_DNA- adduct formation in aflatoxin B_1 exposed rat supplemented with dietary vitamin C. Maryam *et. al.*[42] reported that vitamin c inhibits *Aspergillus parasiticus* growth in the culture and suppresses the production of aflatoxin by inhibiting aflatoxin gene expression. Thus in the present investigation decrease in the adverse effect of aflatoxin on the biochemical parameters of the fish by vitamin C is probably due to suppression of growth of the mold and decrease in expression of gene responsible for aflatoxin production, suppression of aflatoxin–DNA adduct formation, decreased lipid peroxidation through prevention of glutathione depletion and regeneration of other antioxidants in their reduced form during stress and also due to decrease in reactivity of free radicals produced as a result of aflatoxin.

5. CONCLUSION

Dietary aflatoxin B_1 showed significant adverse effects on the parameters investigated in the present studies. Dietary administration of Vitamin C along with the aflatoxin significantly reduces the adverse effect of aflatoxin B_1 . The magnitude of reduction of adverse effects was dependent upon the dose of the vitamin C. 600 mg of vitamin C per kg of feed revealed best results which were quite close to those of control and thus diminishes the effects of aflatoxin B_1. According to the present result we may conclude that Vitamin C may be used in fish feed during aquaculture to get a better yield.. The present finding may also help in reducing the health risk in humans as well who consume the fish as food, because aflatoxin B_1 has the potential to accumulate and stay in the tissues of animals for long period of time.

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7. AUTHORS' CONTRIBUTION STATEMENT

Both Authors contributed equally in collection of references, interpretation of data and conceptualizing of the whole investigation processes.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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