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RESEARCH ARTICLE

Efficacy of *Chlorella pyrenoidosa* to Ameliorate the Hepatotoxic Effects of Aflatoxin B_1 in Broiler Chickens

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ABSTRACT

Chlorella pyrenoidosa has been used safely in humans and in other animals as an immune stimulant. The aim of this study was to evaluate the dietary supplementation with *Chlorella pyrenoidosa* to ameliorate the effects of subacute intoxication with aflatoxin B_1 (AFB_1) in broiler chickens. Hepato-protective assessment of 7-d-old chicks was performed on a total of 120 chickens (Hubbard) divided into six groups having two replicates with 10 birds each: Control, AFB_1 (350 ppb), *Chlorella pyrenoidosa* ethanolic extract (CP) at 250 mg; 500 mg and co-treatment of CP (250 mg; 500 mg) and AFB_1. The experiment was conducted for 35 days. The contents of AFB_1 and AFB_1+CP diets were 350 ppb throughout the experimental period. The results indicated that diet contaminated with low level of AFB_1 significantly ($P<0.05$) reduced the average weight gain and daily food intake during the entire experiment and decreased ($P<0.05$) the serum contents of total protein, albumin and globulins. Moreover, a dietary low level of AFB_1 not only increased ($P<0.05$) lipid peroxidation but also reduced ($P<0.05$) total antioxidant capability, catalase, glutathione reductase and peroxidase activity in liver. Furthermore, addition of CP (500 mg) to AFB_1 contaminated diet counteracted these deleterious effects, indicating that supplementation of *Chlorella pyrenoidosa* to the broiler diet was safe, quiet cheaper provides 16% cost benefit ratio as compared to standard feed supplement, promoting beneficial effects (500 mg) in poultry health with respect to the toxic effects of 350 ppb diet.

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INTRODUCTION

Food security is still a significant Millennium Development Goal in most of the developing countries. Poultry production is one of the means being used to accomplish food security. Aflatoxins are the potent mycotoxins produced as secondary metabolites of *Aspergillus* fungi including *Aspergillus flavus, Aspergillus paraciticus and Aspergillus nomius* (Varga *et al.*, 2015; Khan *et al*., 2017; Saleemi *et al*., 2017). These fungi abundantly occur in tropical and sub-tropical region countries, where temperature and humidity are optimal for the fungal growth and production of mycotoxins (Jalili *et al.*, 2015). There are four aflatoxins (aflatoxin B_1, B_2, G_1 and G_2) that are known to be carcinogenic to both animals and human, of which aflatoxin B_1 is the most potent carcinogenic and hepatotoxic agent. They are chemically stable compound and it is very difficult to incapacitate them through chemical and physical methods (Cardoso *et al*., 2016).

High level of aflatoxins provokes acute aflatoxicosis with impaired immunity, necrosis of hepatocytes, visceral hemorrhages, biliary epithelial hyperplasia, vertigo, subcutaneous edema, coma and death (Resanovic *et al*., 2009; Hameed *et al*., 2017). Consumption of contaminated feed with low level of aflatoxin induces chronic aflatoxicosis in poultry, generally characterized with reduced weight gain, feed intake, feed conversion ratio, decreased egg, meat production and altered visceral organ weights (Kumar *et al*., 2015; Bhatti *et al*., 2017).

Prevention, detoxification and decontamination of feed ingredients contaminated with AFs are of particular significance. The ever-growing importance of phyto-planktons with medicinal properties and their respective

constituents in the animal feed has opened a new perspective of research on different phytogenic compounds added either as dry or in extract form, or as an essential oil to the diets of animals. These additives may act as immune stimulant or performance enhancer, improving the quality of the feed for respective animal species.

Chlorella is a unicellular fresh water green algae, rich source of micro and macronutrients, being used as an immune stimulant for centuries. It is a good source of protein and essential amino acids, chlorophylls, ω-3 and ω-6 polyunsaturated fatty acids, relatively low in cellulose and revealing a wide spectrum of physiological properties (Nakano *et al*., 2007). Its dietary supplementation moderates the lipid absorption, prevent hyperlipidemia and atherosclerosis (Cherng and Shih, 2005). Several studies and animal trial revealed the fact that it possesses the antioxidant activity (Arthur and Ramazanov, 2006). Chlorella has been reported as antiviral and antitumor in animal trials. Additive Chlorella also has been stated to improve the liver function in rat and mice model (Halperin *et al*., 2003). It also enhances the effect of antibody titer after influenza immunization in humans (Azocar and Diaz, 2013).

The aim of the present study was to evaluate the effects of aflatoxin B_1 and *Chlorella pyrenoidosa*, applied either independently or together on growth performance (live body weight, feed intake, FCR), serological biomarkers, antioxidant status and histopathological studies of the hepatic tissues in broiler chickens.

MATERIALS AND METHODS

Production of AFB_1: Pure culture of *A. flavus* (CECT 2687) was used for the production of AFB_1 through fermentation of 100 g long basmati rice in 1000 mL flat wide bottom (Erleynmeyer) flasks, following the method of Shotwell *et al.* (1966). AFB_1 was extracted and quantified through HPLC according to Association of Official Analytical Chemists (AOAC, 2000).

***Chlorella pyrenoidosa* collection and extract preparation:** Organic CP powder was purchased from Shaanxi Fuheng (FH) Biotechnology Cooperation Limited, China with batch No. FH131210. The powder (2 Kg) was extracted with 4.0 litres of 80% ethanol with occasional shaking and filtered. The filtrate was evaporated through rotary vacuum evaporator at 40°C to obtain *Chlorella pyrenoidosa* ethanolic extract (CPEE).

Birds housing and feed: A total of 120, a-day old broiler chicks procured from local hatchery were kept on rice litter husk material under standard management conditions. The basal broiler feed (comprising of soymeal and corn having 22% total protein and 3100 Kcal/Kg metabolize energy) was prepared without addition of any toxin binder and antibiotics. After 6 days of acclimatized period the birds were divided into 6 groups having two replicates with 10 birds each.

Experimental Design: Feeding of test diets commenced at 7th day of bird age and continued till the termination of experiment at six weeks of age. The chicks were assigned to the following treatment groups: 1); CON (control), 2); AF (aflatoxin B_1 at 350 ppb), 3); CP_{250mg} (*C. pyrenoidosa* ethanolic extract at 250 mg), 4); CP_{500mg} (*C. pyrenoidosa* ethanolic extract at 500 mg), 5); AF_{350ppb}+ CP_{250mg} (Aflatoxin B_1 and *C. pyrenoidosa* ethanolic extract at 350 ppb and 250 mg, respectively), 6); AF_{350ppb}+ CP_{500mg} (Aflatoxin B_1 and *C. pyrenoidosa* ethanolic extract at 350 ppb and 500 mg, respectively). Broiler chicks were provided feed and water *ad libitum* throughout the study.

Parameters studied

Body weight and feed conversion ratio: Body weight and feed conversion ratio (FCR) were determined as traditional measures at the end of each week throughout the experiment.

Serum biochemical parameters: The blood was collected from the wing vein of the birds prior to slaughter on the 35th day of the experiment, allowed to clot to separate the serum. The collected serum was used to quantify the serum glucose, AST, ALT, ALP, total protein and albumin. The assays were determined with clinical chemistry assay kits (AMP diagnostics) according to manufacturers recommended procedure. The serum globulin concentration was determined by subtracting the serum albumin concentration to that of total proteins.

Metabolic enzymes and antioxidant indices in liver: Liver tissue (1 g) was cut into small pieces and homogenized in cold saline buffer (0.85%, pH=7.4) (1:9 w/v) with an Ultra-Taurex to form homogenates at a concentration of 0.1 g/mL for further analysis. Liver homogenates were centrifuged at 1,000 g for 15 min at 4ºC and the supernatant was collected. The supernatant was used for the assay of reduced glutathione (GSH), glutathione- S- transferases (GST), glutathione reductase (GR), glutathione peroxidase (GSH-Px), catalase, lipid peroxidation (LP) and total antioxidant capacity (TAC). The assays for the above detections were carried out through spectrophotometer according to the detection kit instructions (SIGMA).

Histopathological studies: The degrees of hepatic damage were observed with hematoxylin and eosin staining as described by Bancroft and Gamble (2007).

Statistical analysis: The data obtained were subjected for analysis of variance (ANOVA) by using General Linear Model procedure and Duncan's new multiple range test was applied to compare means by using SPSS version 22. Significant differences in all analysis were based on $P<0.05$ (Steel and Torrie, 1993).

RESULTS

Growth performance and FCR: At the time of initiation, it was found that all chicks were almost in similar range and uniform. They were provided the same feed to acclimatize them according to open shed environment. After first week, the chicks were divided into groups of similar body weight. From 2 week of age and later, the weights of chickens that received AFB_1 in their diet were lower as compared to the control (Table 1). In groups CP at 250 mg and 500 per kg body weight, significant difference was observed as compared to control group. In AFB_1+CP, at both levels adverse effects

of aflatoxin which was seen in AFB_1 group significantly ($P<0.05$) restored in dose dependent manner. Though, CP (500 mg) has shown the highest BW (2202 g) which stands for the best body weight among all other treatments used in this experiment. Other parameters such as cumulative feed consumption and FCR significantly altered in chicks that received AFB_1 alone. AF elicited an increase in FCR as compared to control group, however, daily weight gain decreased, indicating the toxic effects of mycotoxin. These effects were partially ameliorated by CP in dose dependent manner as compared to control.

Table 1: Effect of *Chlorella pyrenoidosa* against AFB_1 on body weight, cumulative feed consumption and FCR at 6 week of age

Treatments	42 day Body weight (g)	Cumulative feed consumption (g/bird)	FCR
CON	2150.90±2.75^{b}	4345.25±0.41^{a}	2.02±0.14^{d}
AF_{350ppb}	1884.20±1.58^{e}	4127.77±0.61^{d}	2.18±0.21^{a}
CP_{250mg}	2133.05±6.96^{b}	4287.05±0.56^{c}	2.00±0.36^{e}
CP_{500mg}	2202.30±10.1^{a}	4301.10±0.41^{b}	1.95±0.48^{f}
CP_{250mg}+AF_{350ppb}	1916.60±5.34^{d}	4123.21±0.34^{e}	2.15±0.41^{b}
CP_{500mg}+AF_{350ppb}	1964.15±5.48^{c}	4103.21±0.50^{f}	2.10±0.76^{c}

Mean±SE within a row with different superscript letters were significantly ($P<0.05$) different CON (control), AF (aflatoxin B_1 at 350 ppb), CP_{250mg} (*Chlorella pyrenoidosa* at 250 mg), AF_{350ppb}+ CP_{250mg} (Aflatoxin B_1 and *Chlorella pyrenoidosa* at 350 ppb and 250 mg, respectively), CP_{500mg} (*Chlorella pyrenoidosa* at 500 mg), AF_{350ppb}+CP_{500mg} (Aflatoxin B_1 and *Chlorella pyrenoidosa* at 350 ppb and 500 mg, respectively).

Serum biochemical parameters: The effect of dietary treatments on serum biochemical parameters are shown in table 2. Aflatoxin B_1 adversely affect the serum glucose, total protein, albumin and globulin levels, are significantly ($P<0.05$) depleted among aflatoxin treated birds as compared to control group. Simultaneous administration of AFB_1 and CP significantly ($P<0.05$) improved the effect on glucose level in dose dependent manner as compared to AFB_1 treated bird group. At low dose (250 mg), it partially ameliorate ($P<0.05$) the toxic effect of AFB_1 on the serum glucose, total protein, albumin and globulin levels but as the dose increased from 250 mg to 500 mg the glucose, total protein, albumin and globulin levels altered significantly($P<0.05$). However, there is no significant ($P>0.05$) difference noticed in glucose level among CP (250; 500 mg) groups at both levels as compared to control group. However, significant ($P<0.05$) difference found in total protein, albumin and globulin among control and *C. pyrenoidosa* treated groups.

The ALT, AST and ALP activities were found to be significantly ($P<0.05$) disturbed in aflatoxin fed group, which specifying the interference in liver function. The combination between AFB_1 and CP resulted in significant ($P<0.05$) improvement in AST, AST and ALP enzymes compared to AFB_1 group. Results indicated that CP had a positive effect on ALT, AST and ALP in treated groups. Increasing the level of CP from 250 to 500 mg did not affect ($P>0.05$) ALT, AST and ALP enzymes activities as compared to control group. *Chlorella pyrenoidosa* at 500 mg was more effective in improving AST and ALT enzymes as compared to the CP at 250 mg among AFB_1 intoxicated groups.

Antioxidant index: Broilers fed the contaminated diet with AFB_1 had significantly ($P<0.05$) reduced the hepatic GSH, GST, GR, CAT, GSH-Px and TAC activities in the liver as compared with those in the control group (Table 3), wherase a significant higher ($P<0.05$) level of lipid peroxidation observed in AFB_1 treated group. Concurrently administration of AFB_1 and CP exhibited ($P<0.05$) restorative effect on GSH, GST, GR, CAT, GSH-Px and TAC activities in liver in dose dependent manner as compared to AFB_1 treated group. Similar trend found for lipid peroxidation among *C. pyrenoidosa* treated groups as compared to control.

The results of histological alterations of the hepatic tissues of the respective groups are illustrated in Figure 1. There was no lesion observed in hepatic parenchyma of the control and *C. pyrenoidosa* (250 mg; 500 mg) groups. However, liver from the birds fed with the diet containing 350 ppb of dietary AFB_1 showed lesions such as lymphocytes infiltration, interlobular bile duct hyperplasia and mild vacuolar degeneration (Fig. 1b). The effects of AFB_1 intoxication remain persistent in AFB_1 plus CP 250 mg group in terms of cellular infiltration and vacuolar degeneration (Fig. 1d). But, livers from broilers consuming the AFB_1 plus CP 500 mg diet showed slight vacuolar degeneration and slight lymphocytes degeneration (Fig. 1f). These results suggest a protective effect of *C. pyrenoidosa* on aflatoxicosis.

Table 2: Effect of *Chlorella pyrenoidosa* against AFB_1 on serum biochemical parameters in broiler chicken

Group	Glucose (g/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
CON	160.07±0.74^{a}	39.92±1.45^{d}	186.87±1.38^{e}	40.01±1.15^{d}	28.07±1.39^{b}	14.15±0.66^{b}	13.92±0.45^{c}
AF_{350ppb}	146.62±0.34^{d}	53.45±1.44^{a}	224.50±1.25^{a}	67.90±1.53^{a}	21.50±1.09^{d}	11.05±0.14^{d}	10.45±0.17^{e}
CP_{250mg}	159.25±0.59^{a}	38.79±1.73^{d}	191.50±2.04^{de}	38.14±1.41^{d}	29.01±1.05^{b}	14.61±0.31^{b}	14.40±0.26^{b}
CP_{500mg}	160.25±0.63^{a}	38.88±1.70^{d}	194.34±1.26^{d}	39.01±1.52^{d}	31.95±1.22^{a}	16.68±0.21^{a}	15.27±0.55^{a}
CP_{250mg}+AF_{350ppb}	150.57±0.85^{c}	49.02±1.26^{b}	218.25±1.22^{b}	60.51±1.33^{b}	25.85±1.42^{c}	12.45±0.32^{cd}	12.40±024^{d}
CP_{500mg}+AF_{350ppb}	156.25±0.43^{b}	42.40±1.58^{c}	201.05±1.01^{c}	52.13±1.21^{c}	26.95±1.21^{c}	13.08±0.49^{c}	13.87±0.19^{c}

Data are expressed as Mean±SE. AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase. Mean±SE within a row with different superscript letters differ significantly ($P<0.05$).

Table 3: Effect of *Chlorella pyrenoidosa* against AFB_1 on antioxidant defense system in liver among broiler chicken

Group	GSH (mg/g prot)	GST (Mmol CDNB/ min/mg prot)	GR (U/g prot)	GSH-Px (U/mg prot)	LPO (nmol MDA /mg prot)	Catalase (U/mg prot)	TAC (U/mg prot)
CON	3.10±0.06^{c}	6.56±0.26^{a}	4.46±0.11^{c}	43.15±0.86^{a}	2.02±0.01^{cd}	8.14±0.07^{c}	2.21±0.11^{d}
AF_{350ppb}	2.92±0.03^{f}	4.32±0.13^{d}	3.72±0.31^{d}	36.90±0.45^{c}	2.56±0.04^{a}	7.36±0.16^{d}	2.05±0.41^{e}
CP_{250mg}	3.19±0.01^{b}	6.57±0.21^{a}	4.98±0.24^{b}	43.56±0.36^{a}	1.96±0.02^{d}	9.01±0.41^{ab}	2.26±0.27^{c}
CP_{500mg}	3.26±0.04^{a}	6.59±0.36^{a}	5.67±0.17^{a}	43.97±0.21^{a}	1.89±0.03^{e}	9.89±0.23^{a}	2.34±0.29^{a}
CP_{250mg}+AF_{350ppb}	2.96±0.08^{e}	4.98±0.23^{c}	4.47±0.81^{c}	37.01±0.19^{b}	2.43±0.04^{b}	7.98±0.18^{b}	2.24±0.46^{cd}
CP_{500mg}+AF_{350ppb}	3.01±0.02^{d}	5.52±0.41^{b}	4.86±0.44^{bc}	37.51±0.71^{b}	2.13±0.07^{c}	8.02±0.13^{c}	2.30±0.21^{b}

Data are expressed as group mean values. GSH; reduced glutathione, GST; glutathione- S- transferase, GR; glutathione reductase, GSH-Px; glutathione peroxidase, LP; lipid peroxidation, TAC; total antioxidant capacity. Mean±SE within a row with different superscript letters differ significantly ($P<0.05$).

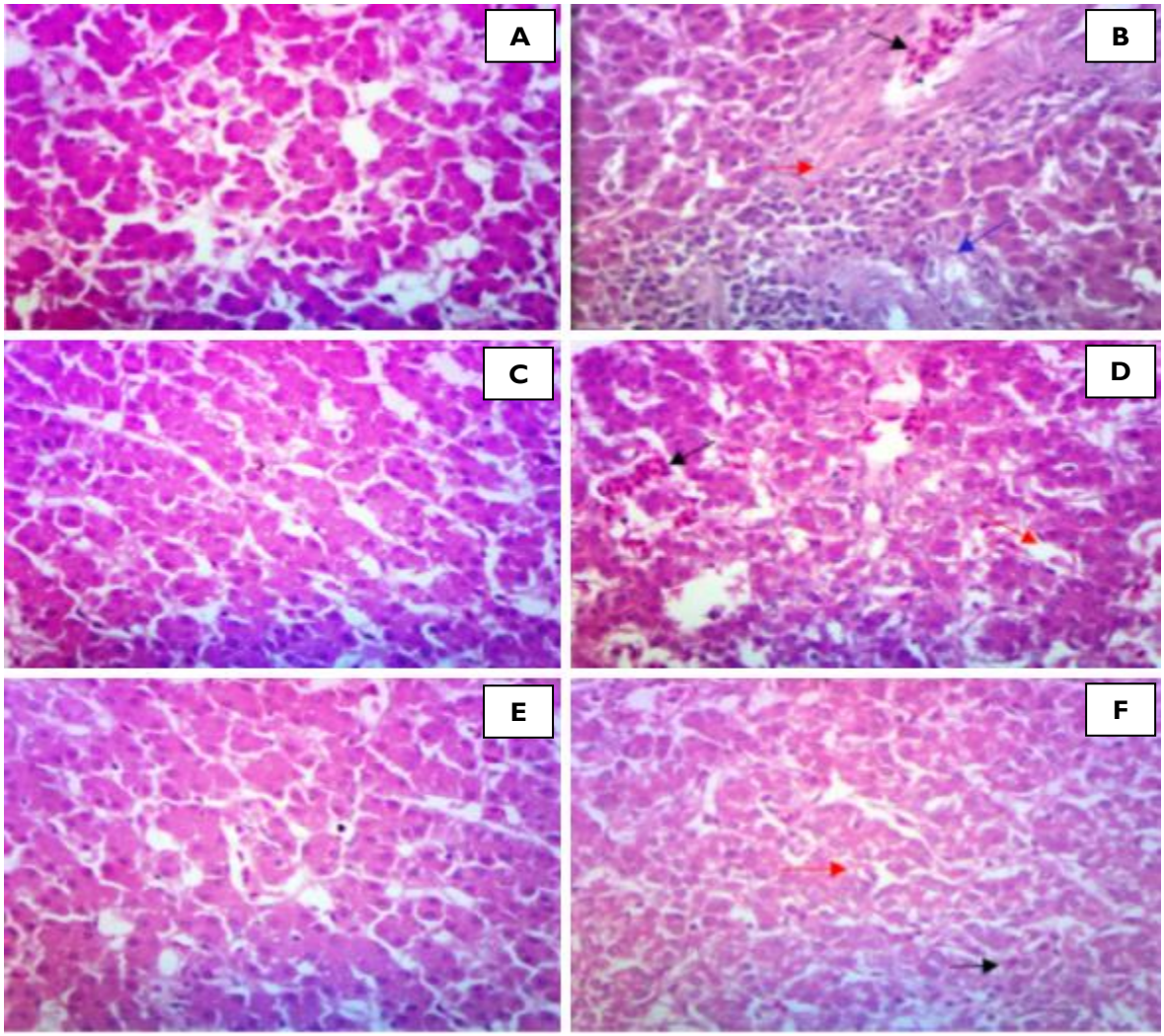

Fig. 1: Photomicrographs (optical microscopy) of broiler liver sections from different treatments. Normal liver architecture was observed in control (a) *Chlorella pyrenoidosa* at 250 mg (c) *Chlorella pyrenoidosa* at 500 mg (e) treated animals. Liver lesions such as interlobular bile duct proliferation (blue arrow), cellular infiltration (black arrow) and mild vacuolar degeneration (red arrow) were observed in AFB_1 treated chicks (b). Cellular infiltration (black arrow) and vacuolar degeneration (red arrow) were persistent in AFB_1 plus CP 250 mg (d) while, Slight lymphocytes infiltration (black arrow) and slight vacuolar degeneration (red arrow) were visible in AFB_1 plus CP 500 mg (f) (H & E Staining; 200x).

DISCUSSION

Aflatoxin B_1 is the prevailing metabolite produced by notorious fungi in most of the animal feed. Broilers fed on the diet composed of numerous ingredients, of which produced under diverse agrometeorological conditions. The toxicogenic threats of aflatoxins depend on their dose and duration of intake, in addition to, animal species, age and nutritional status. It is directly associated with their rapid absorption through gastrointestinal tract (GIT) and instant binding to serum proteins, such as albumin. In poultry, intoxication of AFB_1 at high level instigating heavy economic losses in terms of health and production, that not only induce clinical toxicosis but also interfere with vaccine induced immunity and reduce the resistance to diseases (Saleemi *et al*., 2010).

Now-a-days researchers are exploiting chemicals that are nontoxic, act as antioxidants and not detrimental for health. Unicellular, microalgae, Chlorella gained more attention these days, due to its nutraceutical profile. It is best known feed supplement currently available, having beneficial effects like growth, antioxidant activity, tissue re-building and immune modulation (Buono *et al*., 2014). Due to presence of high crude protein index (78.1%) since it contains all essential aminoacids (Waghmare *et al*., 2016), it could replace soybean meal and fish meal 5% to 10% without any side effects on growth performance and FCR in growing broilers. It is documented that dietary supplementation of chlorella improved growth performance, affected intestinal microbial diversity and modulated immune responses (Janczyk *et al.,* 2009). Later on, it was found that *C. vulgaris* induced growth promoting effects and positively affect the humoral immune response in broiler chicks (An *et al*., 2016). To the best of our knowledge, it is the first study on the effects of dietary supplementation of *Chlorella pyrenoidosa* against AFB_1 on growth performance, serum biochemical parameters, antioxidant capacity and lipid peroxidation in liver of broiler chickens.

In present study, diet contaminated with AFB_1 (350 ppb) induced a significant decrease in body weight of the broilers and adversely effects the overall performance of broiler chickens. Similar detrimental effects reported earlier (Liu *et al.,* 2016) have also been reproduced in the experimental feed of AFB_1 in the current study. No mortalities have been recorded in any group during this experimental study.

Liver is primary target site for the bio-activation of AFB_1 to aflatoxin 8, 9 epoxide that generously bind to proteins and DNA, and form adducts that ultimately damaging the liver structures and increasing the relative liver weight. Fatty liver accompanied with altered glucose levels is the major outcome of aflatoxicosis in poultry. It is foremost reason to take the concern about glucose level among aflatoxin treated birds. AFB_1 depleted the glucose concentration among aflatoxin treated group as compared to the control group. Similar results were reported in broiler (Sridhar *et al.*, 2015). Supplementation of *C. pyrenoidosa* partially reverted this effect in dose dependent manner. The obtained data in this study are in agreement with Senthilkumar *et al.* (2012) who treated diabetic nephropathy with *C. pyrenoidosa* and received positive impact on health of albino rats.

ALT is liver specific enzyme, whereas AST derived from most organs such as heart, skeletal muscles, kidney, brain, pancreas, lungs, leukocytes and erythrocytes other than liver. Damage to hepatic tissue may cause leakage of these enzymes into blood stream. During aflatoxicosis, activity of AST, ALT and ALP were increased in AFB_1 treated group as compared to control group. The change in the activity of these enzymes from the control may be due to the disruption of hepatic cells as a result of necrosis or a magnitude of transformed membrane permeability which in turn leads to leakage of enzymes into circulation. The results are in agreement with the findings of Jayasri and Srikanth (2016). *C. pyrenoidosa* found to be hepato-protective as it ameliorate the toxic effects of AFB_1 and results of current study are in agreement with Peng *et al.* (2009) who reported the hepatoprotective effect of ethanolic extract of *C. sorokiniana* against CCl_4 induced oxidative damage in rats.

Serum total protein works as an indicator of protein synthesis and decreased TP by dietary AFB_1 may also contribute to low level of immunoglobulins. AFB_1 documented as immunosuppressive in birds, and it has been observed that diets containing 300 µg/kg AFB_1 significantly reduced the IgG, IgM and IgA of broilers. The present study also revealed the significant reduction in TP, ALB and Ig. Concurrent administration of *C. pyrenoidosa* along AFB_1 has shown growth promoting effect in dose dependent manner. Similar results for the supplementation of *C. vulgaris* on TP, ALB and Ig has been reported by Khani *et al.* (2017) in Koi (*Cyprinus carpio*).

Body naturally comprising of the defense system, consisting of enzymatic CAT, GR, GSH-Px and non-enzymatic components like vitamin C, E and GSH (Delles *et al*., 2014). As an outcome of pathological response, the oxidative stress raised up ultimately this defense system provokes the regulation and promotion of enzymatic and non-enzymatic components. Previously, it has been reported that dietary AFB_1 could alter the responses of these antioxidants enzymes and non-enzymatic antioxidants in broiler (Fan *et al*., 2015). In this study, it has been found that diet contaminated with low level of AFB_1 (350 ppb) could suppress the antioxidant capacity of the broilers, *C. pyrenoidosa* could ameliorate these deleterious effects in dose dependent manner. Furthermore, *C. pyrenoidosa* as a feed additive reproducing valuable defense in broilers.

Toxic effects elicited by AFB_1 related to the generation of reactive oxygen species (ROS). Production and accumulation of ROS are central to oxidative stress mediated metabolism. The increase ROS could enhance lipid peroxidation which will impair membrane function by reducing membrane fluidity and changing the actions of the membrane bound receptors and enzymes (Arulselvan and Subramanian, 2007). In current study, the content of LPO was significantly increased in liver of broilers indicating the fact that low level of AFB_1 (350 ppb) could enhance the oxidative stress in liver. Similar toxic effects of AFB_1 on the oxidative stress were reported in liver of broilers (Liu *et al*., 2016). Lower oxidative status and higher antioxidant capacity were noticed in broilers fed on a diet supplemented with *C. pyrenoidosa.* In current study, indicating the fact that CP could protect broilers from oxidative damage induced by AFB_1. These results are similar to earlier report documented the chemo-protective effect of *C. vulgaris* against 7, 12 dimethyl benzene-anthracene induced oxidative stress (Amin, 2008). Naturally occurring bioactive compounds have the potential to subside the biochemical balance induced by aflatoxin associated ROS, provide protection without any side effects considered as effective therapeutic drug.

Reduced antioxidant capacity, elevated LPO and hepatic enzyme activities may result from different kind of degenerations in hepatic tissues among AFB_1 treated broiler chickens. However, hepatic lesions with varying amount of fatty and vacuolar degeneration was observed in liver cells during histological studies, confirming the observation of others (Yogeswari *et al*., 2012). Among CP treated groups, a dose dependent improvement in the hepatic architecture was experientially detected. *C. pyrenoidosa* (500 mg) administration to the broilers with hepatic damage induced by AFB_1 resulted only in minimal disruption of hepatic cellular structure.

In sum, prolong intoxication of AFB_1 to broilers caused hepatic lesions, characteristics of aflatoxicosis, including retarded growth performance and disruption in serum enzymatic activities. Daily supplementation with ethanolic extract of CP (500 $mg.Kg^{-1}$ BW) to broiler chickens ingesting AFB_1 (350 ppb) ameliorate the toxic effects of hepato-toxin. Thus, ethanolic extract of *Chlorella pyrenoidosa*, green algae, as food supplement found to be safe, protect and help to improve the health of broiler chickens. To the best of our knowledge, it is the first study reported that *C. pyrenoidosa* used to ameliorate the hepatotoxic effects of aflatoxin B_1 in broiler chicken.The fact that *C. pyrenoidosa* supported liver function in conjunction with AFB_1 toxicity challenge is encouraging and warrants further research.

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Authors contribution: ZS, MS and JAK equally participated in execution of the project, designing research methodology. ZS performed laboratory and statistical analysis and wrote manuscript, while FH contributed in laboratory analysis and manuscript preparation. MS and JAK critically reviewed the manuscript.

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