

THE BIOCHEMICAL PROTECTIVE ROLE OF SOME HERBS AGAINST AFLATOXICOSIS IN DUCKLINGS: I. TURMERIC

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Abstract

Objective - The aim of this study was planned to throw the light on the hepatotoxic effects of aflatoxin in duck and to evaluate the effects turmeric (*Curcuma longa* ground roots) for protection against aflatoxicosis and to explore if they can decrease the aflatoxin production by *Aspergillus flavus* toxigenic strain on poultry diet. **Design** - in vitro and in vivo experimentation, malondialdehyde (MDA), Glutathione (GSH) and aflatoxin residues. **Results** - Aflatoxin (AF) from *Aspergillus flavus* toxigenic strain was selected as a potent and widely distributed hepatotoxin that induces much health and economic hazards in animal and human. *Aspergillus flavus* was cultivated on rice to produce the aflatoxin that is used in the present study. Pekin ducklings were allotted to control, aflatoxin treated, turmeric and aflatoxin and turmeric groups. Total proteins, albumin, alanine aminotransferase (ALT) (EC 2.6.1.1) and aspartate aminotransferase (AST) (EC 2.6.1.2), cholesterol and triacylglycerols were measured in serum. The levels of hepatic Malondialdehyde and Glutathione levels and aflatoxin residues were also measured. **Conclusions** - From the obtained results, it can conclude that aflatoxin has hepatotoxic effects through decrease of total proteins, albumin and glutathione. Moreover, increase ALT, AST, cholesterol, triacylglycerols and lipid peroxidation levels. In addition, aflatoxin induced histopathological changes of liver and residues of aflatoxin were measured. Addition of turmeric to duckling's ration were induced a protective effect against aflatoxicosis. So, we advice to use turmeric as a feed additive in poultry farms.

Key words: *Aspergillus flavus*; Aflatoxin; Turmeric; Aflatoxin residues

INTRODUCTION

Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development. Mycotoxins of greatest public health hazards include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids [1]. AFs are the most naturally occurring mycotoxin that found on foods such as corn, peanuts, various other nuts and cotton seed. Since their discovery in the 1960s, they have been demonstrated to be carcinogenic in many animal species, including rodents, non- human primates and

fish. AF produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* are major contaminants of common feed ingredients used in poultry rations [2]. AFB₁ is the most biologically active form of aflatoxins and causes poor performance, liver lesions, and immunosuppression in poultry [3].

Plant compounds like coumarins, flavonoids, and curcuminoids have inhibitory action on biotransformation of AF to their active epoxide derivatives. Turmeric (*Curcuma longa*), a medicinal plant native to the Asian subcontinent, is known to possess antimicrobial and antioxidant properties. The powder of dried roots and rhizomes of

turmeric is used as one of the spices in many countries. The curcuminoids, yellowish pigments present in turmeric powder, have shown protective effects against AFB₁ [4]. Supplementation of curcumin in the diet normalized the altered activities of LDH and ALT induced by AF. At molecular level, curcumin significantly reduced AFB (1)-N (7)-guanine adduct excretion in the urine, DNA adduct in the liver and albumin adduct in the serum [5]. Supplementation of turmeric powder in diets in chicks fed AF contaminated diets improves the antioxidant, biotransformation, and immune system genes in livers of chicks fed AFB₁ [6].

MATERIAL AND METHOD

The present study was carried out on hundred Pekin ducklings of average body weight about (200±50 g). They were received ration and water *ad libitum*. All birds were housed in the same place and the basal diet for two weeks before the beginning of the experiment for acclimatization and to ensure a normal growth and behavior.

Curcuma longa roots were purchased, (Department of Plant, Faculty of Agriculture, Damanhour University), washed, dried, purified finally ground in mortar and perfectly purified then added to the duckling's diet. Part of identified turmeric were kept at -20C as a reference.

Basal diet contains (63.1% ground yellow corn, 28.2% soya been meal (44% CP), 4.3% corn gluten meal (60% CP), 0.6% vegetable oils, 1.8% dicalcium phosphate, 1.1% ground limestone, 0.4% common salts, 0.3% mineral and vitamin premix, 0.1% lysine and 0.1% methionine) was used for feeding the duckling.

Hundred ducklings were allotted into two equal groups each group was arranged as triplicate. G1 (Control group) contains 50 ducklings fed basal diet and corn oil per os. G2 (Aflatoxin treated group) contains 50 ducklings fed basal diet and corn oil contains aflatoxin (30 µg /kg duck per day) for 2 weeks per os (seven ducklings were die during this period). At 4th week 5 ducklings from each group were scarified and blood samples and liver samples were taken and kept after separation of serum and washing of livers at -20C till biochemical analysis were

done. The remaining birds of each group were allotted into two groups: the above G1 group contains 50 ducklings was allotted into G3 (Control-ve) fed basal diet of 20 ducklings and G4 (Turmeric group) contains 25 ducklings fed basal diet containing 1.0% turmeric powder. In addition, G2 (Aflatoxin treated group) was allotted into G5 (aflatoxicated birds) contains 18 ducklings fed basal diet and G6 (AF+Turmeric) contains 20 ducklings fed basal diet containing 1.0% turmeric powder.

Production of aflatoxin on corn

Spores of *Aspergillus flavus* isolate were kept in physiological salt solution containing 0.01% Tween 80 (PST); they were inoculated onto Czapek's Dox plates and incubated at 30C for 7 days. After this period, the spores were recovered by washing the Czapek's Dox surface with sterile PST. The harvested spores were enumerated and used as inoculums of (5x10³/ml) spore count [7]. Cultivation of *Aspergillus flavus* on corn; this process was done by using the method of [8] with some modifications as following: T₁ (Control): 50 g of crushed yellow corn and *Aspergillus flavus* inoculum; T₂: 50 g of crushed yellow corn, turmeric powder 0.5% and *Aspergillus flavus* inoculum; T₃: 50 g of crushed yellow corn, turmeric powder 1.0% and *Aspergillus flavus* inoculum and T₄: 50 g of crushed yellow corn, turmeric powder 2.0% and *Aspergillus flavus* inoculum.

Extraction and measurement of aflatoxin

AFB₁ and AFB₂ in corn were determined using the method of [9] with some modifications. Then AF extracts were measured by using high performance liquid chromatography (HPLC).

Production of aflatoxin on rice for *in vivo* experiment

Aspergillus flavus was cultured on rice for production of aflatoxin which will be produced for *in vivo* experiment by the method [8]. The extracted aflatoxin has been dissolved in chloroform then adding the corn oil with continuous mixing on magnetic stirrer at 60°C for evaporation of chloroform.

Blood and liver samples

Blood samples were collected from wing vein in clean dry tubes and centrifuged at 3000 rpm for 10 min. Separated serum

samples were collected and subjected to biochemical analysis.

Immediately after ducklings were scarified the abdomens were opened and the livers were removed and washed by cold saline to remove remaining blood then blotted by filter paper to remove remaining saline, In addition, part of liver was kept in 10% neutral buffered formalin solution (BFS) for histopathological examination; The second part was used for measurement of malondialdehyde (MDA) and glutathione (GSH) and the last part was used for quantification of AF residues.

Biochemical analysis

Collected serum samples were subjected to measurement of total proteins and albumin [10], Alanine aminotransferase (ALT) (EC 2.6.1.1) and Aspartate Aminotransferase (AST) (EC 2.6.1.2) [11], Cholesterol [12] and triacylglycerol [13]. Lipid peroxidation product (malondialdehyde) was measured spectrophotometrically after the reaction with TBA according to the method described by [14]. Glutathione level was assayed colorimetrically according to the method described by [15].

Histopathological studies

Liver specimens were rapidly fixed in 10% neutral buffered formalin solution for at least 24 hours. The fixed specimens were processed through the conventional paraffin embedding technique (dehydration through ascending grades of ethanol, clearing in chloroform and embedding in paraffin wax at 60°C. Paraffin blocks were prepared from which 5 microns thick sections were obtained and stained by

hematoxyline and eosin (H&E) according to the method described by [16].

Statistical analysis: All calculations and analysis was done by [17].

RESULTS AND DISCUSSIONS

In vitro experiment

Table (1) and Fig. (1) showed that AFB₁ production levels were decreased as compared to control by inhibition percentages of 35.27%, 53.9%, and 59.17%, respectively. Also AFB₂ production inhibition percentages were 27.02%, 45.95%, 59.46% respectively when compared with control +ve flasks. In the past decade, interest on the topic of antimicrobial plant extracts has been growing. Various spices and herb extracts have been used for the purpose of food preservation and medicinal purposes [18]. Turmeric used as a feed additive as they were added to yellow corn in different concentrations to investigate the effects of these plants on aflatoxin production levels by *Aspergillus flavus* toxigenic strain. Turmeric by a concentrations of 0.5%, 1.0% and 2.0% were decreased the aflatoxin production by *Aspergillus flavus*. This result is agreed with that of [19] who reported the antifungal activity of turmeric volatile oil. About 90% reduction in aflatoxin production at a 5–10 mg/ml concentration of turmeric, an effect attributed to the antioxidant curcumin in turmeric [20]. In addition, turmeric inhibited the spore count and the aflatoxin production by *Aspergillus flavus* [21].

Table 1: Effect of Turmeric on aflatoxin production by *Aspergillus flavus*

	AFB ₁ production level (ppb)	AFB ₁ production inhibition %	AFB ₂ production level (ppb)	AFB ₂ inhibition%
T ₁	1063±35.12		37±5.36	
T ₂	688±18.30**	35.27	27±4.29*	27.02
T ₃	490±20.47**	53.90	20±5.13*	45.95
T ₄	434±15.24**	59.17	15±4.54*	59.46

* Values are significant compared to control (P< 0.05).

** Values are highly significant compared to control (P< 0.01).

T₁ (Control +ve), T₂ (Turmeric 0.5%), T₃ (Turmeric 1.0%), T₄ (Turmeric 2.0%)

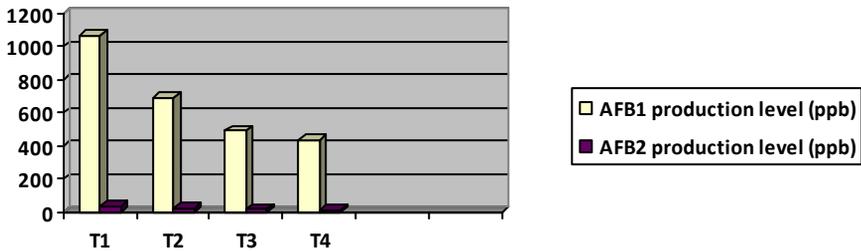


Fig. 1: Effect of Turmeric on aflatoxin production by *Aspergillus flavus*.

In vivo experiment

At the 4th week of experiment total proteins and albumin were significantly decrease. While, ALT, AST, cholesterol and triacylglycerol were significantly increased (Table, 2). These changes might be attributed to the hepatotoxicity induced by aflatoxin. The data illustrated in Table, (3) and Fig. (3) a significant increase in total serum protein levels were recorded. While, AST, cholesterol triacylglycerol were significantly decreased by addition of turmeric as diet supplement (G4). In G5 total protein and albumin levels were significantly decreased. But ALT, AST, cholesterol and triacylglycerol were significantly increased. At 8th week in G4 serum total proteins and albumin were significantly increased While, cholesterol and triacylglycerol were significantly decreased. In contrast, in G5 total proteins and albumin were significantly decreased and ALT, AST, cholesterol and triacylglycerol were significantly increased (Table, 4 and Fig. 4).

In aflatoxin treated group, serum total proteins and albumin have been significantly decreased after two weeks treatment in comparable to control one. Many studies confirmed that observation; aflatoxin decreased the total serum proteins and albumin levels in broiler chicks [22]. The decrease in total serum proteins might be contributed to the binding of aflatoxin to DNA. Therefore, aflatoxin hinder

transcription and translation in return decrease the protein synthesis, as the exo-epoxide product of aflatoxin metabolism reacted with N₇-guanine in DNA [23]. The data recorded in Table, (2) showed a significant increase in ALT, AST, cholesterol and triacylglycerol in aflatoxin treated group. This result comes in accordance with that, aflatoxin was increased serum ALT activity of ducks [24], broilers [25] and Japanese quail [26].

Turmeric was significantly increased total serum proteins at (6th and 8th weeks) and albumin at (8th week) While, AST, cholesterol and triacylglycerol were significantly decreased. These results were in accordance with the study on broiler given a diet mixed with turmeric for 45 days [27]. Supplementation of ducklings with turmeric after aflatoxin treatment increased the total serum proteins level than that of (aflatoxicated birds) group. This might be due to the antioxidant effect of them against aflatoxin or due to that turmeric increase the total serum proteins level [28]. Curcumin admixed with the diet (0.5% w/w) decreased serum cholesterol by about 21% and LDL-cholesterol by 42.5%, but it increased serum HDL by 50% [29]. In G6, turmeric was reverted the reduction in (total proteins and albumin) levels and the elevation in (ALT, AST, cholesterol and triacylglycerol) which induced by AF as turmeric opposed the AF hepatotoxicity.

Table 2: Effect of aflatoxin on some serum parameters of ducklings in G₁ (control) and G₂ (Aflatoxin treated) at 4th week of experiment

	Total protein (g/dl)	Albumin (g/dl)	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	Triacylglyceride (mg/dl)
G1	7.1±0.22	4.29±0.43	26±1.24	22±2.41	120.6±6.25	148.24±7.12
G2	5.01±0.08*	3.07±0.07*	42±2.37*	49±0.46*	176±1.27*	182.04±8.40*

* Values are significant compared to control (P< 0.05).

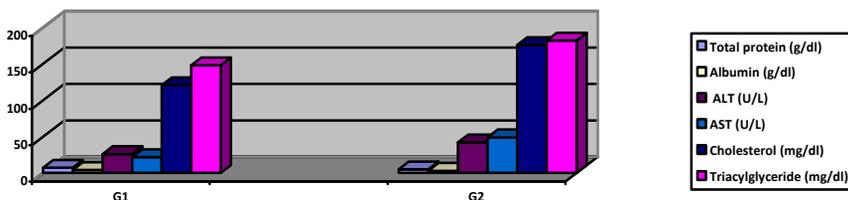


Fig. 2: Effect of aflatoxin on some serum parameters of ducklings in G₁ (control) and G₂ (Aflatoxin treated) at 4th week of experiment

Table 3: Effect of turmeric on some serum parameters of ducklings in G₃ (control-ve), G₄ (Turmeric group), G₅ (aflatoxinicated birds) and G₆ (AF+Turmeric) at 6th week of experiment

	Total protein (g/dl)	Albumin (g/dl)	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	Triacylglycerol (mg/dl)
G3	6.07±0.13	4.01±0.24	26±0.78	23±1.45	150.75±7.31	141.8±4.12
G4	7.96±0.23*	4.51±0.62	25±0.12	26±0.20*	121.15±0.41*	122.82±5.47*
G5	5.34±0.25*	3.08±0.12*	38±1.08*	38±2.30*	173.05±2.20*	174.64±1.10*
G6	6.50±0.54	3.99±0.41	31±2.11	29±1.78	160.3±1.10	140.01±0.32

* Values are significant compared to control (P< 0.05).

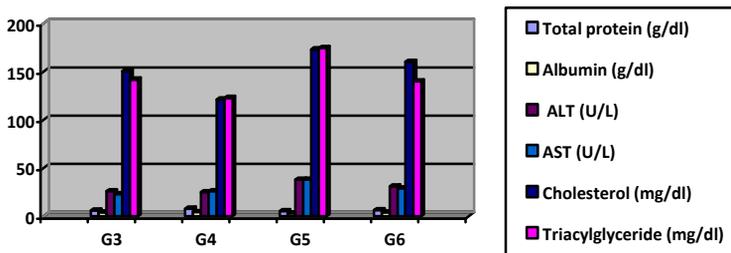


Fig. 3: Effect of turmeric on some serum parameters of ducklings in G₃ (control-ve) G₄ (Turmeric group) G₅ (aflatoxinicated birds) and G₆ (AF+Turmeric) at 6th week of experiment

Table 4: Effect of turmeric on some serum parameters of ducklings in G₃ (control-ve) G₄ (Turmeric group) G₅ (aflatoxinicated birds) and G₆ (AF+Turmeric) at 8th week of experiment

	Total protein (g/dl)	Albumin (g/dl)	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	Triacylglycerol (mg/dl)
G3	6.49±0.25	3.05±0.58	28±0.96	22±1.36	134.17±10.3	142.12±1.14
G4	8.34±0.05*	5.25±0.23*	28±2.17	22±1.42	103.95±5.40*	111.56±0.12*
G5	5.60±0.33*	2.60±0.17*	34±2.64*	32±1.58*	160.6±17.33*	161.13±5.42*
G6	6.07±0.24	3.03±0.43	30±1.69	25±1.40	145.35±20.23	147.78±6.14

* Values are significant compared to control (P< 0.05).

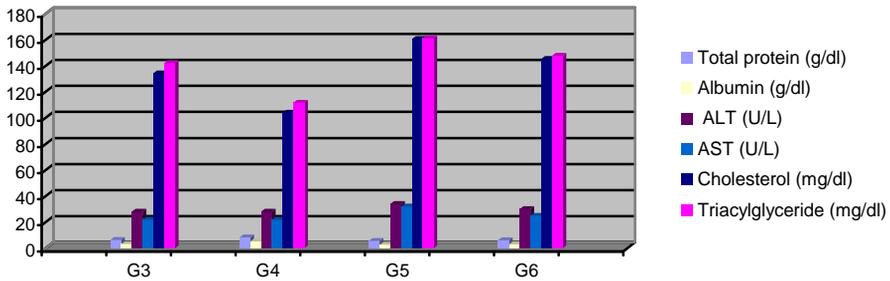


Fig. 4: Effect of turmeric on some serum parameters of ducklings in G₃ (control-ve) G₄ (Turmeric group) G₅ (aflatoxicated birds) and G₆ (AF+Turmeric) at 8th week of experiment

The data obtained in the Table, (5) showed that hepatic malondialdehyde levels were significantly increased and glutathione level was significantly decreased in G₂ at 4th week of experiment when compared with G₁. MDA levels were significantly decreased in G₄ at (6th and 8th weeks) and significantly increased in G₅ at (6th and 8th weeks) in comparison to G₃. In contrary, GSH levels were significantly increased in G₄ at (6th and 8th weeks) and significantly decreased in G₅ at (6th and 8th weeks) in comparison to G₃. The results of our study indicated that administration of aflatoxin to ducklings induced an increase in lipid peroxidation in liver which evidenced by the elevation in malondialdehyde level. The increased lipid peroxidation in aflatoxin treated birds is agreed by [30] who stated that treatment of rats by aflatoxin increased lipid peroxidation level in comparable to control one. Moreover, Aflatoxin treatment caused a significant increase in MDA in rabbit's liver [31], plasma MDA of quail [32] and rats [33]. Malondialdehyde level in liver non-significantly decreased in turmeric fed group

in comparison to control untreated one. This result is agreed by [34] who reported a significant decrease in lipid peroxidation level with concomitant decrease in lead levels in all the brain regions of curcumin fed group. In addition, curcumin exerted a significant decrease in the lipid peroxidation and an improvement of antioxidant status [35].

Obtained data evidenced the protective effect of turmeric against the oxidant adverse effect induced by AF. Curcumin's antioxidant properties might not be only due to its chemical nature as a free radical scavenger, but also due to its ability to induce GSH-linked defense mechanisms against oxidative stress as well as increases in the activity of γ -glutamyl cysteinyl synthase, the rate limiting step in glutathione synthesis [36]. Moreover, induces de novo synthesis of GSH by stimulating the activity and gene expression of glutamate-cysteine ligase [37]. Turmeric as it increases the synthesis of glutathione as the antioxidant mechanisms against any xenobiotic including aflatoxin [28].

Table 5: Effect of aflatoxin and turmeric on MDA level (nM MDA / g wet liver) and glutathione level (μ M / g wet liver) of G₁ (control), G₂ (Aflatoxin treated), G₃ (control-ve), G₄ (Turmeric group), G₅ (aflatoxicated birds) and G₆ (AF+Turmeric)

	4 th week			6 th week		8 th week	
	MDA	GSH		MDA	GSH	MDA	GSH
G ₁	115.54±0.31	6.26±0.13	G ₃	114.64±1.23	4.67±0.36	114.27±2.49	4.82±0.11
			G ₄	100.29±0.56*	16.14±0.45*	96.94±0.37*	17.43±0.31 *
G ₂	188.09±23.46*	3.67±0.24*	G ₅	172.61±3.25*	3.14±1.23*	140.33±1.41*	2.29±0.45*
			G ₆	125.13±2.33	6.88±0.79	120.50±1.63	6.73±1.36

* Values are significant compared to control (P< 0.05).

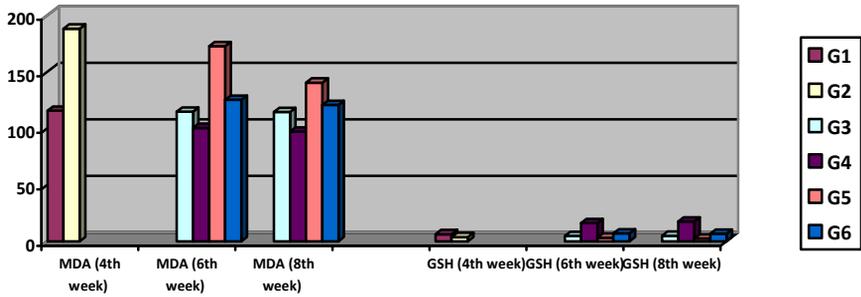


Fig. 5: Effect of aflatoxin and turmeric on MDA level (nM MDA / g wet liver) and glutathione level (µM / g wet liver) of G₁ (control), G₂ (Aflatoxin treated), G₃ (control-ve), G₄ (Turmeric group), G₅ (aflatoxicated birds) and G₆ (AF+Turmeric).

At 6th week AFB₁ residues in liver were decreased from 2.21 ppb to 0.69 ppb (G₅) and to 0.24 ppb (G₆). In addition, it was decreased to 0.24 ppb (G₅) and 0.13 ppb (G₆) at 8th week. AFB₂ were decreased to non detectable levels at both 6th and 8th weeks (Table, 6). Our results indicated that administration of aflatoxin to ducklings led to accumulation of aflatoxin in liver, which extracted and measured by HPLC. In some studies, however, correlations had been made between dietary aflatoxin concentrations and the subsequent concentrations of tissue residues. Fed a variety

of domestic poultry species with diets containing 30 µg for kg AFB₁ for a 7day period and showed that levels of AFB₁ and its metabolites were greater in liver than in muscle for all bird species tested [38]. AFB₁ residues were observed only in muscle and liver of birds given dietary AFB₁ [25]. Turmeric, in which turmeric caused a decrease in aflatoxin residues in liver of G₆ at 6th and 8th weeks these decreased might be concerned to the antioxidant effect of turmeric and enhancement of phagocytosis in liver by turmeric.

Table 6: AFB₁ and AFB₂ residues of duckling's liver, and the effect of turmeric on its level

	4 th week			6 th week		8 th week	
	AFB ₁	AFB ₂		AFB ₁	AFB ₂	AFB ₁	AFB ₂
G ₁	ND	ND	G ₃	ND	ND	ND	ND
			G ₄	ND	ND	ND	ND
G ₂	2.21 ppb	0.27 ppb	G ₅	0.69 ppb	ND	0.24 ppb	ND
			G ₆	0.24 ppb	ND	0.13 ppb	ND

G₁ (control), G₂ (Aflatoxin treated), G₃ (control-ve), G₄ (Turmeric group), G₅ (aflatoxicated birds), G₆ (AF+Turmeric), ND (non detectable level)

Histopathological studies

Fig., (6) showing normal healthy hepatocytes. While Fig., (7) showing the effect of oral administration of aflatoxin (30 ug/kg b.w.) for two weeks, the slide showing a diffuse hydropic degeneration. Histopathologically, addition of turmeric showing mild hydropic degeneration in the hepatocytes at 8th week While Fig., (9). In G₅ at 6th week the liver picture showing diffuse hydropic degeneration While Fig., (10). At 8th week the deleterious effects of aflatoxin is still found as the While Fig., (11) showing

degenerative changes in the hepatocytes with granular cytoplasm. In While Fig., (12) turmeric after aflatoxin treatment was induced a hepatocellular swelling and moderate hydropic degeneration of the majority of hepatic cells at 6th weeks. While Fig., (13) showing congestion and hydropic degeneration of some hepatocytes of liver of duck G₆ at 8th week.

In the present study, the histopathological changes induced by AF is evidenced by the study that reported, aflatoxin induced a liver injury of ducklings such as bile duct

proliferation and fatty degeneration [38]. Large fat droplets that displaced the nucleus in chicks given dietary aflatoxin [39]. Variable degrees of regional massive necrosis, sometimes involving many confluent lobules, scattered individual hepatocyte necrosis, multilobular degeneration, areas of hepatic degeneration showing fatty changes, hyperplasia of the

epithelium and severe bile duct proliferation in ducklings fed an aflatoxin-contaminated diet [40]. Addition of turmeric to poultry ration by percentage of 1.0% induced mild hydropic degeneration in the hepatocytes at 6th and 8th weeks. In contrary, turmeric decreased the histopathological adverse effects induced by AF due to the effect of active principles of turmeric.

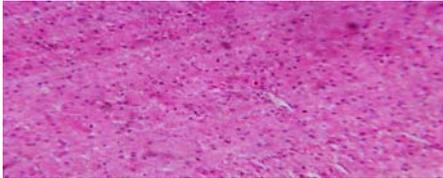


Fig. 6: liver of duck in the G₁ (control) showing normal healthy hepatocytes. H&E (X250).

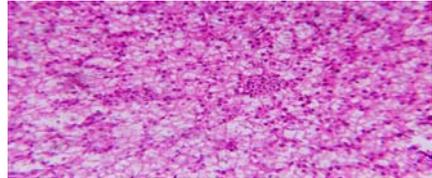


Fig. 7: liver of duck exposed to aflatoxin (G₂) for two weeks showing diffuse hydropic degeneration. H&E (X250).

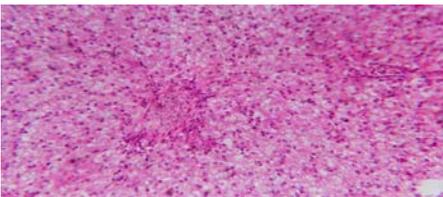


Fig. 8: liver of duck in G₄ at 6th week. H&E (X 250).

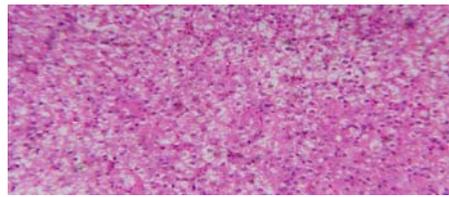


Fig. 9: liver of duck in G₄ at 8th week showing mild hydropic degeneration. H&E (X 250).

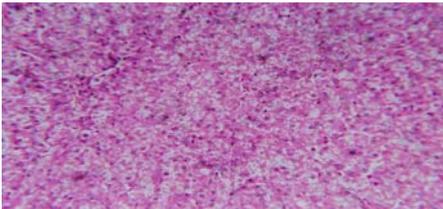


Fig. 10: liver of duck G₅ at 6th week showing diffuse hydropic degeneration. H&E (X 250).

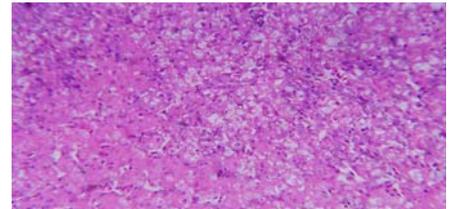


Fig. 11: liver of duck G₅ at 8th week showing degenerative changes in the hepatocytes with granular cytoplasm. H&E (X 250).

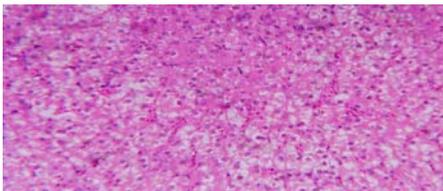


Fig. 12: liver of duck G₆ at 6th week showing hepatocellular swelling and moderate hydropic degeneration of the majority of hepatic cells. H&E (X 250).

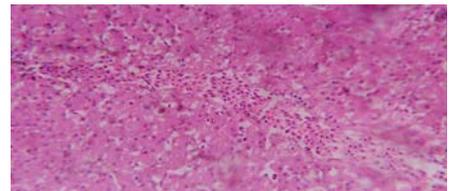


Fig. 13: liver of duck G₆ at 8th week showing congestion and hydropic degeneration of some hepatocyte.

CONCLUSIONS

From the obtained results, it can conclude that addition of turmeric as a feed additive in poultry diet products the hepatotoxicity induced by aflatoxin as turmeric inhibit the growth and aflatoxin production by *Aspergillus flavus*.

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