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Biochemical, Histological and Ultrastructural Studies on the Effect of Citric acid Supplementation on Aflatoxins-intoxicated Japanese Quail

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Abstract

For poultry farmers and quails' producers', one of the biggest challenges is dealing with natural diet contaminants like mycotoxins. Worldwide, mycotoxins are present in all feed sources, primarily in corn, and they significantly reduce the health, immune function, and performance of birds. For this purpose, the effect of citric acid (CA) supplementation on diet contaminated with Aflatoxins (AFL) was investigated in male Japanese quail (*Coturnix coturnix*) through studying hepatic biochemical, histological, and ultrastructural changes. Influences of experimental diets were assessed in 3 replications of 6 birds each (n=18 per treatment). Quails two weeks old were assigned into 4 equal groups. The control quails fed only basal diet, AFL group quails were given basal diet contaminated with 2.5 mg AFL/kg diet, citric group quails fed basal diet with 10 g citric acid/Kg, and AFL/citric group quails fed basal diet contaminated with 2.5 mg AFL /Kg and augmented with 10 g citric acid/Kg. After four weeks, feeding AFL to quails induced hepatotoxicity as evidenced by significant decline in body weight, serum albumin and total protein while it significantly increased serum ALT, and AST activities. AFL also induces liver oxidative stress by the elevation of lipid peroxidation and reducing GPx, ADH, SOD and catalase activities. Descriptive hepatic histological and ultrastructural alteration were also noted in the AFL group. Treatment with CA induced an increase in total protein, albumin, SOD, GPx, ADH and significantly decreased ALT and AST activities and MDA level. Moreover, it also improved the histological and ultrastructure alternations induced in the liver of AFL group. It was concluded that supplementation of CA into the AFL polluted diets lessened the adverse influences of AFL on quail's liver.

KEYWORDS
Aflatoxins, Citric acid, Hepatoprotective, Hepatotoxicity, Quails

INTRODUCTION

The mycotoxins corruption of feeds is a serious health concern in production of animal all over the world (Abdallah *et al*., 2015). Mycotoxins are byproducts formed by many fungi species that grow and colonize different crops causing toxic effects when consumed and led to mycotoxicosis in both animals and humans (Awuchi *et al*., 2021). There is a wide diversity of toxic mycotoxins (Moretti *et al*., 2017). The most communal mycotoxins is the group of aflatoxins (AFL) which is produced by *Aspergillus parasiticus* and *Aspergillus flavus* (Priesterjahn *et al*., 2020). AFL affect many plants including cereal grains which are the main animal feed (Winter and Pereg, 2019). Chicken, ducks, turkey and quails are the most susceptible species affected by AFL (Diaz and Murcia, 2011). Aflatoxins significantly affect performance, reducing feed intake and growth rate of quail (Mahrose *et al*., 2021), which affects reproductive capacity and the immune system, causing obvious direct or indirect economic losses in most countries. AFL is very difficult to remove or destroy, so it enters the feed chain with preserving its toxic criteria (Čolović *et al*., 2019). During the course of AFL metabolism, cytochrome P450 is enthused, and hydrogen peroxide as well as superoxide ions as reactive oxygen species (ROS) are generated, that can provoke as status of oxidative stress (Mates, 2000). Furthermore, increased ROS levels generated by AFL can extinguish hepatocytes of liver (Eftekhari *et al*., 2018).

A wide range of chemicals (include oxidizing agents as ozone or hydrogen peroxide, reducing agents as bisulfites, acids as hydrochloric acid or acetic acid, bases as ammonium or sodium hydroxide and reagents as formaldehyde) shown to react with AFL and turn them to compounds less toxic or could destroy them (Čolović *et al*., 2019). Unfortunately, most of these substances have confines, owing to their unsafety, and their reduction for the nutritious value and functional criteria of the feed (Rasane *et al*., 2015). Interestingly, using organic acids (CA or lactic acid) has improved the nutritional properties of feeds thus improving their health value in animal and human nutrition (Abdel-Fattah *et al*., 2008). Moreover, Safara *et al*. (2010) proved the detoxifying property of CA on feeds contaminated with AFL. However, little research has examined the influence of adding CA to the contaminated AFL diet on the male quail's liver.

MATERIALS AND METHODS

Chemicals

Serum albumin, total protein, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using commercial kits (Biodiagnostic Co., Egypt). Alcohol dehydrogenase (ADH), Cytochrome 450 reductase, superoxide dismutase

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(SOD), glutathione peroxidase (GPX), malondialdehyde (MDA) and catalase, in liver homogenate were done using commercial kits (Cell Biolabs, Inc).

Experimental birds

A total of 72 male Japanese quails (*Coturnix coturnix japonica*) were obtained from a local farm. Water and feed were allowed ad libitum until two weeks of age (55-62g), with controlled temperature (22-31°C) and ventilation. Constant light conditions were employed (24 hours lighting). Quails were inspected three times daily for water, feed, and mortality. They were fed basal diet that formed to satisfy their nutritional requirements according to Abdelrazek *et al*. (2015). The vitamin and mineral supplements were free from antioxidant and antibiotic substances.

Ethical Approval

The study was conducted according to approval REC161/2022 from the Scientific Research Ethics Committee of the Faculty of Science, Suez Canal University and all efforts were made to minimize birds suffering during the handling.

Aflatoxin and citric acid

Aflatoxins obtained through the fermentation of the toxic fungus strain *Aspergillus parasiticus* NRRL 2999 on rice that was previously parboiled. The procedures were carried out following *Shotwell et al. (1966).* With a total AFL content of 6.5 mg/kg, rice had levels of AFL B1.The AFL mixture was added into quails' basal diet to produce an AFL concentration of 2.5 mg/kg following Abdelrazek *et al*. (2015). Citric acid used (CAS Number: 77-92-9) was with purity > 98.5% obtained from Sigma Aldrich.

Experimental design

The experimental quails were assigned into 4 groups randomly, 6 birds each and repeated three times. Control group quails nourished only basal diet. AFL group quails nourished basal diet contaminated with 2.5 mg AFL/kg diet for 4 weeks (Abdelrazek *et al*., 2015; Khaleghipour *et al*., 2020). Citric group quails fed basal diet with added dietary 10 g citric acid/Kg for 4 weeks (Fikry *et al*., 2021). AFL/Citric group quails fed basal diet contaminated with 2.5 mg AFL /Kg diet with 10 g/Kg dietary CA for 4 weeks. Body weight was determined weekly.

Samples collection

Quails were slaughtered after 4 weeks experimental duration. The blood was collected in plain tubes for separation of sera. The sera were stored at -20ºC for biochemical examinations. Liver samples from each group were quickly collected, weighed, and inspected for any lesion then divided into three parts. Part of the liver stored at -20ºC for biochemical examinations. Another part of the liver was fixed in 10% formalin solution for 48 h for paraffin sectioning. The last part of liver was fixed in 3% glutaraldehyde solution at 4°C overnight for transmission electron microscope (TEM) examination.

Biochemical analysis

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed according to Reitman and Frankel (1957). Albumin and total protein contents were measured as reported by Kingsley (1939) and Rodkey (1965), respectively. Alcohol dehydrogenase (ADH), Cytochrome 450 reductase, superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), and catalase followed the manufacturer's directives.

Histopathology

The fixed liver tissues with 10% neutral formalin solution were used to make the paraffin sections. The paraffin sections were subjected to be stained with Hematoxylin and Eosin stain (H&E) (Slaoui and Fiette, 2011) or the periodic acid Schiff stain (PAS) (Fu and Campbell-Thompson, 2017).

Ultrastructural study

Ultrastructural preparations were carried out according to Skovorodin *et al*. (2019) and El Hak *et al*. (2022). Fresh liver parts were subjected to be cut into small cubes about 1 mm^3. The samples were then fixed by rapid immersion in 3% glutaraldehyde solution for an overnight period at 4°C, rinsed in sodium cacodylate buffer at a temperature of 4°C, put in 1% osmium tetroxide 1h at 4°C for post fixation, dehydrated in ascending series of alcohols then implanted in Araldite resin. Semi-thin sections obtained for microscopic examination while ultra-thin sections were mounted in copper mesh grids, stained with lead citrate and uranyl acetate then examined by TEM.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data followed by post hoc Duncan test using SPSS. Data results considered significant at P value < 0.05. Data were denoted as mean ± standard error of the mean.

RESULTS

Clinical symptoms, mortalities, and postmortem lesions

Birds fed diets containing AFL showed mild clinical symptoms such as ruffled feathers, and reduced feed intake while no symptoms were recorded in other groups. Postmortem examination of slaughtered quails belonging to AFL group showed enlargement of livers with friable texture and appeared slightly yellowish in color. These lesions were mild in AFL/Citric acid group. There was no mortality in the treated groups during the experiment.

The quail body weight, weight gain and relative liver weight

The final body weights and weight gains of the quails fed the AFL-contaminated diet were lower than those of the control quails fed the normal diet ($P<0.05$). However, quails fed CA contained diet and AFL showed a significantly ($P<0.05$) increase in their weight gain and final body weight in comparison to AFL group (Table 1).

The relative liver weights were elevated ($P<0.05$) in quails fed with contaminated diet with AFL than control group. However, quails fed with diet contained CA and AFL significantly ($P<0.05$) decreased the relative hepatic weights in comparison to AFL group (Table 1).

Biochemical Analysis

The serum biochemical parameters results were demonstrated in Table 2. The mean values of AST and ALT were upsurged

(P<0.05) while albumin and total protein contents were significantly (P<0.05) downregulated in AFL group compared to control group. Quails treated with both AFL and CA revealed significant (P<0.05) decline in AST and ALT while significant increase in albumin and total protein concentrations compared to AFL group.

The mean values of hepatic ADH, cytochrome 450 reductase, GPX, SOD, and CAT activities were reduced significantly (P<0.05) in AFL group compared to quails in the control. However, those values were significantly (P<0.05) upsurged in AFL/Citric acid group when matched to AFL group. MDA showed significant (P<0.05) increase in AFL group compared to the control group, while downregulated significantly (P<0.05) in AFL/Citric acid group compared to AFL group.

Histopathological examination of liver

Histopathology examination of the liver of control quails and those fed with normal diet supplemented with CA showed normal structure of liver tissue (Fig. 1a & b). Liver of AFL group revealed severe fatty degeneration surrounded the central vein and damaged portal area (Fig. 1c). Liver examination of AFL/Citric acid group revealed normal histological structure (Fig. 1d).

The quantitative estimation of carbohydrates PAS-positive material in the liver of control and other experimental groups

Table 1. Effect of addition of citric acid (CA) supplement on contaminated food diet with AFL on the quail body weight, weight gain and relative liver weight.

	Groups			
	Control	Citric acid	AFL	AFL/Citric acid
Initial weight (g)	60.16±3.00	59.66±2.60	61.78±2.90	56.63±1.81
Final weight (g)	158.00±2.50^{b}	164.00±6.00^{a}	130.50±2.81^{c}	146.00±4.13^{b}
Weight gain (g)	97.83±1.10^{a}	104.33±5.00^{a}	69.05±1.91^{c}	86.86±4.22^{b}
Relative liver weight (%)	2.04±0.10^{b}	1.73±0.10^{b}	2.38±0.06^{a}	1.98±0.11^{b}

Data are expressed as means ± SEM, n=18. Data with different superscripts in the same row are significantly different at p≤0.05.

Table 2. Effect of addition of citric acid (CA) supplement on contaminated food diet with AFL on serum biochemical parameters of Japanese quails.

	Groups			
	Control	Citric acid	AFL	AFL/Citric acid
ALT (U/L)	14.92±0.30^{b}	14.03±0.81^{b}	26.59±1.80^{a}	16.37±0.41^{b}
AST (U/L)	130.96±4.02^{c}	126.56±2.08^{c}	300.51±4.71^{a}	186.85±7.96^{b}
Total protein (g/dl)	4.38±0.06^{b}	4.93±0.05^{a}	2.57±0.07^{d}	3.85±0.08^{c}
Albumin (g/dl)	2.79±0.01^{a}	1.72±0.13^{b}	1.48±0.12 ^{b}	2.67±0.05^{a}
ADH (nmol/g tissue)	3.04±0.40^{a}	3.03±0.40^{a}	1.64±0.40^{b}	2.61±0.50^{a}
CYP450R (nmol/ g tissue)	2.77±0.03^{a}	2.85±0.03^{a}	1.35±0.21^{c}	2.07±0.07^{b}
GPX (nmol/ g tissue)	18.55±0.18^{a}	18.75±0.24^{a}	13.51±0.32^{c}	16.54±1.10^{b}
SOD (µ/ g tissue)	10.98±0.04^{b}	11.53±0.20^{a}	6.82±0.20^{d}	10.40±0.20^{c}
CAT (µ/ g tissue)	29.42±0.30^{a}	29.74±0.40^{a}	15.65±0.30^{c}	27.66 ±0.60^{b}
MDA (nmol/ g tissue)	0.44±0.01^{c}	0.38±0.02^{c}	0.76±0.03^{a}	0.63±0.01^{b}

Data are expressed as means ± SEM, n=18. Data with different superscripts in the same row are significantly different at p≤0.05. ALT: alanine aminotransferase, AST: aspartate aminotransferase, ADH: alcohol dehydrogenase, CYP450R: Cytochrome P450 reductase, GPX: Glutathione peroxidase, SOD: Superoxide Dismutase, MDA: Malondialdehyde.

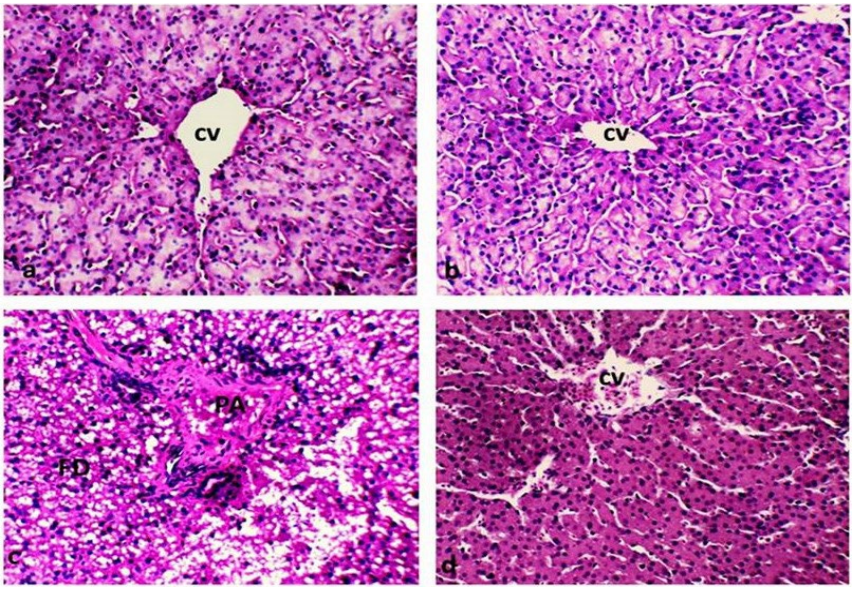

Fig. 1. Photomicrograph of a) Liver of control group revealed normal liver structure surrounded the central vein (CV). b) Liver of citric acid (CA) group demonstrated normal liver structure surrounded the central vein (CV). c) Liver of AFL group showed that liver lost its normal structure with fatty degeneration of hepatocytes (FD) surrounded the damage portal area. d) Liver of AFL/Citric acid showed normal hepatic structure surrounded the central vein. (H&E, 400X).

is shown in Figure 2. Liver sections of control and CA groups revealed a strong cytoplasmic PAS reaction. Livers of quails treated with AFL showed decreased PAS reaction, while livers of quails treated with AFL/Citric acid showed positive PAS reactions have been restored.

Ultrastructure examination of liver

Ultrastructure observation by TEM of the hepatic tissue of the control and the CA group displayed a definite eccentric nucleus with obvious nucleolus and a well-defined nuclear envelop (its inner membrane contained large amounts of light euchromatin alternated with areas of dense heterochromatin). The cell membrane was intact. The cytoplasm contained endoplasmic reticulum. Free ribosomes were also found either scattered or aggregated in groups within the cytoplasm. The mitochondria were numerous and medium-sized exhibited spherical or elongated shapes and electron dense matrices filled with tubular cristae (Figure 3a & b). The ultra-structure examination of liver of AFL treated quails showed varied ultra-structural changes. Nucleus showed margination of chromatin (condensation of chromatin around the nuclear envelop). Moreover, most of cells displayed complete disappearance of cellular organelles including mitochondria and ER and cell membrane rupture. Also, lamellar myelin figure was recorded (Figure 3c). Liver of AFL/Citric acid group showed normal nucleus of the liver and cellular organelles, with some lipid droplets appear (Figure 3d).

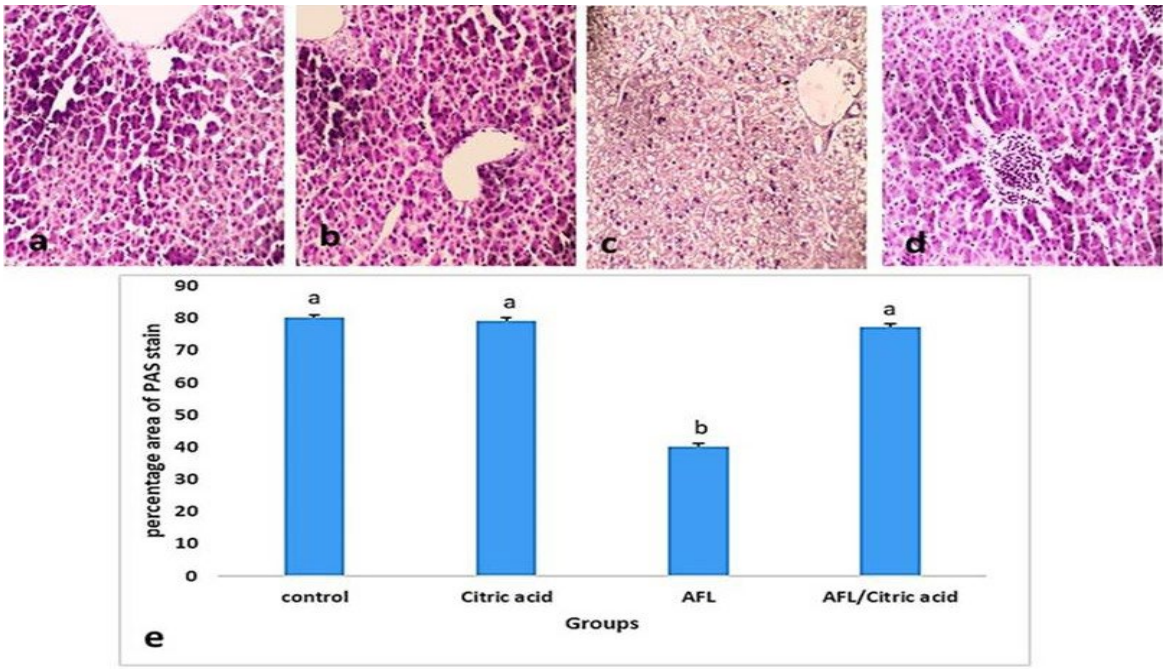

Fig. 2. Photomicrograph of liver sections stained with PAS stain of (a) control group with hepatocytes showing positive PAS reaction, (b) Citric acid (CA) group showing normal positive PAS. (c) AFL treated group with decreased PAS reaction, (d) AFL/Citric acid group showed positive PAS (PAS staining technique, 200X). (e) Percentage of PAS-stained area in the treated group. Data were expressed as means ± SEM, n=18. Data were statically analyzed using One-way ANOVA followed by Duncan multiple comparisons test. Different letters showed data of different row which is statistically significant p≤0.05.

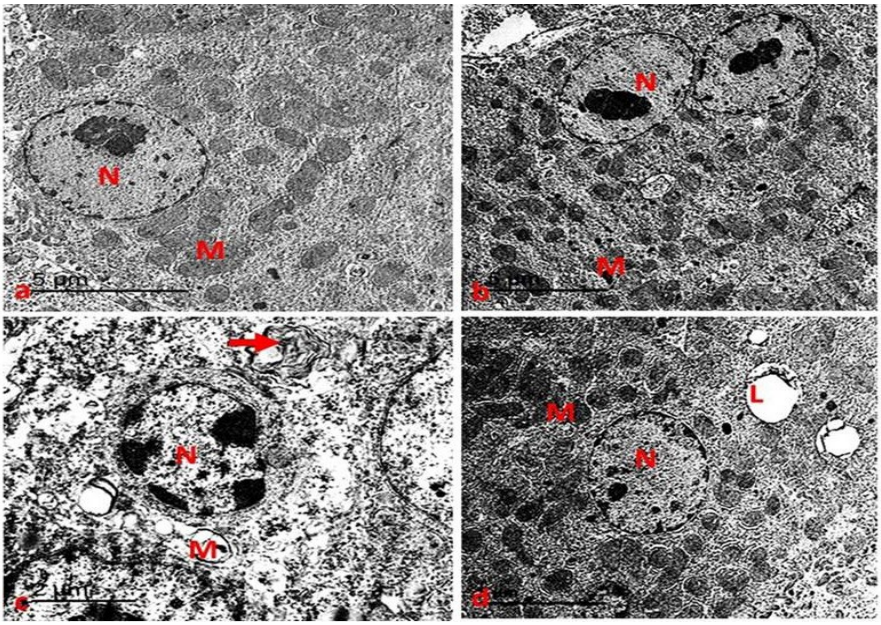

Fig. 3. TEM micrograph of liver of (a&b) normal ultrastructure of liver tissue (nucleus (N) and mitochondria (M)) of control and citric acid (CA) groups. (c) AFL group showed altered the ultrastructure of liver tissue including margination of chromatin in the nucleus (N), myelin figure (arrow) and dissolved cellular organelles especially the mitochondria (M). (d) Normal liver ultrastructure of AFL/Citric acid group showed restored the normal ultrastructure of liver tissue (nucleus (N) and mitochondria (M)) with some lipid droplets (Li).

DISCUSSION

Mycotoxins deem to be one of the most serious contaminants of animal feed as it is very hard to be eliminated and could lead to considerable health issues which could ended with death (Santos Pereira *et al*., 2019). Different approaches were used for mycotoxins detoxification, including chemical, biological and physical methods (Liu *et al*., 2022). The current study investigated the protective effect of CA supplement in contaminated AFL diet against hepatotoxicity induced by AFL in male quails.

In the current experiment, birds fed diets containing AFL showed mild clinical symptoms such as ruffled feathers, and reduced feed intake. The necropsy of slaughtered quails belonging to AFL group, enlargement of liver with friable texture and slight yellowish in colour were noted. These findings matched with Elbasuni *et al*. (2022). Histopathological inspection of the liver further confirmed that lesion due to presence of fatty degeneration of hepatocytes. Using CA was able to reduce the adverse effect of AFL on the birds' liver as CA possesses detoxification action versus AFL-contaminated materials (Méndez-Albores *et al*., 2007).

The present results showed that ingestion of male quail to diet contaminated with AFL caused significant decrease in weight gain and final body weight. These results were in agreement with Harvey *et al*. (1993). Aflatoxin detoxification necessitates glutathione utilization, which is made up of methionine and cysteine. As a result, this detoxification mechanism may diminish the metabolic availability of methionine, leading to a decreased growth rate (Hassan *et al*., 2019). Feeding contaminated diet with AFL contained CA significantly increases quails' weight gain compared to quails fed diet contaminated with AFL. This result is in harmony with Mishra and Swain (2022) who reported that CA positively affected Japanese quails and enhanced poultry growth. Melaku *et al*. (2021) suggested that increased in the body weight may be due to the stimulation of the proteolytic enzymes and modulation of protein digestibility, besides decreasing the intestinal pH that enhances the birds' performance.

The AFL resulted in an elevation in the relative liver weights of the quails which could be due to the lesions observed in the histopathological section of the current study. The elevations in the relative liver weights of the quail is a pointer of the uproar of hepatic metabolic activities (Emam *et al*., 2018). However, quail fed with diet contain CA and AFL significantly decreased the relative liver weights than those AFL group. The results of effect CA supplementation on quails' relative liver weights in the current study are in line with Tanpong *et al*. (2021).

In our study, the activities of AST and ALT revealed a significant increase in the AFL intoxicated group. This could be due to hepatocytes' membrane disruption or necrosis caused by mycotoxins, which causes hepatocytes degeneration and the leakage of such enzymes into the circulation (Barati *et al*., 2018; Kepekçi *et al*., 2013). Most AFL are bioactivated in the liver to the reactive 8,9-epoxide form, which has been shown to muddle proteins and DNA, causing damage to liver constructions and increasing the weight of liver (Bailey *et al*., 2006; Miazzo *et al*., 2005; Pasha *et al*., 2007). AFL B1 has also been linked to mitochondrial damage and hepatocytes dysfunction in broilers, which leads to apoptosis (Liu and Wang, 2016).

A significant decrement in albumin and total protein levels in the AFL-intoxicated group were found. AFL interfere with protein synthesis by preventing mRNA transcription or amino acid transfer (Kubena *et al*., 1993). On the other side, the AFL/Citric group showed a significant reduction in serum ALT and AST while increasing serum total protein and albumin as matched to the control quails. Fikry *et al*. (2021) demonstrated that ALT activity was lower in the citric acid-fed quails in comparison to the control, AST activity remained unchanged, but serum total protein levels were significantly higher. Acidic solutions were discovered to be capable of destroying mycotoxins (Jalili *et al*., 2011). As a result, CA treatment is effective in declining the harmful adverse effects of AFL on mutagenicity, toxicity, and carcinogenicity while preserving the organoleptic and nutritional qualities of the feed (Méndez-Albores *et al*., 2005).

ADH enzyme system serves in glycolysis and gluconeogenesis as well as breakdown of toxic alcohols with the reduction of nicotinamide adenine dinucleotide into useful ketone, aldehyde or alcohol groups during metabolic process (Edenberg, 2007). Therefore, reduction of ADH in AFL group denoted the adverse influence of AFL on hepatic metabolism and detoxification that was ameliorated by CA addition.

The current study found that feeding AFL to quails increased lipid peroxidation and oxidative stress, as evidenced by upregulated hepatic MDA levels and reduced antioxidants levels of (CAT, SOD and GPX). The liver is the key target organ of AFL, which can cause dysfunction in the hepatic mitochondrial antioxidants and enormous ROS production to cause oxidative stress, resulting in an inequity between the oxidative and antioxidative defense systems (Denli *et al*., 2009; Liu and Wang, 2016). The effects of AFL were clearly improved when combined with CA, as evidenced by a marked increase in the activities of catalase, GPX, and SOD while decreasing MDA levels in the AFL/citric acid group. These findings are consistent with the results of Fikry *et al*. (2021), who found that dietary CA supplementation to quails significantly increased SOD and GPx as matched to the control group. Parallel to the elevated oxidative stress and lipid peroxidation parameters, the AFL significantly reduced cytochrome P450 reductase level. Whereas it is a membrane bound enzyme that was liberated and become labial to proteolytic enzymes. Such enzyme was crucial for reduction of NADPH to activate cytochrome P450 and other heme proteins by donating electron (Pandey and Flück, 2013). The addition of CA to AFL diet induced significant improvement of lipid peroxidation concurrent with preserving cytochrome P450 reductase therefore it denoted positive influence of CA on AFL induced retrograded metabolism.

Histopathological and ultrastructure report confirmed that the AFL contaminated diet produced damaging effect in the liver of quail than the combined toxicant with CA, thus supporting the observed biochemical observations. The liver is the chief organ intricated in detoxifying AFL (El-Agamy, 2010). The contemporary research showed that AFL induced histopathological alteration in liver of quails including induction of fatty degeneration and portal area inflammatory cells infiltrations. These results coincide with Chen (2016) that found quails fed AFL contaminated diet revealed liver hepatocytes necrosis, fatty changes, and bile duct hyperplasia beside lymphocytes aggregation. According to Ferramosca and Zara (2014) fatty degeneration of hepatocytes occurs due to imbalance in fat metabolism. Pereira *et al*. (2022) suggested that AFL limits fat digestibility by lowering enzyme activity and bile acid needed for fats digestion and absorption. Citric acid showed normal liver structure. This finding agreed with Eissa (2004) who reported CA induced improvement the histopathological changes in liver tissue of the quail.

The existing study revealed a reduction in the hepatocytes' glycogen contents as appeared in the PAS staining sections of quails fed AFL contaminated diet this result harmonized with Ruby *et al*. (2014) who declared that AFL leads to the liberation of glucose from glycogen, so blood glucose elevates while liver glycogen contents declines. The quails fed diet with CA and AFL promoted the hepatic glycogen contents as demonstrated by the elevated PAS stained percentage. This assured the glycogen storing belongings of the citric acid.

The present results showed that AFL intoxicated quails revealed varied ultrastructure changes including chromatin condensation and margination, lipid droplets, degeneration of mitochondria and RER and cell membrane rupture. This result is in harmony with Nazar *et al*. (2012) who demonstrated several ultrastructure alternations in broiler chicks fed AFL contaminated diet. According to Doonan and Cotter (2008) chromatin condensation is indicative of varying stages of cell death while Shahinfar *et al*. (1991) state that margination of chromatin appears to be a fairly change that occurs in the nucleus after irreversible injury leading to cell death. In this study, the AFL group's degenerat-

ed mitochondria with few cristae were prominent. Inhibition of cell respiration may be a result of these mitochondrial changes, which were seen in the existing study and could be accredited to a change in the mitochondrial transport system (Brand and Nicholls, 2011). While AFL/CA treated group reduced AFL-induced ultrastructure changes.

CONCLUSION

Citric acid supplement added to contaminated diet with AFL has a protecting effect versus hepatotoxicity (structure and function) induced by AFL contaminated diet in quails by improving the liver function, inhibiting oxidative stress and enhance antioxidant activity.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdallah, M.F., Girgin, G., Baydar, T., 2015. Occurrence, prevention and limitation of mycotoxins in feeds. Animal Nutrition and Feed Technology 15, 471-490.

Abdel-Fattah, S.A., El-Sanhoury, M.H., El-Mednay, N., Abdel-Azeem, F., 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7, 215-222.

Abdelrazek, H., Yusuf, M.S., Hassan, M.A., Soliman, M.T.T., El Nabtiti, A.A., 2015. Ameliorative Effect of Oregano Essential Oil on Mycotoxins-Induced Immune Impairments in Growing Japanese Quail. Egyptian Academic Journal of Biological Sciences C, Physiology and Molecular Biology 7, 101-114.

Awuchi, C.G., Ondari, E.N., Eseoghene, I.J., Twinomuhwezi, H., Amagwula, I.O., Morya, S., 2021. Fungal growth and mycotoxins production: Types, toxicities, control strategies, and detoxification. In: Fungal reproduction and growth. Published by Intechopen. DOI: 10.5772/intechopen.100207.

Bailey, C., Latimer, G., Barr, A., Wigle, W., Haq, A., Balthrop, J., Kubena, LF., 2006. Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. Journal of Applied Poultry Research 15, 198-206

Barati, M., Chamani, M., Mousavi, S.N., Hoseini, S.A., Taj Abadi Ebrahimi, M., 2018. Effects of biological and mineral compounds in aflatoxin-contaminated diets on blood parameters and immune response of broiler chickens. Journal of Applied Animal Research 46, 707-713.

Brand, M.D., Nicholls, D.G., 2011. Assessing mitochondrial dysfunction in cells. Biochemical Journal 435, 297-312.

Chen, X., 2016. Impact of dietary modification on aflatoxicosis in poultry. PhD. Dissertation, Purdue University, United States.

Čolović, R., Puvača, N., Cheli, F., Avantaggiato, G., Greco, D., Đuragić, O., Kos, J., and Pinotti, L., 2019. Decontamination of mycotoxin-contaminated feedstuffs and compound feed. Toxins 11, 617.

Denli, M., Blandon, J.C., Guynot, M.E., Salado, S., Perez, J.F., 2009. Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B(1). Poultry Science 88, 1444-1451.

Diaz, G.J., Murcia, H.W., 2011. Biotransformation of aflatoxin B1 and its relationship with the differential toxicological response to aflatoxin in commercial poultry species. Aflatoxins-Biochemistry and Molecular Biology 1, 3-20.

Doonan, F., Cotter, T.G., 2008. Morphological assessment of apoptosis. Methods 44, 200-204.

Edenberg, H.J., 2007. The genetics of alcohol metabolism. Alcohol Research 30, 5.

Eftekhari, A., Ahmadian, E., Panahi-Azar, V., Hosseini, H., Tabibiazar, M.,-Maleki Dizaj, S., 2018. Hepatoprotective and free radical scavenging actions of quercetin nanoparticles on aflatoxin B1-induced liver damage: in vitro/in vivo studies. Artificial cells, nanomedicine, and biotechnology 46, 411-420.

Eissa, O.S., 2004. Protective Effect of Vitamin C and Glutathione Against the Histopathological Changes Induced by Imidacloprid in the Liver and Testis of Japanese Quail. The Egyptian Journal of Hospital Medicine 16, 39-54.

El-Agamy, D.S., 2010. Comparative effects of curcumin and resveratrol on aflatoxin B 1-induced liver injury in rats. Archives of toxicology 84, 389-396.

El Hak, H.N.G., Metawea, S.I., Nabil, Z.I., 2022. Fenugreek (*Trigonella foenum graecum* L.) supplementation safeguards male mice from aflatoxin B1-induced liver and kidney damage. Comparative Clinical Pathology 31, 925-942.

Elbasuni, S.S., Ibrahim, S.S., Elsabagh, R., Nada, M.O., Elshemy, M.A., Ismail, A.K., Mansour, H.M., Ghamry, H.I., Ibrahim, S.F., Alsaati, I., 2022. The Preferential Therapeutic Potential of Chlorella vulgaris against Aflatoxin-Induced Hepatic Injury in Quail. Toxins 14, 843.

Emam, H., Ahmed, E., Abdel-Daim, M., 2018. Antioxidant capacity of omega-3-fatty acids and vitamin E against imidacloprid-induced hepatotoxicity in Japanese quails. Environmental Science and Pollution Research 25, 11694-11702.

Ferramosca, A., Zara, V., 2014. Modulation of hepatic steatosis by dietary fatty acids. World Journal of Gastroenterology: WJG 20, 1746.

Fikry, A.M., Attia, A.I., Ismail, I.E., Alagawany, M., Reda, F.M., 2021. Dietary citric acid enhances growth performance, nutrient digestibility, intestinal microbiota, antioxidant status, and immunity of Japanese quails. Poultry Science 100, 101326.

Fu, D.A., Campbell-Thompson, M., 201). Periodic acid-Schiff staining with diastase. Methods Mol. Biol. 1639, 145-149.

Harvey, R., Kubena, L., Elissalde, M., Phillips, T., 1993. Efficacy of zeolitic ore compounds on the toxicity of aflatoxin to growing broiler chickens. Avian Diseases 37, 67-73.

Hassan, A.A., Hafsa, S.H.A., Elghandour, M.M., Reddy, P.R.K., Monroy, J.C., Salem, A.Z., 2019. Dietary Supplementation with sodium bentonite and coumarin alleviates the toxicity of aflatoxin B1 in rabbits. Toxicon 171, 35-42.

Jalili, M., Jinap, S., Son, R., 2011. The effect of chemical treatment on reduction of aflatoxins and ochratoxin A in black and white pepper during washing. Food Additives and Contaminants Part A, Chemistry, Analysis, Control, Exposure and Risk assessment 28, 485-493.

Kepekçi, R.A., Polat, S., Çelik, A., Bayat, N., Saygideger, S.D., 2013. Protective effect of Spirulina platensis enriched in phenolic compounds against hepatotoxicity induced by CCl4. Food Chemistry 141, 1972-1979.

Khaleghipour, B., Khosravinia, H., Toghiyani, M., Azarfar, A., 2020. Efficacy of silymarin-nanohydrogle complex in attenuation of aflatoxins toxicity in Japanese quails. Italian Journal of Animal Science 19, 351-359.

Kingsley, G.R., 1939. The determination of serum total protein, albumin, and globulin by the biuret reaction. Journal of Biological Chemistry 131, 197-200.

Kubena, L.F., Harvey, R.B., Huff, W.E., Elissalde, M.H., Yersin, A.G., Phillips, T.D., and Rottinghaus, G.E., 1993. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. Poultry Science 72, 51-59.

Liu, L., Xie, M., Wei, D., 2022. Biological detoxification of mycotoxins: Current status and future advances. Int. J. Mol. Sci. 23, 1064.

Liu, Y., Wang, W., 2016. Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis and involves Nrf2 signal pathway in primary broiler hepatocytes. Animal science journal = Nihon chikusan Gakkaiho 87, 1490-1500.

Mahrose, K.M., Michalak, I., Farghly, M., Elokil, A., Zhang, R., Ayaşan, T., Mekawy, A., Fazlani, S., 2021. Role of clay in detoxification of aflatoxin B 1 in growing Japanese quail with reference to gender. Veterinary Research Communications 45, 363-371.

Mates, J., 2000. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153, 83-104.

Melaku, M., Zhong, R., Han, H., Wan, F., Yi, B., Zhang, H., 2021. Butyric and citric acids and their salts in poultry nutrition: Effects on gut health and intestinal microbiota. Int. J. Mol. Sci. 22, 10392.

Méndez-Albores, A., Arámbula-Villa, G., Loarca-Piña, M.G., Castaño-Tostado, E., and Moreno-Martínez, E., 2005. Safety and efficacy evaluation of aqueous citric acid to degrade B-aflatoxins in maize. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 43, 233-238.

Méndez-Albores, A., Del Río-García, J., Moreno-Martinez, E., 2007. Decontamination of aflatoxin duckling feed with aqueous citric acid treatment. Animal Feed Science and Technology 135, 249-262.

Miazzo, R., Peralta, M.F., Magnoli, C., Salvano, M., Ferrero, S., Chiacchiera, S.M., Carvalho, E.C., Rosa, C.A., Dalcero, A., 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poultry Science 84, 1-8.

Mishra, S.K., Swain, B.K., 2022. Aflatoxin Occurrence, Detection, and Novel Strategies to Reduce Toxicity in Poultry Species. In: Aflatoxins-Occurrence, Detection and Novel Detoxification Strategies. IntechOpen, DOI: 10.5772/intechopen.107438.

Moretti, A., Logrieco, A.F., Susca, A., 2017. Mycotoxins: An underhand food problem. Methods Mol. Biol. 1542, 3-12.

Nazar, F.N., Magnoli, A.P., Dalcero, A.M., Marin, R.H., 2012. Effect of feed contamination with aflatoxin B1 and administration of exogenous corticosterone on Japanese quail biochemical and immunological parameters. Poultry Science 91, 47-54.

Pandey, A.V., Flück, C.E., 2013. NADPH P450 oxidoreductase: structure, function, and pathology of diseases. Pharmacology & therapeutics 138, 229-254.

Pasha, T., Farooq, M., Khattak, F., Jabbar, M., Khan, A.J., 2007. Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. Animal Feed Science and Technology 132, 103-110.

Pereira, C., Cunha, S.C., Fernandes, J.O., 2022. Mycotoxins of concern in children and infant cereal food at European level: Incidence and bioaccessibility. Toxins 14, 488.

Priesterjahn, E.-M., Geisen, R., Schmidt-Heydt, M., 2020. Influence of light and water activity on growth and mycotoxin formation of selected isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. Microorganisms 8, 2000.

Rasane, P., Jha, A., Sabikhi, L., Kumar, A., Unnikrishnan, V., 2015. Nutritional advantages of oats and opportunities for its processing as value added foods-a review. Journal of Food Science and Technology 52, 662-675.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology 28, 56-63.

Rodkey, F.L., 1965. Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry 11, 478-487.

Ruby, D.S., Masood, A., Fatmi, A., 2014. Effect of Aflatoxin Contaminated Feed on Energy Reservesof Fish Labeo Rohita (Hamilton). Current World Environment 9, 1037.

Safara, M., Zaini, F., Hashemi, S.J., Mahmoudi, M., Khosravi, A.R., Shojai-Aliabadi, F., 2010. Aflatoxin detoxification in rice using citric acid. Iranian journal of public health 39, 24.

Santos Pereira, C., C. Cunha, S., Fernandes, J.O. 2019. Prevalent mycotoxins in animal feed: Occurrence and analytical methods. Toxins 11, 290.

Shahinfar, S., Edward, D.P., Tso, M.O., 1991. A pathologic study of photoreceptor cell death in retinal photic injury. Current Eye Research 10, 47-59.

Shotwell, O.L., Hesseltine, C., Stubblefield, R., Sorenson, W., 1966. Production of aflatoxin on rice. Applied microbiology 14, 425-428.

Skovorodin, E., Bronnikova, G., Bazekin, G., Dyudbin, O., Khokhlov, R., 2019. Antioxidant influence on poultry liver morphology and hepatocyte ultrastructure. Veterinary World 12, 1716.

Slaoui, M., Fiette, L., 2011. Histopathology procedures: from tissue sampling to histopathological evaluation. Drug Safety Evaluation. Methods Mol. Biol. 691, 69-82

Tanpong, S., Cherdthong, A., Tengjaroenkul, B., Reungsang, A., Sutthibak, N., Wongtangtintharn, S., 2021. A study on citric acid by-product as an energy source for Japanese quail. Tropical Animal Health and Production 53, 1-9.

Winter, G., Pereg, L., 2019. A review on the relation between soil and mycotoxins: Effect of aflatoxin on field, food and finance. European Journal of Soil Science 70, 882-897.