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Hepatoprotective effect of copper chitosan nanocomposites on Aflatoxin B1 contaminated feed in broiler chickens

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ABSTRACT

Aflatoxicosis is still a significant issue in poultry rearing due to immunity reduction, injuries to various tissues, degradation of the quality of carcasses, and increased vulnerability to different infective agents. So, the existing research was assessed to evaluate the effect of copper chitosan nanocomposites (Cu-Chit/NCs) on aflatoxin B1 (AFB1) contaminated feed in broiler chicken on hepatic function, antioxidant status, and hepatic tissues. A total of 60 one-day-old broiler chickens were assigned into six equal groups: group 1 was kept as control, group 2 was fed AFB1 (1 mg/kg basal diet), and groups 3 and 4 were fed AFB1 together with Cu-Chit/NCs at low and high doses, respectively. While groups 5 and 6 (protective groups) were fed Cu-Chit/NCs at low and high doses, respectively, in the 1st week, then AFB1 together with Cu-Chit/NCs at low and high doses, respectively, for the last 3 weeks. The results revealed that there was liver injury in group 2 that was fed AFB1 indicated by significant increase in serum AST, ALT and significant increases in malondialdehyde (MDA) and decrease in the activities of reduced glutathione (GSH) and glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase(CAT), and total antioxidant(TAO). Nevertheless, dietary supplementation of Cu-Chit/NCs alleviated the adverse effect of AFB1 and improved the hepatic functions in broiler chicken' specially in prophylactic groups through their antioxidant properties.

1. INTRODUCTION

Mycotoxins are harmful secondary metabolites produced from certain *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* fungi (Umereweneza *et al*., 2017). These mycotoxins could contaminate agricultural products at several points in the food supply chain (Areo *et al*., 2023). They are known by many different names, including "hidden killers," "natural toxicants," and "inevitable contaminants.". They are regarded as one of the most important immunosuppressive toxins, increasing susceptibility to illnesses and reducing animal productivity. Additionally, they are chemically and thermally stable toxins that are able to withstand different feed processing operations (Pietsch *et al.*, 2013). Exposure to mycotoxins represents one of the major negative impacts that appear in poultry farming, which results in weak performance and reduced quality of the meat and eggs, which has negative economic effects (Rawal *et al*., 2010).

Aspergillus flavus, *A. parasiticus*, and *A. nomius* are the three most significant and well-known species of Aspergillus flavus that produce aflatoxin. Aflatoxin contaminates many crops (groundnuts, pistachio nuts, dried figs, hazelnuts, spices, almonds, rice, melon seeds, Brazil nuts, and maize) (Pickova *et al*., 2021).

Four major Aflatoxins (AFs) have been identified: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2). AFG1 and AFG2 are produced exclusively by *A. parasiticus*, while AFB1 and AFB2 are produced by both *A. flavus* and *A. parasiticus* (Muaz *et al.*, 2022). AFB1 represents the most important mycotoxin that received more attention among all mycotoxins, categorized as a category 1 human carcinogen by the International Agency for Research on Cancer (IARC) (Jakši *et al.*, 2019). Exposure of poultry to AFB1 leads to lower feed consumption, growth complications, an impaired immune system, organ damage, disruption of the gut microbiota's balance, as well as raising death rates in the chicken (Rashidi *et al.*, 2020). Many studies have realized the level of aflatoxins (AFs) in chicken feeds to be with an average from 64% to 100% (Aboagye *et al.*, 2021). Being lipolytic in nature, aflatoxins can be efficiently absorbed by the cell membrane of the respiratory and digestive systems and passed into the circulation, where they are transported to different tissues and to the liver. AFB1 needs to undergo bioactivation in hepatocytes to be toxic (Sarma *et al.*, 2017). AFs are converted into reactive epoxide intermediates via cytochrome P450 enzymes (CYPs) in the liver that are widely distributed in the liver as well as intestinal and respiratory tissues (Yilmaz *et al*., 2017). Additionally, AFB1 is transformed into other intermediates such as AFL, AFB2a, AFQ1, and AFM1 (Dai *et al*., 2022).

There's a global curiosity about the use of nanotechnology across various biomedical domains. Nanoparticles are used in many medication delivery methods, and a range of compounds are used as stimulants or enhancers to increase

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the safety, stability, and effectiveness of treatments (Stapleton and Nurkiewicz, 2014). Metal nanoparticles (NPs) have a considerable role as antibacterial agents; they have a higher surface area over their volume ratio that permits them to become successful at low concentrations (Sundaresan *et al.,* 2012). Copper oxide (CuO) nanoparticles have advantageous properties of copper, such as its thermal and electrical conductivity, which can be reached at a significantly minor cost compared to noble metals like silver and gold (Han *et al.,* 2006). Furthermore, the combination of metal nanoparticles with biopolymers can lead to the development of innovative nanocomposites for biological applications (Soto-Mendoza *et al.,* 2023). Chitosan, a versatile biopolymer composed of linear polysaccharides containing reactive amino groups derived from N-deacetylated chitin, is notably biocompatible and cost-effective in contrast to alternative biopolymers (Veisi *et al.,* 2018). This has prompted the use of chitosan composites and biomolecules made of natural polysaccharides for in-situ synthesis and stabilization of copper nanoparticles (Xu *et al.,* 2022). The incorporation of this two-shell-core configuration enhances the steadiness of nanoparticles of copper against unintended oxidation and self-aggregation (Xu *et al.,* 2022). Combination of copper nanoparticles with chitosan matrices significantly enhances their capacity to destroy both fungi and bacteria (Arjunan *et al.,* 2017). Consequently, the aim of the current study is to determine the ameliorative effect of Cu-Chitosan nanocomposite addition on AFB1-contaminated diets on liver function, oxidative markers, and histopathological changes in the liver of broiler chickens.

2. MATERIAL AND METHODS

Ethical approval:
This study was conducted in compliance with the rules set by the Public and Ethics Health Committee in Egypt. The scientific research committee, Faculty of Veterinary Medicine, Benha University, Cairo, Egypt, approved the use of animals and the associated protocols with an ethical approval number (BUFVTM 09-10-23).

2.1. Preparation of aflatoxin:
Feed samples were obtained from poultry farms suffering from some problems such as growth retardation, anorexia, weakness, ruffled feather and change in fecal consistency. Feed samples were collected in sterile plastic bags and transferred to the laboratory for analysis. The preparation and mycological examination of the feed samples Carried out according to the protocol recommended by Dalcero et al. (1998). Meanwhile, the detection of the produced fungi was described as mentioned by Pitt and Hocking (2009). Then, the Aflatoxigenic *Aspergillus flavus* strains obtained from livestock feed at the Laboratory of Mycology were utilized to produce aflatoxin on yellow corn that previously examined to assure absence of aflatoxin contamination (Smith, 1997). Finally, detection of aflatoxins using chromatography study on thin layer plates (AOAC, 2000). The obtained aflatoxins were stored at 40 °C prior to the experiment.

2.2. Production and characterization of copper chitosan nanocomposites
Copper nanoparticles with a size of 50 nm were synthesized at the Biochemistry, Toxicology, and Feed Deficiency Department of the Animal Health Research Institute, Cairo, Egypt. The characterization of these nanoparticles was conducted at the Central Laboratory of Elemental and Isotopic Analysis at the Nuclear Research Centre, Egypt. Preparation of chitosan-copper nanocomposites was performed according to Du *et al*., 2009.

2.3. Experimental animals
A total of 60 one-day-old broiler chickens were purchased from El-WATANIA Company and housed in clean metal cages under optimal environmental conditions, including appropriate temperature, humidity, and lighting. They were supported with a stabilized diet and an adequate supply of water. Then they were left to acclimatize to their new environment for a week before the startup of the trial. On the 7th day of the experiment, all chickens were vaccinated against Newcastle Disease (ND) and Infectious Bronchitis Virus (IBV) using the Combivac C vaccine via the ocular route.

2.4. Experimental design
Six equal groups of ten chicks each were randomly assigned to the experimental broilers. Group 1 served as a control and was fed a standard balanced diet free from mycotoxins. Group 2 received only AFB1 at a dose of 1 mg/kg basal diet/day (Kaoud, 2013). Group 3 was fed AFB1 (1 mg/kg diet/day) + Cu-Chit/NCs (50 mg/kg basal diet) for 28 days (Wang *et al*., 2011). Group 4 fed AFB1 (1 mg/kg diet/day) + Cu-Chit/NCs (100 mg/kg basal diet) for 28 days. Group 5 was given Cu-Chit/NCs (50 mg/kg basal diet) for a week, then AFB1 (1 mg/kg diet/day) + Cu-Chit/NCs (50 mg/kg basal diet) for the last three weeks. Group 6 was fed Cu-Chit/NCs (100 mg/kg basal diet) for a week, then AFB1 (1 mg/kg diet/day) + Cu-Chit/NCs (100 mg/kg main diet) for the last three weeks. All groups were monitored daily for 28 days.

2.5. Sampling
At the end of the experiment, blood samples were taken from the wing vein "brachial" of each chicken. The clotted blood samples were centrifuged at 3000 rpm for 15 minutes, and serum was then stored at -20°C for biochemical and oxidative markers.

2.6. Serum biochemical studies
ALT and AST were measured utilizing the technique outlined by Reitman and Frankel (1957).

2.7. Antioxidants and peroxidation biomarkers
The plasma levels of oxidative biomarkers were assessed, including malondialdehyde (MDA) as per the scheme established by Ohkawa et al. (1979), total antioxidant capacity (TAC) according to Koracevic et al. (2001), glutathione peroxidase (GPX) tracking the protocol of Paglia and Valentine (1967), superoxide dismutase (SOD) as described by Nishikimi et al. (1972), catalase (CAT) according to Aebi (1974), and reduced glutathione (GSH) based on the method of Ellman (1959).

2.8. Histopathological examination
Liver tissue specimens were collected from different groups and preserved in 10% formalin. Following proper fixation, the samples were dehydrated through a series of ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin. Thin sections of 5 µm were prepared and stained with hematoxylin and eosin according to Banchroft *et al.* (1996).

2.9. Statistical analysis:

The data collected from the various experimental groups were implemented using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test employing SPSS software. Data were expressed as the mean ± standard error (SE). The data were statistically significant at P values <0.05.

3. RESULTS

3.1 Cu-Chit/NCs were produced and identified and the resulting nanocomposites were spherical in shape and 50 nm in size (Figure 1). However, the UV-VIS showed that the highest absorption took place at a wavelength of 500–600 nm.

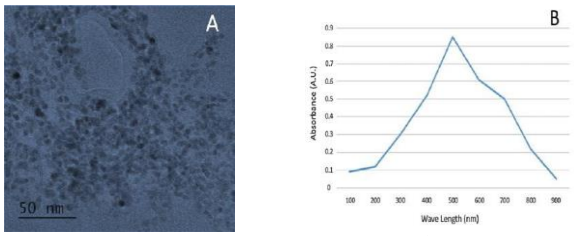

Figure 1.A The morphological characters of copper-chitosan nanocomposites Cu-Chit/NCs under transmission electron microscopy (50 nm in size. B. UV–visible spectrophotometry of Cu-Chit/NCs (the peak was at a range of 500–600 nm,

3.2. liver function tests

The consequences of aflatoxin B1 and Cu-Chit/NCs on liver functions were assessed and exist in figure (2). There was a remarkable elevation in AST and ALT enzymes activity (77.60 ± 3.14 and 43.00 ± 2.7) respectively, within the group2 compared with that detected in the control group1 (31.60 ± 1.07 and 12.2 ± .86). Whereas AST and ALT were considerably decreased in all groups treated with Cu-Chit/NCs (G3, G4, G5, G6) contrasted with AFB1 group 2. In addition, there was a meaningful decrease in AST and ALT in prophylactic groups (G5 and G6) than therapeutic (G3 and G4) in which the prophylactic groups (G5 and G6) showing a clearly decline in AST (41.5 ± 2.04 and 34.00 ± 1.75) respectively, than therapeutic groups (G3 and G4) that recorded (53.20 ± 2.26 and 45.00 ± 2.12) and for ALT the prophylactic groups (G5 and G6) showing marked decrease recorded as (20.7 ± 3.01 and 15.67 ± .91) respectively, compared with that detected in the therapeutic G4 (27.8 ± 2.7).

3.3. Changes in oxidative markers

The effect of AFB1 induced toxicity and treatment with Cu-Chit/NCs on serum antioxidant parameters were illustrated in figure (3)

There was a notably rise in the concentration of MDA (3.477±0.30) in AFB1 group (G2) when compared with control group (G1) (1.907±0.17). While a considerable decrease in values of CAT, GSH, SOD and GPX (0.860±0.170, 2.32±0.17, 29.82±4.37 and 3.910±0.116 respectively) in group (G2) contrasted with the control group (G1) (2.007±0.144, 4.99±0.34, 48.56±4.37 and 7.330±1.053 respectively). Where, the levels of MDA were meaningful fall in all groups handled with Cu-Chit/NCs (G3, G4, G5 and G6) especially groups (G5 and G6) which displayed more improvement in MDA level (2.067±0.22 and 1.787±0.15 respectively) when contrasted to group 2. However, the addition of Cu-Chit/NCs improves the oxidation inhibitor activities in serum by increasing CAT, GSH, SOD and GPX which is more eminent in prophylactic groups (G5 and G6) that demonstrated more increase in serum antioxidant activity. moreover, there was a meaningful decrease in TAO level on aflatoxicated group(G2) (0.89±0.09) when compared with control group1 (2.53±0.12) whereas, there was a significant increase in TAO in all Cu-Chit/NCs treated groups (G3, G4, G5 and G6) especially prophylactic groups (G5 and G6) (2.12±0.12 and 2.29±0.19) more than therapeutic groups (G3 and G4) (1.36±0.07 and 1.73±0.09).

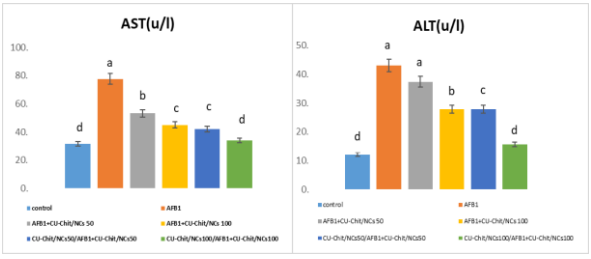

Figure (2): Changes in serum ALT and AST activities of broiler chicken after AFB1 exposure and treatment with Cu-Chit/NCs

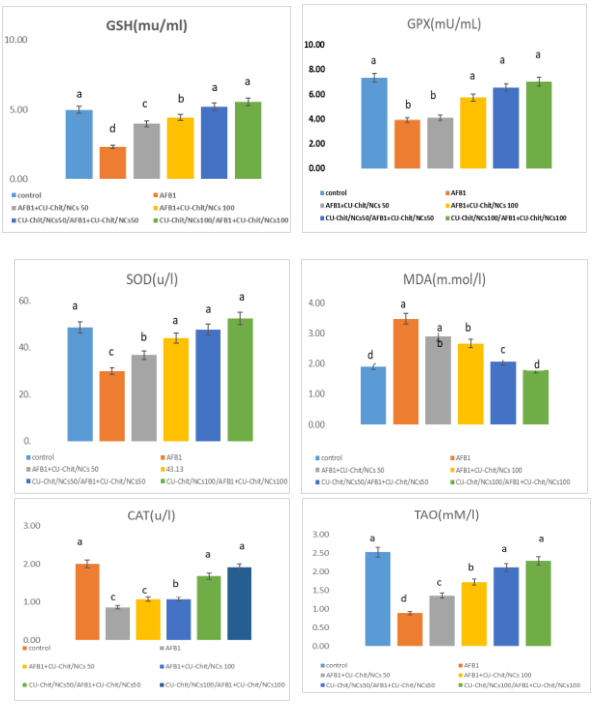

Figure (3): Effect of AFB1 toxicity and treatment with Cu-Chit/NCs on serum antioxidant parameters

3.4. Histological finding

The light microscopy of the control liver showed intact liver parenchyma comprising hepatocytes in a good arrangement along with blood vessels (Figure 4A). Contrary to liver of aflatoxin group showed severe hepatocytes damage characterized by severe hepatic necrosis and atrophy, dilatation with engorgement of the blood vessels, perivascular inflammation mainly with leucocytes and interstitial leucocytes infiltration (Figure 4B). AFB1+Cu/Chit 50 revealed moderate histological lesions inform of focal necrosis, congestion of the central vein and minimal perivascular infiltration (Figure 4C). Histological lesions in AFB1+Cu/Chit 100 was exclusive in congestion of the blood vessels (Figure 4D). However, Chit/NCs50/ AFB1+ Cu-Chit/NCs50 and Cu-Chit/NCs100/ AFB1+ Cu-Chit/NCs100 groups displayed normal liver structures distinguished by normal hepatocytes simultaneously with normal central vein and blood sinusoids (Figure 4E, F).

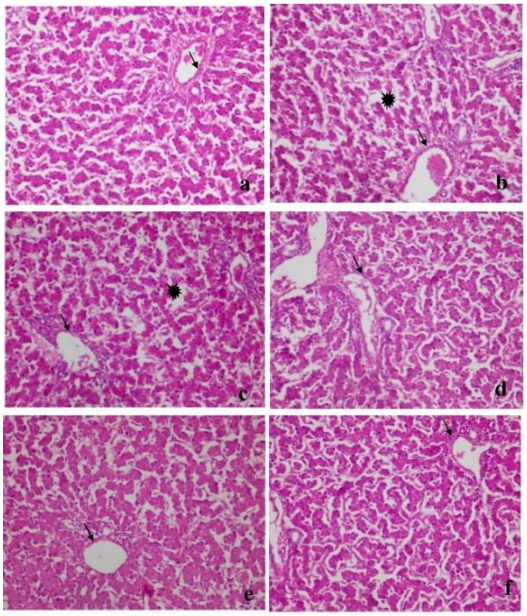

Fig.4-1: H&E-stained liver sections of control (A), AFB1 (B), AFB1+Cu/Chit 50 (C), AFB1+Cu/Chit 100 (D), Cu-Chit/NCs50/ AFB1- Cu-Chit/NCs50 (E), Cu-Chit/NCs100/ AFB1- Cu-Chit/NCs100 (F). A) liver showing intact hepatic cells and blood vessel (arrow). B) liver showing marked congested blood vessel (arrow), besides extensive hepatic necrosis and atrophy (asterisk). C) liver showing congested vein with minimal perivascular infiltration (arrow), focal areas of hepatocellular necrosis (asterisk). D) liver showing dilatation of the portal vein (arrow). E) liver showing normal liver parenchyma with normal portal vein (arrow). F) liver showing intact vasculature (arrow).

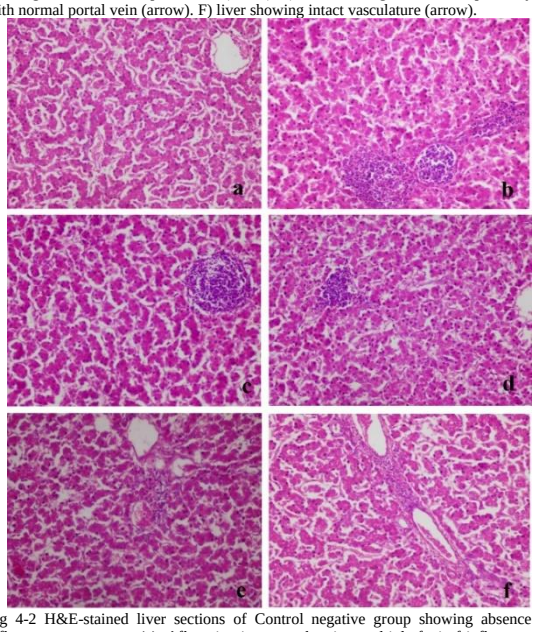

Fig 4-2 H&E-stained liver sections of Control negative group showing absence of inflammatory response (a), Aflatoxicosis group showing multiple foci of inflammatory cells infiltration (b), AFB1- Cu-Chit/NCs50 group showing large foci of inflammatory cells infiltration (c), AFB1- Cu-Chit/NCs100 group showing moderate foci of inflammatory cells infiltration (d). Cu-Chit/NCs50/ AFB1- Cu-Chit/NCs50 group showing focal aggregation of few inflammatory cells (e), Cu-Chit/NCs100/ AFB1- Cu-Chit/NCs100 group showing aggregation of inflammatory cells in the portal area (f) H&E x400

4. DISCUSSION

Aflatoxin B1 is frequently detected in human and animal feed throughout the world, so there is growing concern about the impact of aflatoxins, which are prevalent, on human and animal health (Kovalsky *et al.*, 2016). It will be compulsory to use nutritive additives to counter the adverse effect of such toxins. So, the present research was done to establish the consequences of Cu-Chit/NCs addition to AFB1-contaminated diets on liver function and histopathological alteration as well as the serum antioxidant enzymes.

Progressive new developments in nanotechnology have improved animal production and health. In addition to its numerous uses as food safety additives, disease detection, diagnosis, and treatment, several nanomaterials are being employed as nano antifungals. According to Alghuthaymi et al. (2021), the important therapeutic and preventative properties of nano-antifungals, especially copper nanoparticles, have been assessed against a range of fungal infections and mycotoxicosis in animals.

The current study involved the chemical synthesis and characterization of Cu-Chit/NCs. Scanning electron microscopy revealed that the Cu-Chit/NCs measured 50 nm in size. However, the wavelength range of Cu-Chit/NCs' UV-VIS absorption spectra was 500–600 nm. These results in Figure 1 are similar to the finding of Vanti *et al*. (2020). Currently, the prepared Cu-Chit/NCs are used for ameliorating the hepatotoxicity induced by AFB1 in broilers. After the end of experimental periods, several biochemical parameters were monitored on all treated broilers.

The experimental broiler chicken exposed to AFB1 changed the redox balance in their liver. Redox imbalance is known as the inequality between antioxidants and oxidants, leading to the disturbance of redox signaling and resulting in molecular damage (Pizzino *et al.*, 2017). Several studies have identified redox imbalance as a key pathological mechanism underlying AFB1-caused tissue injury, contributing to both oxidative and inflammatory damage (Zhao *et al.*, 2017). This condition can lead to an increased production of various reactive oxygen species (ROS), like superoxide anions and hydroxyl free radicals, in both humans and animals. ROS can lead to proteins and nucleic acid alterations, generate significant amounts of MDA, which is a final product of lipid peroxidation whose levels reflect the extent of lipid peroxidation inside the body, and induce tissue damage (Bai *et al.*, 2017).

MDA is a byproduct of lipid peroxidation, which has the capacity to form erratic binding with other components, like nucleic acid and proteins. Like this claim, the results of the current investigation showed that MDA concentrations had significantly increased in AFB1 intoxicated chicken, leading to cellular disruption through necrosis or a change of membrane permeability resulting in leaking of liver enzymes into the blood, thus increasing levels of serum AST and ALT (Sang *et al.*, 2023). The present findings were confirmed by histopathological examination which revealed significant hepatocyte injury, as evidenced by profound hepatic necrosis and atrophy, blood vessel engorgement and dilatation, perivascular inflammation mainly involving leucocytes, and interstitial leucocyte infiltration. These results came in sync with the finding of Ashry *et al.* (2022). Conversely, the supplementation of Cu-Chit/NCs to the AF intoxicated diet ameliorates the deteriorating effects of AFB1 on liver enzymes in broiler chickens, with more pronounced improvements observed in the prophylactic groups (groups 5 and 6) than in the therapeutic groups (groups 3 and 4). This may be due to Cu-Chit/NCs effectively counteracting AFB1 by inhibiting its detrimental impact on liver function and may be attributed to the reducing properties of copper nanoparticles, which help in reducing oxidative stress through scavenging free radicals and ROS, which contribute to cellular damage. Antioxidant activity of copper nanoparticles has an essential role in guarding liver cells from the toxic effects of AFs (Kirrella *et al.*, 2023). Additionally, Chitosan has a potential nutritional strategy to maintain liver function in broilers, as stated by EL-Habashy et al. (2024), who found that liver enzymes significantly decreased when Chitosan

was fed with food contaminated with AF, and Lan et al. (2023) also reported that dietary Chitosan supplementation has an ameliorative effect on restoring the hepatic enzymes to almost normal levels in broiler chickens.

Moreover, in the current study, AFB1 was found to increase MDA levels along with decreasing the activities of GSH and GPx, SOD, CAT, and TAC. These findings align with previous studies that demonstrated AFB1's ability to induce oxidative stress in broilers (Nabi *et al.,* 2022). Conversely, there was a significant reduction in serum MDA levels in chicken fed AFB1 contaminated diets supplemented with Cu-Chit/NCs at various dosages, suggesting that Cu-Chit/NCs exhibit notable antioxidant activity in birds. The advantageous outcomes were notably found in prophylactic groups (groups 5and 6). These findings were consistent with earlier studies demonstrating that chitosan and its derivatives possess a strong capacity to scavenge free radicals, including hydroxyl radicals and superoxide anions (Sun *et al.,* 2007). Additionally, Farivar *et al*. (2018) reported that the addition of various doses of chitosan in the diets of laying hens caused a restoration in plasma antioxidant capacity. Moreover, the decline in MDA levels noted in birds fed diets supplemented with Cu-Chit/NCs aligns with Abdullah *et al.* (2022), who reported that birds treated with copper nanoparticles exhibited increased SOD serum activity and decreased MDA levels, indicating a reduction in oxidative stress among the nanoparticle-administered groups. Similarly, Kumar *et al.* (2013) found that plasma MDA levels in broiler chickens decreased when their diets were supplemented with copper sulfate pentahydrate. Furthermore, Kato *et al*. (2007) noted that supplementation of copper, whether in organic or inorganic forms, led to a decrease in activity of MDA, which serves as a guide to cell membrane injury caused by free radical-induced oxidative stress. Also, there was an increase in serum TAC, indicating more response against free radicals, as higher TAC levels reflect the total antioxidant components present (Rubio *et al.,* 2016). There was also an increase in GSH levels, which plays a crucial antioxidant role by engaging directly with ROS or serving as a cofactor for peroxidase enzymes (Cooper *et al.,* 2011).

5. CONCLUSIONS

This study concluded that CuChit/NCs effectively alleviate the adverse effect of AFB1 on the liver of broiler chickens through their antioxidant characteristics by repairing the altered hepatic enzyme and oxidative imbalance.

6. REFERENCES

1. Abdullah, S. S., Masood, S., Zaneb, H., Rabbani, I., Akbar, J., Kuthu, Z. H., Masood, A., Dhakal, R., & Pérez, E. V. B., 2022. Effects of Copper nanoparticle supplementation on growth, intestinal morphology and antioxidant status in broilers exposed to cyclic cold stress. Emirates Journal of Food and Agriculture, 34,*5, 436-445.*
2. Aboagye-Nuamah, F. , Kwoseh, C.K., Maier, D.E., 2021. Toxigenic mycoflora, aflatoxin and fumonisin contamination of poultry feeds in Ghana. Toxicon, 198, 164–170.
3. Aebi, H., 1984. Catalase in vitro. In: Methods in Enzymology, 105, 121-126
4. Alghuthaymi MA, Hassan AA, Kalia A, Sayed El Ahl RMH, El Hamaky AAM, Oleksak P, Kuca K, Abd-Elsalam KA., 2021. Antifungal Nano-Therapy in Veterinary Medicine: Current Status and Future Prospects. J Fungi (Basel). 7,7:494. doi: 10.3390/jof7070494.
5. AOAC, 2000. Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.
6. Areo, O. M., Abafe, O. A., Gbashi, S., Njobeh, P. B., 2023. Detection of multi- mycotoxins in rooibos and other consumed teas in South Africa by a modified QuEChERS method and ultra-high-performance liquid chromatography tandem mass spectrometry. Food Control, 143, Article 109255.
7. Arjunan N, Singaravelu CM, Kulanthaivel J, Kandasamy J., 2017. A potential photocatalytic, antimicrobial and anticancer activity of chitosan-copper nanocomposite. Int. J. Biol. Macromol. ,104 (Pt B), 1774-1782.
8. Ashry A, Taha NM, Lebda MA, Abdo W, El-Diasty EM, Fadl SE, Morsi Elkamshishi M. 2022. Ameliorative effect of nanocurcumin and Saccharomyces cell wall alone and in combination against aflatoxicosis in broilers. BMC Vet Res. 18,1, 178. doi: 10.1186/s12917-022-03256-x.
9. Bai, K., Huang, Q., Zhang, J., He, J., Zhang, L., Wang, T., 2017. Supplemental effects of probiotic Bacillus subtilis fmbj on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult. Sci. 96,1, 74–82.
10. Banchroft, D.J, Cook, C.H., Stirling, R.W., Turner, D.R., 1996. Manual of histopathological techniques and their diagnostic application. (rd.ed[3]) Chuchill Livingston, Edinburg.
11. Cooper, A. J. L., Pinto, J. T., Callery, P. S., 2011. Reversible and irreversible protein glutathionylation: Biological and clinical aspects. Med. Sci. Monit. 7.7, 891–910.
12. Dai C., Tian E., Hao Z., Tang S., Wang Z., Sharma G., Jiang H., Shen J., 2022. Aflatoxin B1 toxicity and protective effects of curcumin: Molecular mechanisms and clinical implications. Antioxidants.11:2031. doi: 10.3390/antiox11102031.
13. Dalcero, A., Magnolt, C., Luna, M., Ancast, G., Reynoso, M.M., Chiacchiera, S., Miazzo, R., Palacio, G., 1998. Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia ,141,1, 37–43.
14. Du WL, D., Niu SS, Xu YL, Xu ZR, Fan CL., 2009. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. Carbohydrate Polymers, 75,3, 385-389.
15. EL-Habashy A, El-Sherbini, E., Abd El Dayem G., El-Adl, M., 2024. The synergistic Effect of Both Mannan and Chitosan Oligosaccharides on Broilers Intoxicated with Aflatoxicosis Egypt. J. Vet. Sci., 55,3, 759-774.
16. Ellman, G. L. 1959. Tissue sulfhydryl groups. Archives of biochemistry and biophysics, 82,1, 70-77.
17. Farivar, A., Saber, N., Ahan, Z., Serbester, U., Celik, L., 2018. Effects of high degree deacetylated chitosan supplementation on yolk and blood immune status of laying hens. J. Anim. Sci., 96, 296–297.
18. Han WK, Choi JW, Hwang GH, Hong SJ, Lee JS, Kang, S.G.2006. Fabrication of Cu nano particles by direct electrochemical reduction from CuO nano particles. Appl Surface Sci 252: 2832-2838
19. Jakši´c, D., Kocsubé, S., Bencsik, O., Kecskeméti, A., Szekeres, A., Jeli´c, D. Kopjar, N., Vágvölgyi, C., Varga, J., Šegvi´c , Klari´c, M., 2019. Aflatoxin production and in vitro toxicity of Aspergilli section Flavi isolated from air samples collected from different environments. Mycotoxin Res. 35, 217–230.
20. Kato, J., Ruram, AA., Singh, SS., Devi, SB., Devi, TI., Singh, WG., 2007. Lipid peroxidation and antioxidant vitamins in urolithasis. Indian J Clin Biochem., 22, 1,128-130.
21. Kaoud, H., 2013. Innovative methods for the amelioration of aflatoxin (AFB1) effect in broiler chicken. Sci J App Res., 1 1, 16-21.
22. Kirrella, A., Al-Wakeel, R., El-Naggar, K., Mohamed, R., Abdo, S., El-Mehaseeb, I., El-Magd, M., El-Kassas, S., 2023. Copper Nanoparticles Improve Growth Performance and Modulate Biochemical and Lipid Profiles in Broilers Exposed to Chronic Heat Stress. Egyptian Journal of Veterinary Sciences. 54, 167-179.

23. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V., 2001. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 54,5,356-61.
24. Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T. 2016. Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished. Toxins. 8(363): 1-29
25. Kumar, P., Biswas,A. , Bharti,VK. , , Srivastava,RB. 2013. "Effects of dietary copper supplementation on performance and blood biochemical parameters in broiler chickens at cold desert region in India." J. Vet. Sci. Photon 114,166-172.
26. Lan R, Luo H, Wu F, Wang Y, Zhao Z., 2023.Chitosan Oligosaccharides Alleviate Heat-Stress-Induced Lipid Metabolism Disorders by Suppressing the Oxidative Stress and Inflammatory Response in the Liver of Broilers. Antioxidants (Basel).12, 8:1497. doi: 10.3390/antiox12081497.
27. Muaz, K., Riaz, M., Oliveira, C.A.F.D., Akhtar, S., Ali, S.W., Nadeem, H. ,Balasubramanian, B. ,2022. Aflatoxin M1 in milk and dairy products:Global occurrence and potential decontamination strategies. Toxin Re-views, 41,2, 588–605.
28. Nabi F, Tao W, Ye R, Li Z, Lu Q, Shang Y, Hu Y, Fang J, Bhutto ZA, Liu J. 2022. Penthorum Chinense Pursh Extract Alleviates Aflatoxin B1-Induced Liver Injury and Oxidative Stress Through Mitochondrial Pathways in Broilers. Front Vet Sci. 2;9: 822259. doi: 10.3389/fvets.2022.822259.
29. Nishikimi, M., Rao, N.A., Yagi, K.J.B., 1972. Communications br: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Comm., 46(2, 849-854.
30. Ohkawa, H., Ohishi, N. and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95,92,351-358.
31. Paglia, D.E., Valentine, W.N., 1967. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. Journal of Laboratory and Clinical Medicine, 70, 158-169.
32. Pietsch, C., Kersten, S., Burkhardt-Holm, P., Valenta, H., Danicke, S., 2013. "Occurrence of deoxynivalenol and zearalenone in commercial fish feed: an initial study," Toxins. 5,1, 184–192
33. Pitt, JJ., Hocking, AD., 2009. Fungi and Food Spoilage. 3rd Ed. Published by Spriiger Dordrech Heidelberg, London, New York.
34. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., 2017. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017. doi: 10.1155/2017/8416763. Epub 27.
35. Pickova, D., Ostry, V., Malir, F. 2021. A Recent Overview of Producers and Important Dietary Sources of Aflatoxins. Toxins , 13, 186. https://doi.org/10.3390/ toxins13030186
36. Rashidi, N., Khatibjoo, A., Taherpour, K., Akbari-Gharaei, M., Shirzadi, H., 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to Aflatoxin-B (1). Poult. Sci. 99,5896–5906.
37. Rawal, S., Kim, J. E., Coulombe, R., 2010. Aflatoxin B1 in poultry: toxicology, metabolism and prevention. Res Vet Sci.89,325–31.
38. Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol.,28,56-63.
39. Rubio, C. P., Hernández-Ruiz, J., Martinez-Subiela, S., Tvarijonaviciute, A. and Ceron, J. J. 2016. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: An update. BMC Vet. Res. 12, 166. DOI:10.1186/s12917-016-0792-7 Corpus ID: 4663395
40. Sang, R., Ge, B., Li, H., Zhou, H., Yan, K., Wang, W., Cui, Q., Zhang, X., 2023. Taraxasterol alleviates aflatoxin B1-induced liver damage in broiler chickens via regulation of oxidative stress, apoptosis and autophagy. Ecotoxicology and Environmental Safety, 251, 114546. doi: 10.1016/j.ecoenv.2023.114546.
41. Sarma, UP., Bhetaria, PJ., Devi, P., Varma, A., 2017. Aflatoxins: Implications on Health. Indian J Clin Biochem. 32, 2,124-133.
42. Smith, JE., 1997. Aflatoxins. In Handbook plant and fungal toxicant. Edited by J.P. Fle-xinx, D'Mello, CR (Press, Boca Raton, New York. pp. 269-285.
43. Soto-Mendoza, A., Larrañaga-Tapia, M., Robles, E., Martínez Sanmiguel, J., Truong, L., Medina, D., Cholula-Díaz, J., Mostafavi, E. (2023). Natural biopolymers combined with metallic nanoparticles: a view of biocompatibility and cytotoxicity. 10.1016/B978-0-323-99853-6.00004-8.
44. SPSS,14,2006. Statistical Package for Social Science, SPSS for Windows Release 14.0.0,12june, 2006.Standard Version, Copyright SPSS Inc.,1989-2006.SPSS Inc.
45. Stapleton, P.A., Nurkiewicz, T.R., 2014. Vascular distribution of nanomaterials. Wiley Interdisciplinary Rev. Nanomed. Nanobiotechnol. 6, 4, 338–348.
46. Sun, T., Zhou, D. Xie, J., Mao, F., 2007. Preparation of chitosan oligomers and their antioxidant activity. Eur. Food Res. Technol. 225, 451–456.
47. Sundaresan, K., Sivakumar, A., Vigneswaran, C., Ramachandran, T., 2012. Influence of nano titanium dioxide finish, prepared by sol-gel technique, on the ultraviolet protection, antimicrobial, and self-cleaning characteristics of cotton fabrics. J Indust Text 41, 259-277.
48. Umereweneza, D., Kamizikunze, T., Muhizi, T.,2017. Assessment of mycotoxins types in some foodstuff consumed in Rwanda. Food Control. 85. 10.1016/j.foodcont. 10.015.
49. Vanti, GL., Masaphy, S., Kurjogi, M., Chakrasali, S., Nargund, VB., 2020. Synthesis and application of chitosan-copper nanoparticles on damping off causing plant pathogenic fungi. International Journal of Biological Macromolecules. 156, 1387-1395.
50. Veisi, H., Najafi, S., Hemmati, S., 2018. Pd(II)/Pd(0) anchored to magnetic nanoparticles (Fe3O4) modified with biguanidine-chitosan polymer as a novel nanocatalyst for Suzuki-Miyaura coupling reactions Int. J. Biol. Macromol. 113, 186–194.
51. Wang, C., Wang, M. Q., Ye, S. S., Tao, W. J., Du, Y. J.2011. Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poultry Science. 90,2223–2228.
52. Xu, D., Li, E., Karmakar, B., Awwad, N.S., Ibrahium, H.A., Osman, H-E.H., El-kott, A.F., Abdel-Daim, M.M., 2022. Green preparation of copper nanoparticle-loaded chitosan/alginate bio-composite: Investigation of its cytotoxicity, antioxidant and anti-human breast cancer properties. Arabian Journal of Chemistry, 15,3, 103638. https://doi.org/10.1016/j.arabjc.
53. Yilmaz, S., Kaya, E., Kisacam, M. A., 2017. The Effect on Oxidative Stress of Aflatoxin and Protective Effect of Lycopene on Aflatoxin Damage. InTech. doi: 10.5772/intechopen.69321.
54. Zhao, W., Wang, L., Liu, M., Jiang, K.Y., Wang, M.Q., Yang, G., Qi, C.C., Wang, B.J., 2017. Transcriptome, antioxidant enzyme activity and histopathology analysis of hepatopancreas from the white shrimp Litopenaeus vannamei fed with aflatoxin B1 (AFB1). Dev. Comp. Immunol. 74, 69–81.