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Experimental Trail for Controlling Ochratoxicosis in Poultry Using Nano Titanium Dioxide

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Abstract

THE mycotoxin ochratoxin A (OTA) poses the risks of nephrotoxicity, hepatotoxicity, teratogenicity and immunotoxicity in animals. Nanoparticles from different minerals are often incorporated into chicken feed to serve as an alternative to antibiotics, promote growth, development, and a robust immune system. This research investigated the effects of 30 ppb of dietary OTA on broiler chickens' growth, blood indicators, biochemical measures, and oxidative stress, and the ability of nano-titanium dioxide (TiO2) to counteract its effect. Fifty one-day-old Cobb broiler chicks were divided into five groups each group containing 10 chicks: Gp1: control group, Gp2: fed diet containing 30 ppb of OTA Gp3: fed diet containing 30 ppb OTA plus 20% of nano-titanium dioxide (TiO2), Gp4: fed diet containing 30 ppb OTA plus 40% of TiO2 , and Gp5: fed diet containing 30 ppb OTA plus 80% TiO2. Our results showed significant decrease in body weight gain, Hb concentration, PCV%, WBCs, heterophiles and lymphocytic count. Levels of pro-inflammatory cytokines, malondialdehyde, catalase, and nitric oxide, liver and kidney serum enzyme activity, reduced glutathione and total antioxidant capacity of OTA intoxicated broiler.increased significantly. Chickens in non-treated groups that received ochratoxin only had noticeable levels of ochratoxin residues in the liver and muscle tissues. However, TiO2 NPs addition to diet improved all the previous parameters. In conclusion, nano-titanium dioxide (especially with a concentration of 40 %) minimized the harmful effects of OTA in broilers and improved its elimination from liver and muscle tissues.

Keywords: Nano titanium dioxide, poultry, Electrophoresis, hematology, ochratoxin-A residue.

Introduction

Ochratoxins are a class of mycotoxins produced by various Penicillium or Aspergillus molds. These include several members of the Aspergillus ochraceus group, along with *Penicillium verrucosum* types I and II [1].Ochratoxinsincludecompounds A, B, and C. Ochratoxin-A (OTA) is the most harmful of the three. These compounds are present in food or feedstuffs because it could pollute susceptive agricultural goods [2]. Poultry, among animals raised for food, is highly vulnerable to OTA contamination. Studies have demonstrated that maize, a key ingredient in poultry feed, can be tainted with OTA,

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particularly during the processes of handling and storage [3]. Feeding OTA, at levels between 130 µg and 3.9 mg in poultry feed, leads to a reduced feed conversion ratio, slower growth, impaired kidney function, and eventually, death[4-5]. Furthermore, providing OTA to birds negatively impacts their development. This is likely because it leads to a lower body mass and poor feed conversion ratio[6].It strongly impacts the avian immune system, ultimately leading to a reduction in white blood cells [7]. The LD50 in chickens ranges from 2–4 mg/kg BW [8]; however, it ranges from 3.6 ppm to 16.5 ppm/kg BW for 3-week-old Japanese quails [9].

Nanotechnology focuses on nanoscale materials and their varied applications. Nanoparticles (NPs), typically atomic clusters spanning 1-100 nm, exhibit novel and enhanced characteristics, dependent on their size, arrangement, and shape when contrasted with larger particles of the same bulk materials [10]. Nanoparticles' high surface area-to-volume ratio makes them highly efficient in chemical and biological reactions. To clarify, these particles measure between 1 and 100 nm. At this size, materials exhibit altered, often unexpected, characteristics in terms of physics, chemistry, and biology. This size range offers greater possibilities compared to larger materials, allowing for less material usage [11].

These nanoparticles exhibit increased bioactivity because of their large surface area relative to their volume. The greater surface area compared to their size is responsible for the particles' impressive effectiveness in chemical reactions and biological processes. Nanotechnology methods appear to be a promising, dependable, and economical means to lessen the health impacts of mycotoxins. Research employs three core approaches: preventing mold growth, capturing mycotoxins, and minimizing toxic effects through nanoparticles[12]. In general, adsorptive compounds can diminish the impact of mycotoxins [13]. The first development of nanotechnological applications for the removal or detection of mycotoxins has been carried out in 2009 [14].

Titanium oxide nanoparticles (TiO2NPs) offer cell protection because they readily absorb ultraviolet radiation, thereby lessening undesirable skin reactions[15]. It has been reported that TiO2 NPs are characterized by their high inhibition capacity and heat-resistant against both pathological bacterial strains and fungi [16], so they play an important role in microbial inhibition. TiO2 is a substance that is safe for people and the environment.AlthoughTiO2 is chemically stable, its' chemical activity increases dramatically and significantly when exposed to a light source such as ultraviolet radiation. This phenomenon is called photoactivity [17].

The present study was done to evaluate the toxic effects of ochratoxin alone on body performance, hematological-biochemical and oxidative stress in broiler chickens and modulation this toxic effect using different concentrations (20%, 40%, and 80%) of nano titanium dioxide (0.06 mg/kg)

Material and Methods

Chemicals and treatments

Titanium dioxide nanoparticle preparation

The green synthesis of titanium nanoparticles was performed using mint leaf extract as a natural reducing and capping agent. Fresh mint leaves were first washed thoroughly with deionized water to remove impurities, followed by cutting them into small pieces, and 20 g of leaves were boiled in 200 mL of distilled water at 80°C for 30 minutes to obtain the extract. After cooling to room temperature, the extract was filtered through Whatman No. 1 filter paper and stored at 4°C. The synthesis was initiated by adding the mint extract dropwise to titanium tetrachloride ($TiCl_4$) solution under continuous stirring at room temperature, maintaining a pH of 7. The color change from pale yellow to dark brown indicated the formation of titanium nanoparticles. The reaction mixture was stirred continuously for 5 hours to ensure complete reduction and stabilization. The resulting nanoparticles were separated by centrifugation at 12,000 rpm for 20 minutes, washed repeatedly with deionized water and ethanol to remove any unreacted materials and organic residues, and finally dried in a vacuum oven at 70°C for 24 hours to obtain titanium nanoparticle powder [18-19- 20].

Characterization of the prepared TiO2NPs was performed using Zetasizer, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). The average size of nanoparticles measured by Zetasizer was 56.13 nm. XRD confirmed the anatase crystalline structure of synthesized Titanium dioxide nanoparticles. SEM analysis showed that the morphology of synthesized nanoparticles was spherical and results of AFM indicated that the nanoparticles are smooth [21-22].

Preparation of TiO_2 Treatments

Pure titanium dioxide (TiO_2) nanopowder (100% concentration) was used for the preparation of the intended concentration for groups 3 (20%), 4(40%) and 5(80%). The powder was dissolved in water.

Ochratoxin A:

Ochratoxin A (OTA) was provided by mycology department; Animal Health Research Institute. It was provided in the concentration of 30 ppb.

Ochratoxicated feed preparation:

Ochratoxin-A (OTA)–contaminated feed ingredients were prepared following the method

described by Sansing et al. (23), through artificial contamination of sterilized yellow corn with Aspergillus ochraceus. The OTA-contaminated corn was thoroughly mixed with broiler feed to achieve a final concentration of 30 µg/kg of diet. The concentration of the mycotoxin was determined using immune affinity chromatography as outlined by Hansen (24) and Truckess et al. (25).

Vaccines

Vaccination protocol All birds were vaccinated with Newcastle disease virus (NDV) (HitchnerB1 and La-Sota at age of 7 and 18 days, respectively), and with infectious bursal disease (IBD) vaccine at age of 14 days. NDV vaccines were purchased from Intervet Boxmeer Company, Holland. Gumboro vaccine was purchased from Rhone-Merieau Company, France.

Experimental animal:

Fifty (unsexed) one-day-old Cobb broiler chicks were obtained from the Faculty of Veterinary Medicine, Zagazig University (laboratory Animals Housing Unit). The animals were clinically healthy, kept under hygienic conditions, shaving as bedding. They were maintained on a balanced diet. The animals were fed commercial ration purchased from Feed Mix Company. The animals were accommodated to the laboratory conditions for one week before the beginning of the experiment.

On day 8, the chicks were weighed and then divided into 5 groups each containing 10 chicks. The experimental feeding was designed as follows: (Gp1) fed a healthy diet and served as the control group. (Gp2)fed diet with 30 ppb diet ochratoxin according to Awaad *et al*. [26] only for 28 days.(Gp3) fed diet with 30 ppb diet ochratoxin + 20% nano titanium dioxide for 28 days. (Gp4) fed diet with 30 ppb diet ochratoxin + 40%nano titanium dioxide for 28 days. (Gp5) fed diet with 30 ppb diet ochratoxin + 80% nano titanium dioxide for 28 days. Ochratoxin was added to feed while nano titanium dioxide was administrated orally to the chicken.

Body weight:

The animals were weighted individually at the beginning of the experiment to obtain the average initial body weight and then body weight was recorded every week for calculation of the average body weight development in each group.

Sampling:

On day 35 of the experiment, two blood samples were collected by wing vein from each chicken. The first sample was collected in a tube containing EDTA as an anticoagulant for hematological profile analysis, meanwhile the second tube was without an anticoagulant for obtaining serum. Then, chicks were sacrificed by neck dislocation, and liver, and muscle tissue samples were collected. Concerning tissues, both the liver and muscle were excised and prepared for ochratoxin residue. The liver tissue was divided into three sections, one for antioxidant assessment, and the other for ochratoxin residue. Tissue samples were kept at -20°C till the examination.

Serum preparation:

The collected blood samples were allowed to coagulate at 37C° for 30 minutes and then centrifuged at 3000 rpm for 15 minutes.The separated serum was aspirated by automatic pipette into clean well dried Witherman tubes and stored at -20 C° till used for hormonal and biochemical analysis.

Hematological studies:

The erythrocytic count (RBCs) hemoglobin concentration (Hb %) and packed cell volume (PCV) were determined. The erythrocytic indices {mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)} were calculated. Moreover, the total and differential leukocytic counts were conducted [27].

Biochemical analysis:

Serum total protein and its electrophoretic pattern were determined by Spectrum kit CAT. NO 310 001 and chemical preparation of polyacrylamide gel electrophoresis using the continuous buffer system of Kaplan and Szalbo [28] and Davis [29] respectively, and the calculated based on SynGene S. No. 17292*14518 sme*mpcs program using Scie Plas TV100 Mini Vertical Gel Unit UK with Power Supply Consort EV714, Belgium.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by spectrum kit CAT. NO 260 001 and 265 001 respectively. Levels of creatinine and urea in serum were determined by using spectrum kit CAT. NO 235 004 and 319 005 respectively. Additionally, nitric oxide (NO) and total antioxidant capacity (TAC) were estimated by using Bio-diagnostic kit CAT. No. NO 25 33 and TA 2513 respectively. Liver tissue of malonaldehyde (MDA), reduced glutathione (GSH), and catalase (CAT) were estimated by using Bio-diagnostic kit CAT.NO MD 25 29, GR 25 11 and CA 25 17 respectively.

Moreover, serum Tumor necrotic factor-α (TNF-α) and Interleukin 6 (IL-6) were determined by commercially available ELISA kits based on manufacturers' instructions (R and D Systems; Minnesota; Minneapolis; USA).

Measurement of total ochratoxin residues

Liver and muscle tissues were subjected to direct examination using high-performance liquid chromatography. to determine total ochratoxin residues, according to Zhang, *et al*. [30].

Statistical analysis

The obtained data were analyzed using statistical package for social science (SPSS,17 Software, 2004) for obtaining mean and standard error, the data were analyzed using one way ANOVA to determine the statistical significance of difference among groups. Means considered significance at $p < 0.05$ %

Results

The results in Table 1 show that both the final weight and body weight gain were significantly decreased in OTA intoxicated broiler compared with all groups. However, treatment of OTA-intoxicated group with TiO2 NPs 40% concentration resulted in a significant increase in final weight and body weight gain compared to all groups.

In the current work, Ochratoxin resulted in a non-significant decrease in RBCs count with a significant decrease in Hb concentration, PCV %, total WBCs, heterophils and lymphocytic count the ochratoxicated broiler chicken compared to control group. Moreover, treatment with nano-titanium 40% induced significant increase in Hb concentration, PCV % and lymphocytic count besides an insignificant increase in total WBCs and heterophils counts compared to the ochratoxicated group as shown in Table 2.

Regarding the biochemical studies, the obtained results demonstrated in Table 3 revealed that serum AST, ALT, ALK, urea, and creatinine concentrations were significantly elevated in OTA-intoxicated broilers when compared with the control group. Meanwhile, significant decreases of all previous parameters were reported in all treated groups with the TiO2 NPs when compared with ochratoxicated broilers. The obtained results revealed that treated group (OTA+ TiO2 NPs with a concentration of 40 %) showed a significant decrease of previous parameters compared with other concentrations of 20%, and 80%.

The obtained data established in Table 5 revealed a significant increase in serum TNF-α, IL-6, MDA, CAT, and NO with a marked decrease in GSH, and TAC were observed in OTA exposed Broilers in comparison with treated groups (OTA + TiO2 NPs) and control group. However, significant decreases of serum TNF-α, IL-6, and NO were recorded in the treated group (OTA+TiO2 NPs 40%) when compared with other treated groups.

Table 6 illustrates ochratoxin residue in the liver and muscle of non-treated groups that received ochratoxin only and had noticeable levels of ochratoxin residues in the liver and muscle tissues. However, treatment with different concentrations of nano-titanium resulted in significant decrease in ochratoxin residues especially the group treated with 40% nano titanium dioxide which exhibited marked improved ochratoxin elimination in the liver and muscle tissues.

Table (5a), showed OTA significantly reduced total albumin ($p < 0.05$) versus control. nanoTiO2at 20% and 40% restored albumin to control-like values, while 80% showed partial recovery but remained lower than control. OTA also significantly reduced α-globulin; nanoTiO_2 at 20% and 40% improved levels but remained below control, with 80% showing moderate improvement. β-globulin was unaffected by OTA, and all nanoTiO2groups-maintained control-like values. γ-globulin decreased significantly with OTA; 20% and 40% nanoTiO2 restored values to control levels, while 80% improved but remained lower. Total globulin, A/G ratio, and total protein were all significantly reduced by OTA, with all nanoTiO2 treatments improving values; 40% was closest to control.

Table (5b), illustrated that OTA markedly reduced pre-albumin all nano TiO2 groups improved it, though 80% remained lower than control. Albumin levels decreased with OTA but were fully restored by all nanoTiO2 doses. α_1- and α_2-globulins were significantly reduced($p < 0.05$) by OTA; nanoTiO2improved both, with 40% and 80% approaching control. β_1- and β_2-globulins decreased with OTA; 20% and 40% nanoTiO2restored β_1 to intermediate levels, while 40% and 80% nearly normalized β_2. OTA lowered γ_1-globulin, but all nanoTiO2 groups increased it; 40% and 80% matched control. No significant changes occurred in γ_2-globulin among any groups.

Liver Ochratoxin Residue (µg/kg) OTA group showed the highest toxin accumulation, indicating significant ochratoxin deposition in the liver.

G 3 caused a significant reduction ($p < 0.05$) compared to OTA alone.G4 (8.53 ± 0.2333) showed the greatest reduction, with toxin residues dropping to less than half of the OTA group.G 5 showed partial reduction, but still significantly higher than the 40% group.

Muscle Ochratoxin Residue (µg/kg): OTA group had the highest residues in muscle. G 3 showed a significant decrease compared to OTA.G4 achieved the best detoxification effect, with minimal ochratoxin residues detected.G 5 decreased residues compared to OTA but was less effective than 40%.

Discussion

The present study evaluated the ameliorative effects of nano titanium dioxide (nano-TiO2 at dietary inclusion levels of 20%, 40%, and 80% on ochratoxicosis-induced biochemical changes in chicken serum. Ochratoxicosis, primarily caused by ingestion of ochratoxin A (OTA), is a significant mycotoxicosis in poultry that disrupts liver and kidney functions and induces oxidative stress, resulting in impaired performance and health [31].

OTA fed birds (G2) showed various clinical signs and behavioral alterations as anorexia, depression, increased water intake, diarrhea and ruffled feathers where severity of signs in G2 increased gradually throughout the experimental period. No mortalities were recorded throughout the study. In treated-groups with TiO2 NPs the signs were mild and no mortality was recorded.

Ochratoxin caused a considerable decrease in both the final weight and weight gained compared to every other group. Nevertheless, treating the OTA-poisoned group with TiO2 NPs at a 40% concentration led to a considerable rise in final weight and weight gained compared to all groups. The decrease in weight gain during ochratoxicosis might be because of the unfavorable effects of ochratoxin on the intestinal tract, which reduces feed absorption, subsequently leading to alterations in weight gain[32].The decrease in weight gain caused by ochratoxicosis matched earlier findings in studies that used ochratoxin in the diet of broiler chickens [33].

The main economic issues arising when chicks consumed diets polluted by OTA included slower growth, reduced appetite, and ineffective feed utilization[4-5-32-34-35-36].Duringa study of Elaroussi*et al.*[5], birds consuming contaminated food (0.4 and 0.8mg OTA/kg) showed a reduction in feed intake and body mass that was related to the OTA dose and their feed conversion also became worse as the dose increased.

Regarding the heamatological results, non-significant decrease in RBCs count with significant decrease in Hb concentration, PCV %, total WBCs, heterophils and lymphocytic count were recorded in the ochratoxicated broiler chicken.Analyzing blood parameters in animals exposed to a threat can help diagnose mycotoxicosis. Since these measures are more responsive than things like performance, this finding would suggest the poisoning before other signs appear [37].

However, a marked decrease in the overall count of red and white blood cells in broiler chickens given a diet tainted with OTA (1 mg OTA/ kg) was noted bySawale*et al.*[38]. Similar results have been found in other studies [34 –39]. Also,Elaroussi*et al.*[5] demonstrated a decrease in red and white blood cell counts in broiler chickens after they were given two diets that were tainted with 0.4 and 0.8 mg OTA per kilogram.

Lowered red blood cell counts and hemoglobin levels can occur because of a decrease in iron in the blood during ochratoxicosis [40].A decline in the overall number of white blood cells circulating, occurring in ochratoxicosis, stems from reductions in lymphocytes and monocytes[41]. Across a spectrum of OTA exposure levels beginning at 0.5 ppm [40] and up to 4.0 parts/10^6[41], a form of anemia was documented, showing a considerable drop in packed cell volume (PCV%) and hemoglobin (Hb) concentration. This was thought to be caused by either a lack of iron or a problem within the blood-producing system. A reduction in the white blood cell count was observed, mainly due to fewer lymphocytes, with monocytes and heterophils also contributing, but to a lesser degree [34-41]. A low lymphocyte count can be a valuable and helpful sign of ochratoxin poisoning, potentially stemming from its direct impact on the germinal centers within lymphoid tissues. This also suggests a change in the immune system's operation. OTA's harmful impact on white blood cell counts was also observed in young male turkeys. They were given diets containing OTA at levels of 4 and 8 mg/g of food, starting right after they hatched and continuing for three weeks [41], and in Japanese quail received OTA via tube into their esophagus at a dosage of 50 mg per bird daily, for 60 days[43].

Our results revealed that serum AST, ALT, ALK, urea, and creatinine concentrations were significantly elevated in OTA-intoxicated broilers when compared with the normal control group. Since OTA metabolism primarily occurs in the liver, and it's eliminated via the kidneys, poisoning from it caused harm to the liver cells [44] and nephrotoxiceven when exposed to small amounts of OTA, which causes changes in the structure and function of the kidneys and liver[45].The results showed a marked rise in serum AST, ALT, and Alk levels, along with urea and creatinine values, in broilers given OTA (0.0م mg / kg of diet) compared to normal group. These elevated values were highly critical serum biomarkers in determining hepatocyte's function and damage in OTA supplementation [46].Severe harm to the liver cells resulted in an increased release of liver enzymes [47].

Liver damage usually stems from free radicals. These radicals, produced during the processing of harmful substances, start the toxic effects [45]. In the current work, the obtained result revealed an increase of serum TNF-α, IL-6, MDA, No, MDA and CAT values in OTA Broilers. Oxidative stress is strongly linked to inflammation. When oxidative stress occurs, cells release large amounts of ROS, which trigger the NF-κB inflammatory signaling pathway and also increase the expression of pro-inflammatory factors [49].

Inflammation is a serious indicator of liver damage triggered by harmful physical or chemical substances. This inflammation plays a crucial part in healing the liver [50].TNF-α is the initial inflammatory factor released during the inflammatory response. This can trigger the release of other cytokines, impact NF-κB (a primary regulator of inflammation), increase free radical production, and worsen liver injury[51]. During liver injury, TNF-α and IL-6 become active and release.

This action results in neutrophils accumulating in liver cells. Consequently, cytokine expression increases [52**].**Research has shown that mycotoxins, including OTA, might amplify the production of TNF-α in rats [53]. As shown in the table (5) G2 (OTA only fed) showed a significant increase in MDA and a decrease in GSH as all groups throughout the study.

MDA is a persistent byproduct of lipid peroxidation (LP), which is a chain reaction triggered by free radicals [54]. By adding OTA to broilers ration, MDA levels might be increased [55]. Because it causes the creation of free radicals (ROS). Using TiO2 NPs in the diet and its compatibility with living organisms led to boosted No, CAT, and MDA activity, with no oxidative harm observed [56]. In the current study, an increase in MDA (as an oxidative stress marker) level was observed in ochratoxicosis group when compared with the controlgroup.This result convention with [57-58-59] suggested that, over two weeks of OTA administration (0.5 mg/kg body weight) to rats, there was DNA damage in lymphocytes, kidneys, and liver. Additionally, a decrease in GSH levels was accompanied by an increase in SOD activity.

Also, serum CAT, MDA, and No activity significantly increased in OTA treated broilers group as compared to the control group. These results are nearly similar to Soyöz, *et al.,*[60] and Özçelik, *et al.,*[61]who reported that CAT increased in the blood of rats given OTA (0.289 mg kg−1 for four weeks). CAT is most prevalent in liver cells and cooperates with SOD[62].

The obtained results illustrated that OTA decreased the concentration of serum TAC, and GSH. There is a balance between ROS production and ROS degradation with total antioxidants in the cells. Elevated levels of either ROS or antioxidants can lead to an irregular condition of oxidative stress **[63].**The buildup of free radicals in the kidney or elsewhere leads to tissue damage. This is caused by the oxidative interaction with crucial intracellular components, like GSH (which has thiol groups), and lipid peroxidation of cell membranes. This latter process may significantly contribute to the cell's self-destruction (apoptosis)[64].

The obtained result revealed that treated OTC groups with TiO2NPS significance decrease in AST, ALT ,ALK , Creatinine and Urea compared with the OTC broiler group similar to Hosseini*et.al.,*[65] who recorded that, decreased values of AST, ALP, ALT, creatinine, and BUN in Propylthiouracil Se-NPs treated groups versus in rats. The protective effect of TiO2-NPs on the liver and kidneys was linked to higher serum levels of GSH and TAC in all Se-NPs treated groups, relative to the OTA group.

OTA's harmful effects could stem from its release of reactive oxygen species (ROS), leading to oxidative stress in different organs. The production of ROS and lipid peroxidation in cell membranes can disrupt membrane fluidity. This disruption can also elevate membrane permeability or alter membrane potential, subsequently triggering enzymes to leak out of cells[66]. Elevated cell damage and increased cell membrane permeability tied to excessive release of liver cell contents and bile duct blockage will raise ALT, AST levels, and also Alk in blood [67-68].

Currently, the application of nanotechnology model to acute toxicity during OTA-intoxication is under-explored. Thus, an integrated analysis of oxidative stress (OS) biomarkers and biochemical tests was conducted in this study to clarify OTA toxicity.

TiO2 NPs have shown antioxidant or anti-inflammatory effects [15]. At the nanoscale, nanoparticles (NPs) act as mycotoxin-absorbing materials that can bind and prevent mycotoxins from being absorbed in the gut [66].Various nanoparticles (NPs) are currently used as feed additives to bind or reduce the damaging effects of mycotoxins through adsorption in animals.

Several NPs demonstrated a strong ability to eliminate OTA mycotoxin [70].The protective hepatorenal influence of TiO2-NPs was associated with the increased serum values of GSH and TAC in all TiO2-NPs treated groups with the control group. In the present work, the supplementation of TiO2-NPs improved the damage induced by OTA as noted via increased GSH, TAC, and reduced MDA values compared with the OTA group which is expressed as a result of macrophage activity revealed a significant increase in OTA group only while all birdsTiO2 NPs received showed a significant decrease than other both control groups. These wonderful effects of TiO2 either low or high, not only ameliorated the toxic effect of OTA on macrophage activity but also modulated microphage activity. The increase expressed NO post TiO2NPs therapy is attributed to the redox-active NPs which tend to modulate innate and adaptive immunity, and this ability is extensively observed within blood cells and immune-related organs, like the liver [71].Furthermore, TiO2-NPs have been shown to safeguard the liver by stabilizing liver function indicators and lessening antioxidant enzyme activity, including CAT, within the treated group

The results showed that TiO2NPs significantly decrease TNF-α and IL-6 values. This result is agreed with Duan *et al.*[72]. TiO2-NPs might have anti-inflammatory effects by modulating the production of pro- and anti-inflammatory cytokines. This effect could be partly due to their ability to lessen the activation of NF-κB and p38 mitogen-activated protein kinases (p38 MAPKs) [73]. Also, Sojka et al., [74] observed a reduction in IL-10 activity in rats exposed to TiO2NPs; this could be

linked to a decline in the lymphocyte population. The possible recovery following the discontinuation of TiO2NP exposure could be due to a partial restoration of lymphocyte activity after a period of complete cessation.

Consistently, our findings suggested that TiO2 NPS inhibited the release of the cytokines TNF-α and IL-6, decreased the level of MDA, but increased the concentrations of the antioxidants GSH.

The present study evaluated the ameliorative effects of nano titanium dioxide (nano-TiO_2) at dietary inclusion levels of 20%, 40%, and 80% on ochratoxicosis-induced biochemical changes in chicken serum. Ochratoxicosis, primarily caused by ingestion of ochratoxin A (OTA), is a significant mycotoxicosis in poultry that disrupts liver and kidney functions and induces oxidative stress, resulting in impaired performance and health [28]. serum hypoalbuminemia in OTA intoxication table(5a, b,) reflects a multifactorial interplay between hepatic insufficiency, renal albumin loss [75]., inflammatory suppression of synthesis [76] and altered vascular permeability [77].Measurement of serum albumin levels in OTA exposure thus provides valuable insight into the extent of organ dysfunction and systemic inflammatory status, and it may serve as a useful biomarker in clinical and toxicological evaluations Moreover, the reduction of albumin level may be due to reduced formation of protein in the liver or loss of protein formation from the alimentary tract [78].

The increase in serum beta-globulin levels in OTA toxicity reflects a composite effect of chronic inflammation, acute phase protein synthesis C3 and C4, transferrin, and beta-2 microglobulin [77], impaired renal protein clearance [75] and sustained immune activation IgA and IgM [79]. These alterations in serum protein profile may serve as useful biomarkers for assessing the extent of OTA-induced organ damage and systemic immune response. The decline in serum globulin concentrations associated with OTA intoxication is attributable to a complex interplay of suppressed immunoglobulin synthesis, impaired hepatic production, and enhanced renal losses, compounded by systemic inflammatory changes. These alterations reflect OTA's multifaceted toxicity profile and underscore the utility of serum globulin measurements as indicators of immune competence, hepatic function, and renal integrity in both experimental and clinical assessments of OTA exposure.

Elevated pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha during OTA toxicity may alter protein metabolism and tissue distribution, leading to dynamic shifts in serum protein composition [77].

OTA exposure in this investigation led to marked decreases in serum total protein, albumin concentrations, and globulins with concurrent elevations in beta globulin fractions. These alterations reflect impaired hepatic protein synthesis due to OTA-induced hepatotoxicity and heightened immunoglobulin production secondary to chronic inflammation [31]. Total proteins decrease may be due to the fact that OTA is a protein synthesis inhibitor that has an effect on mitochondrial oxidative enzyme activity [80], or might be due to the imbalance between the rate of protein synthesis and the rate of its degradation in liver[81].

The observed hypoalbuminemia and reduced albumin/globulin (A/G) ratio are consistent with OTA's inhibition of mRNA translation and its capacity to elicit oxidative stress and tissue damage [82] the increase in serum beta-globulin levels in OTA toxicity reflects a composite effect of chronic inflammation, acute phase protein synthesis C3 and C4, transferrin, and beta-2 microglobulin[77], impaired renal protein clearance [75]and sustained immune activation IgA and IgM [80]. These alterations in serum protein profile may serve as useful biomarkers for assessing the extent of OTA-induced organ damage and systemic immune response.

Dietary supplementation of nano-TiO_2 exhibited a dose-dependent protective effect against OTA-induced protein fraction abnormalities. This suggests that nano-TiO_2 at this dose mitigated oxidative damage and modulated immune responses, likely through its high adsorption capacity and antioxidant properties [83]. Reduced oxidative and inflammatory stress allows hepatocytes to restore normal albumin synthesis, improving serum total protein and albumin levels. TiO_2 NPs may minimize protein loss through urine and stabilize globulin fractions. TiO_2 NPs can adsorb toxins and reduce their bioavailability, thus diminishing systemic toxicity [84].

TiO_2 NPs, due to their high surface area and redox activity, can effectively scavenge free radicals, thus protecting cellular components from oxidative damage. Abdel-Wahhab *et al.* [85] demonstrated that dietary supplementation with TiO_2 NPs significantly reduced oxidative stress markers and improved antioxidant enzyme activity in OTA-. Additionally, TiO_2 NPs possess significant anti-inflammatory properties), which further impair hepatic and immune functions [86].

Ochratoxin A (OTA) builds up in poultry meat, presenting a risk to people who eat it. This is because OTA remains in the body for a long time and is tough to break down with heat or chemicals [87] and Its existence in large amounts presents health hazards to exposed individuals, ranging from allergic responses up to death [88-89].

The main reason to monitor this mycotoxin in feed is because of worries about transmitting OTA to poultry and its later spread in their edible parts. In this regard, Pozzo *et al.*[36] performed an experiment in which thirty-six day-old male broiler chicks were given a diet supplemented with 0.1 mg OTA/ kg. Following this, the levels of OTA were assessed in blood serum, liver, kidney, breast, and thigh samples. The mycotoxin was found in the serum, liver, and kidney, with concentrations of 1.2, 0.4 ng/mL, 1.9, 0.2µg/kg, and 3.6,0.9µg/kg. It wasnot found in breast and thigh.Similarly, in the study by Birò et al., [90] in broiler chicks fed with feed containing 0.5 mg/kg OTA, the distribution was: liver > kidney > plasma > muscle.

Our results revealed that treatment with different concentrations of nano-titanium resulted in significant decrease in ochratoxin residuesespecially the group treated with 40% nano titanium dioxide which exhibited marked improved ochratoxin elimination in the liver and muscle tissues.

Nanotechnology provides three primary ways to fight mycotoxins: directly stopping the fungus, absorbing mycotoxins, and lessening their poisonous impacts. Carbon-based nanomaterials, like graphene, strongly bind to mycotoxins [91].

Adsorption is extremely important for removing mycotoxins. Research indicates that nanoparticles (NPs) can also participate in mycotoxin detoxification via an adsorption process [92].Nanoparticles, at a tiny scale, act as mycotoxin-absorbing compounds. They attach to mycotoxins, thus preventing their absorption in the gut[69].Several nanoparticles (NPs) are now utilized as dietary supplements. They work to capture or reduce the detrimental impact of mycotoxins by adsorption in livestock. Certain NPs exhibited significant mycotoxin removal effectiveness in dealing with OTA [70] because of their particular qualities, like their small size, a high surface area relative to volume, surface charge, and high catalytic activity, they can adsorb more effectively. This, in turn, gives them a greater ability to bind mycotoxins, leading to improved absorption [93]. Therefore, the adsorption method can be used to remove mycotoxins from poultry feed [61].

Some scientists proposed that metallic oxide nanoparticles diminish mycotoxins due to an electrostatic pull. They suggested the negatively charged analytes (COO-) and positively charged metal attract, as the electron exchange between the substances was sped up by Ti4+ from TiO2. The high absorption capability of the used nanoparticles enables the formation of coordinate and electrostatic bonds by titanium cations with the OTA's β-dicarbonyl system [69].

Conclusion

While few studies have examined nanomaterials' direct impact on mycotoxins, existing studies mostly focus on nanomaterials' influence on fungi, potentially preventing toxin production. This research demonstrated that ochratoxicosis in broilers resulted in poor weight gain and, significant decrease in Hb concentration, PCV%, WBCs, heterophiles and lymphocytic count. On the other hand, there was significant increase in pro-inflammatory cytokines, malondialdehyde, catalase, and nitric oxide , liver and kidney serum enzyme activity, reduced glutathione and total antioxidant capacity of OTA intoxicated broiler. Moreover, 40% TiO2 NPs effectively minimized the harmful effects of OTA in broiler chickens and improved ochratoxin elimination in the liver and muscle tissues. Nanotechnology approaches appear promising, dependable, and affordable for lessening mycotoxins' health effects. Therefore, applying this technique for treating in feed is suggested to minimize mycotoxin.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The experimental protocol was approved by the Animal Health Research Institute (AHRI) in conformity with the Agriculture Research Center (ARC) and IACUC Committee (ARC, AHRI, IACUC, 146 /24) in Egypt.

TABLE 1. The effect of nano-titanium dioxide treatment on growth performance in ochratoxicated broilers chickens.

Group	Unit (Kg)		
	Initial weight	Final weight	Body weight gain
Control (G1)	45 ± 1.29	1792.5^{b} ± 21.74	1747.5^{b}± 21.74
OTA(G2)	44 ± 1.32	1110 ^{e}± 24.832	1065.5^{e} ± 23.73
OTA +TiO2 20%(G3)	43 ± 1.29	1575 ^{c}± 32.27	1532^{c} ± 32.041
OTA + TiO2 40%(G4)	45 ± 1.87	1952^{a}± 29.54	1907.5^{a} ± 29.60
OTA + TiO2 80%(G5)	43.75 ±0.629	1482.5^{d} ± 27.80	1439^{d} ± 27.15

Data are presented as (Mean ± SE). n= 3. Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

TABLE 2. The effect of nano-titanium dioxide treatment on blood picture in ochratoxicated broilers chicken.

Parameter	Group				
	Control (G1)	OTA (G2)	OTA + TiO2 20%(G3)	OTA + TiO2 40%(G4)	OTA + TiO2 80%(G5)
RBCsX10^6/cmm	3.33 ± 0.31	2.69 ± 0.18	3.05 ± 0.29	3.15 ± 0.34	2.84 ± 0.23
Hbg/dl	10.30 ± 0.42 ^{a}	8.04 ± 0.37 ^{b}	8.85 ± 0.38 ^{b}	9.56 ± 0.24 ^{a}	8.32 ± 0.33^{b}
PCV %	32.20 ± 0.98 ^{a}	25.22 ± 0.71 ^{c}	28.88 ± 0.82 ^{bc}	30.55 ± 0.82 ^{ab}	27.44 ± 0.79 ^{c}
MCVFl	96.70 ± 2.05	93.75 ± 2.14	94.69 ± 1.72	96.98 ± 2.03	96.62 ± 1.94
MCHPg	30.93 ± 1.10	29.89 ± 1.14	29.02 ± 1.08	30.35 ± 1.17	29.30 ± 1.26
MCHCg/dl	31.99 ± 1.24	31.88 ± 1.09	30.64 ± 1.28	31.29 ± 1.37	30.32 ± 1.52
WBCsX10^3/cmm	22.84 ± 1.02^{a}	18.44 ± 1.11 ^{b}	19.31 ± 1.06 ^{b}	20.76 ± 0.96 ^{ab}	19.42 ± 1.03 ^{b}
Lymphocytes	12.81 ± 0.61^{a}	10.09 ± 0.73 ^{c}	10.62 ± 0.69 ^{bc}	11.57 ± 0.64 ^{ab}	10.78 ± 0.58 ^{bc}
Heterophils	7.85 ± 0.44 ^{a}	6.40 ± 0.31 ^{b}	6.68 ± 0.43 ^{ab}	7.08 ± 0.42 ^{ab}	6.64 ± 0.54 ab
Monocytes	1.59 ± 0.22	1.45 ± 0.19	1.48 ± 0.20	1.55 ± 0.23	1.49 ± 0.18
Eosinophils	0.36 ± 0.05	0.31 ± 0.07	0.33 ± 0.06	0.35 ± 0.05	0.32 ± 0.06
Basophils	0.23 ± 0.03	0.19 ± 0.02	0.20 ± 0.03	0.21 ± 0.02	0.19 ± 0.01

Data are presented as (Mean ± SE). n= 5. Mean values with different superscript letters in the same row are significantly different at ($p < 0.05$).

TABLE 3. The effect of nano-titanium dioxide treatment on serum liver marker enzymes (AST, ALT, and ALK) activities and kidney function tests (urea and creatinine) concentrations in ochratoxicated broilers.

Parameter	Group				
	Control (G1)	OTA (G2)	OTA + TiO2 20%(G3)	OTA + TiO2 40%(G4)	OTA + TiO2 80%(G5)%
ALT u/l	10.5e±0.58	18.500a±0.47	15.166c±0.58	11.033d±0.028	17.24b±0.006
AST u/l	85c±0.5773	124a±0.5773	91b±0.5773	86c±0.5773	90b±0.5773
ALK u/l	395e±0.5773	482a±0.577	471c±0.577	421d±0.5773	460b±0.3333
Urea mg/dl	21b±0.05773	45a±0.05773	22.1b±0.05773	21.6b±0.05774	22.2b±0.05774
Creatinine mg/dl	0.38d±0.006	1.4a±0.00577	0.9b±0.00577	0.8bc±0.00577	0.75c±0.00577

Data are presented as (Mean ± SE). n= 3. Mean values with different superscript letters in the same row are significantly different at ($p < 0.05$).

TABLE 4. The effect of nano-titanium dioxide treatment on serum pro-inflammatory cytokines and oxidative stress/antioxidant markers in ochratoxicated broilers.

Parameter	Group				
	Control (G1)	OTA (G2)	OTA + TiO2 20%(G3)	OTA + TiO2 40%(G4)	OTA + TiO2 80%(G5)%
TNF-α (Pg/ml)	53.2^{e}±0.5774	173.71^{a}±0.4371	90.49^{b}±0.5586	67.6^{d}±0.7788	82.88^{c}±0.5745
IL-6 (Pg/ml)	100.8^{e}±0.9073	257.57^{a}±0.5773	113.5^{c}±0.5959	106^{d}±0.5773	128.4^{b}±0.5773
NO(u/l)	26.707^{c}±0.51522	33.380^{a}±0.5773	27.913^{bc}±0.585	26.37^{c}±0.5234	28.64^{b}±0.5234
TAC (Mm/l)	3.5333^{a}±0.2027	1.27^{c}±0.02906	1.8^{b}±0.05774	1.73^{b}±0.00577	1.716^{b}±0.00577
CAT (U/l)	12^{d}±0.5773	23^{a}±0.5773	16^{b}±0.5773	14^{c}±0.5773	13^{cd}±0.5773
GSH nmol/ml	16.42^{a}±0.5179	12.35^{c}±0.9265	14.3^{b}±0.9073	13.2^{bc}±0.5773	15.00^{ab}±0.5773
MDA (nmol/ml)	1.6^{d}±0.05774	3.7^{a}±0.05774	2.2^{bc}±0.05774	2.3^{b}±0.06351	2.1^{c}±0.05774

Data are presented as (Mean ± SE). n= 3 . Mean values with different superscript letters in the same roware significantly different at ($p < 0.05$).

TABLE 5a,b. Effect of nano-titanium dioxide treatment on serum T. protein and its mean and subfractions electrophoresis in ochratoxicated broilers chicken.

TABLE 5a

Group	t-albumin	T- Alpha	t -Beta	t -Gamma	T globulinl	a/g	T. protein
Control (G1)	1.33a	1.18a	0.68a	1.22a	3.08a	0.43a	4.41a
	±0.03	±0.04	±0.03	±0.04	±0.09	±0.01	±0.11
OTA (G2)	0.87c	0.78c	0.75a	0.93c	2.46d	0.35b	3.32d
	±0.02	±0.02	±0.01	±0.06	±0.05	±0.01	±0.07
OTA + TiO2	1.31a	0.92b	0.73a	1.16ab	2.80bc	0.47a	4.11b
20%(G3)	±0.03	±0.03	±0.01	±0.04	±0.06	±0.02	±0.03
OTA + TiO2	1.33a	0.98b	0.72a	1.17ab	2.87b	0.46a	4.19ab
40%(G4)	±0.01	±0.03	±0.04	±0.05	±0.05	±0.01	±0.04
OTA + TiO2	1.18b	0.94b	0.64a	1.06abc	2.64dc	0.45a	3.82c
80%(G5)	±0.07	±0.03	±0.07	±0.03	±0.06	±0.02	±0.12

Data are presented as (Mean ± SE). n= 3 . Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

TABLE 5b

Group	Pre ab	Albumin	Alpha1	Alpha2	Beta1	Beta2	Gamma1	Gamma2
Control (G1)	0.19b	1.14a	0.41a	0.77a	0.33c	0.35a	1.09a	0.13a
	±0.01	±0.03	±0.03	±0.03	±0.02	±0.01	±0.03	±0.01
OTA (G2)	0.14d	0.72b	0.22c	0.56b	0.58a	0.17c	0.83c	0.10b
	±0.01	±0.02	±0.01	±0.02	±0.01	±0.02	±0.05	±0.01
OTA + TiO2	0.22a	1.09a	0.31b	0.60b	0.46b	0.27ab	1.02a	0.14a
20%(G3)	±0.00	±0.03	±0.02	±0.04	±0.01	±0.00	±0.04	±0.01
OTA + TiO2	0.18bc	1.15a	0.34b	0.64b	0.39bc	0.32a	1.03ab	0.14a
40%(G4)	±0.01	±0.01	±0.01	±0.03	±0.01	±0.05	±0.05	±0.01
OTA + TiO2	0.17c	1.02a	0.32b	0.62b	0.40bc	0.24bc	0.93bc	0.13a
80%(G5	±0.00	±0.07	±0.02	±0.01	±0.05	±0.02	±0.03	±0.00

Data are presented as (Mean ± SE). n= 3 . Mean values with different superscript letters in the same column are significantly different at ($p < 0.05$).

TABLE 6. The effect of nanotitanium dioxide onochratoxin residue in liver and muscle during ochratoxicosis in broiler chicken.

Parameter	Group Unit(PPb)				
	Control (G1)	**OTA (G2)**	**OTA + TiO2 20%(G3)**	**OTA + TiO2 40%(G4)**	**OTA + TiO2 80%(G5)%**
Liver	ND	20.23^{a}±0.4255	15.33^{c}±0.2905	8.533^{d}±0.2333	17.16^{b}±0.4055
Muscle	ND	13.666^{a}±0.4163	6.520^{b}±0.355	2.433^{d}±0.240	8.11^{c}±0.4705

Data are presented as (Mean ± SE). n= 3 . Mean values with different superscript letters in the same ~~column~~ are significantly different at ($p < 0.05$).

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محاوله للتحكم فى الاوكراتوكسيكوزيس فى الدواجن باستخدام نانو ثانى اوكسيد التيتانيوم

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الملخص

تشكل السموم الفطرية الأوكرا توكسين A (OTA) مخاطر على السمية الكلوية، والسمية الكبدية، والتشوهات الخلقية، والسمية المناعية في الحيوانات. وغالبًا ما يتم دمج الجسيمات النانوية من معادن مختلفة في علف الدجاج كبديل للمضادات الحيوية، لتعزيز النمو، والتطور، ونظام المناعة القوي. وقد بحثت هذه الدراسة في تأثير 30 جزءًا في البليون من OTA الغذائي على نمو دجاج التسمين، ومؤشرات الدم، والقياسات البيوكيميائية، والإجهاد التأكسدي، وقدرة أكسيد النانو-تيتانيوم (TiO2) على مواجهة تأثيره. تم تقسيم عدد خمسين كتكوت من سلالة كوب البالغة من العمر يومًا واحدًا إلى خمس مجموعات كل مجموعة احتوت على 10 كتاكيت: المجموعة 1: مجموعة التحكم، المجموعة 2: تلقت طعامًا يحتوي على 30 جزءًا في البليون منOTA ، المجموعة 3: تلقت طعامًا يحتوي على 30 جزءًا في البليون من OTA بالإضافة إلى 20% أكسيد النانو-تيتانيوم ، المجموعة 4: تلقت طعامًا يحتوي على 30 جزءًا في البليون من OTA بالإضافة إلى (40% TiO2، والمجموعة 5: تلقت طعامًا يحتوي على 30 جزءًا في البليون من OTA بالإضافة إلى80% .TiO2. أظهرت نتائجنا انخفاضًا كبيرًا في معدل الزيادة فى وزن الجسم، وتركيز الهيموغلوبين، ونسبة خلايا الدم الحمراء، وخلايا الدم البيضاء، وعدد الخلايا المعتدلة واللمفاوية. كما ارتفعت مستويات السيتوكينات المؤيدة للالتهاب، ومالونديالدهيد، والكاتالاز، وأكسيد النيتريك، ووظائف الكبد والكلى، والغلوتاثيون المختزل، والقدرة المضادة للأكسدة الكلية بشكل ملحوظ في الدواجن التي تم اعطائها .OTA. بينما كانت مستويات متبقيات الأوكراتوكسين ملحوظة في الكبد والعضلات للدجاج في المجموعات غير المعالجة التي تلقت الأوكراتوكسين فقط. ومع ذلك، فإن إضافة جزيئات ثاني أكسيد التيتانيوم النانوية إلى النظام الغذائي حسنت جميع المعايير السابقة. في الختام، أدى ثاني أكسيد التيتانيوم النانوي (خصوصًا بتركيز 40٪) إلى تقليل الآثار الضارة لـ OTA في الدجاج اللاحم وحسن التخلص منها من الكبد والعضلات.

الكلمات الدالة: متبقيات الأوكراتوكسين-أ، نانو ثاني أكسيد التيتانيوم، الدواجن، التحليل الكهربائي، علم الدم.