



## Evaluation of the role of green synthesis silver nanoparticles as adsorbents and protective agents for broilers tissue treated with aflatoxin

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### Abstract

Aflatoxin (AF) is the most crucial problem in the poultry industry, feed contaminants lead to toxicity. This study aims to evaluate the effects of synthetic silver nanoparticles mediated by plant extract on the histopathological alteration in broilers treated with AF. Forty-five chicks were divided randomly into three groups: the control group, the second group of chicks were treated with aflatoxin at a concentration of 70 part per million rations for 21 days, the third group of chicks was treated with AF 70 ppm, and silver nanoparticles 150 ppm. Aflatoxin concentration significantly declines in the ratio of the third group 1.86 ppm in comparison to the second group 1.91 ppm. Histopathological examination in the liver of broilers infected with aflatoxicosis revealed vascular disturbances involving congestion of the central vein, recent thrombus formation, and dilatation of sinusoid with the cell adaptation mainly hyperplasia of epithelial cells lining the bile duct, and this considers pathognomic lesion of aflatoxicosis and hepatic necrosis. The silver nanoparticles exhibit a tissue protective role against aflatoxin, improving the histopathological architecture. The I See an Inside method is a good tool for statistical histopathological analysis, which revealed significant elevation in the score of liver alteration in the second group in contrast to the third and first groups. The study concludes that silver nanoparticles can adsorbent the aflatoxin and reduce its deleterious toxic effect, and ISI is one of the vital techniques for detecting toxic effects in variable parts of the liver.

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### Introduction

*Aspergillus flavus* and *A. parasiticus* are the significant fungal microorganisms that produce aflatoxin, which are secondary toxic metabolites that contaminate poultry feed under high humidity and temperature (1-3). Aflatoxin B1 (AFB1) is the most hazardous of the critical aflatoxins (4). Additionally, it is regarded as a Group I carcinogen. Because the administration of AFB1 has adverse hepatotoxic, mutagenic, carcinogenic, and teratogenic effects on people and many species of livestock, even fish (5), major economic losses are posed by human and animal consumption of feed or food products affected by this toxin (6), the permissible dose for poultry is 20 ppb. The critical economic impact of aflatoxicosis in broilers is the reduction in body weight gain

and growth performance, which is likely brought on by changes in protein metabolism (7-9). Nanotechnology is promising and innovative, with a wide range of applications, and has great potential benefits in the poultry industry (10). Nanoparticles have unique physical and chemical properties, such as their small size ranging from 1-100 nm and large specific surface area SSA, they are more bioavailability, stable, and bioactivity than bulk particles (11), and therefore they are widely used in drug delivery, vaccine preparation, immune stimulation, encapsulated bioactive compound as well as their activity as antifungal, anti-parasitic, antiviral and antibacterial in addition anti mycotoxin (12-15). There are physical and chemical methods for manufacturing nanoparticles, and recently, biological methods based on microorganisms (fungal and bacteria) and plant extracts have

been used. This method is called green synthesis. It is environmentally friendly because it produces nanomaterials that are pure and less toxic compared to chemical methods and less economical (16). Abdel Ghany (17) refers to the robust activity of silver nanoparticles mediated by cinnamon oil as an antifungal and a decline in toxin production.

So, this study aimed to evaluate the silver nanoparticle (Ag NP) activity mediated by peel pomegranate as improving growth performance, biochemical, and repair tissue injury in the broiler with aflatoxicosis.

## Materials and methods

### Ethical prove

Scientific Ethical Committee on Animal Experimentation at College of Veterinary Medicine, University of Mosul, UM.VET.2022.015.

### House and animal management

In this investigation, 45 unsexed broiler Ross 308 1-day-old get from a local hatchery were used and transported to a cage 2x1.5 m<sup>2</sup> in the form of the house care animal in College of Veterinary Medicine- University of Mosul. This farm was declared safe and disinfected with formaldehyde gas (produced by combining 40% formalin with potassium

permanganate powder) and kept in a well-ventilated chamber and electric bulbs 200 watts were used in each partition to keep the room at the light and dark cycle for 23/1 hrs./day. Fresh, clean straw had been placed over each cell to create a deep litter 4 centimeters deep. Moreover, there was an appropriate feeder and waterer in each compartment. The chicks were weight as soon as they arrived farm. Newcastle disease and Evan Influenza were given to birds as vaccines (Intervert -injected S.C.), and water and food were available during the experimental 21 days. Preparation of silver nanoparticles according to Al-Othman *et al.* (18)

### Pomegranate peels aqueous extract PPE

Pomegranate peel was obtained from a local supermarket, washed with deionized distilled water, and the drying at 50 °C for 72 hours, then with a blender, crushed into powder, 100 ml of sterile, deionized water and 10 g of peel powder were combined, heated for 15 minutes, and then filtered using Whatman filter paper No. 1.

### Synthesis of silver nitrate nanoparticles mediated by PPE

100 ml of silver nitrate 1 mM solution was combined with 10 ml of PPE before being incubated at 60-70°C for 15-20 minutes. The characterization result of AgNPs prepared as in table 1 (19).

Table 1: Characterization of AgNPs mediated by PPE (19)

Measurements techniques	Characterization of NPs
Color	Dark-Brown after three days
UV-visible Spectroscopy	The peak at the wavelength of 450 nanometers
FTIR	451.34 cm <sup>-1</sup> is the band for silver nanoparticles binding with O <sub>2</sub> from hydroxyl groups
SEM	The average size was 40nm
Zeta potential	The stability of AgNPs at degree -29 mV

### Prepare a diet contaminated with AFB1

AFB1 was obtained from inoculated *Aspergillus flavus* in the rice medium and incubated at 28°C for at least two weeks. Following a successful fermentation, the rice was steam heated to inhibit fungal growth (20), then the contaminated rice was dried and ground, then mixed with diet according to the Al-Shawabkeh *et al.* (21).

### Experimental design

The birds were randomly divided into 3 groups, each subdivided into three replicates, 5 birds /replicate. First treatment: Basal starter diet. Second treatment: Basal diet with 70 ppm AFB1. Third treatment: Basal diet with 70 ppm AFB1 with 150 ppm silver nanoparticles (19).

### Quantitative estimation of aflatoxin

ELIZA technique has been done to detect the AFB1 concentration in the ratio according to Al-Sawaf and Abdullah (22), Al-Dabbagh (23).

### Histological analysis

The cervical displacement method was used to sacrifice the anesthetized broilers, and sections of the liver were fixed in 10% formalin for histological analysis (24).

### I See Inside (ISI) technique

This method depends on the analysis of histopathological alteration in the liver according to Domingues *et al.* (25), who explains the ISI depends on the following evaluation: impact factor (IF), which means the histological alteration if it is reversible or irreversible, it is range from 1- 3, and each lesion take the score as in the table 2. ISI = IF× Score value (Table 2).

### Statistical analysis

The liver histopathological section in the variable group was analyzed. Shapiro-Wilk Test was first used for data normality, followed by Kruskal -Wali's test at P≤ 0.05.

Table 2: Score for the percentage histopathological area %

Histopathological area involvement %	Score
Absence of pathological alteration 0%	0
Histopathological alteration up to 25%	1
Histopathological alteration from 25 -50%	2
Histopathological alteration up to 50%	3

**Results**

**Quantitative estimation of aflatoxin**

The statistical analysis of the results of this experiment revealed there were significant differences in the concentration of aflatoxin in the experimental diets, as the significant increase of aflatoxins in the diet of the second group to which the toxins were added at a concentration of 70 parts per million and the average was 1.91 parts per gram, in contrast, to control (first) group, in the third group in which the silver nanoparticles were added to ration at 150 ppm, there was decline significantly in the aflatoxin concentration to the 1.85 ppm (Figure 1).

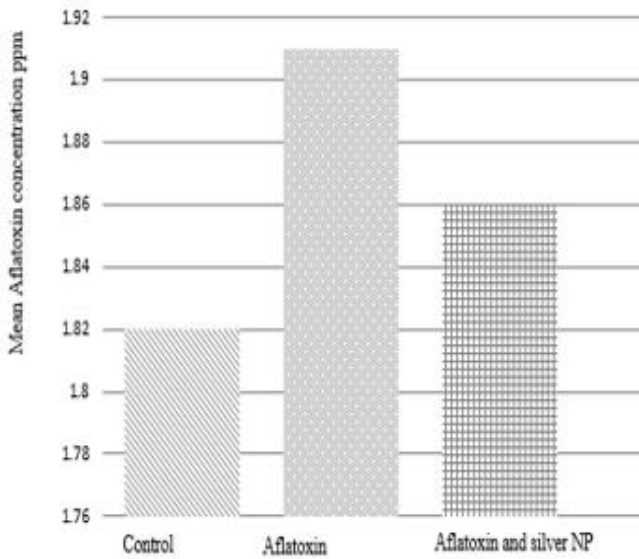


Figure 1: Effects of Silver nanoparticles on the Aflatoxin concentration (ppm) in the broiler ratio.

**The microscopic examination**

The histopathological examination for the broiler liver treated with aflatoxin 70 ppm for 21 days shows variable alteration characterization by recent thrombus and proteinaceous deposition in the central vein and congestion. Dilatation in the sinusoid (Figure 2) with hepatic necrosis (Figure 3), the hyperplasia of epithelial cells lining the bile duct and sloughing to the lumen (Figure 4). Furthermore, the edema and hemorrhage are one of the hepatic circulatory disturbances and severe necrosis in the hepatic tissue (Figure

5). The silver nanoparticles interfere with the pathological effects of aflatoxin in liver architecture, so a microscopic examination of liver feeding with a diet treated with aflatoxin and 150 ppm of silver nanoparticles revealed congestion in the hepatic central vein (Figure 6) and dilatation of sinusoids with slight edema (Figure 7).

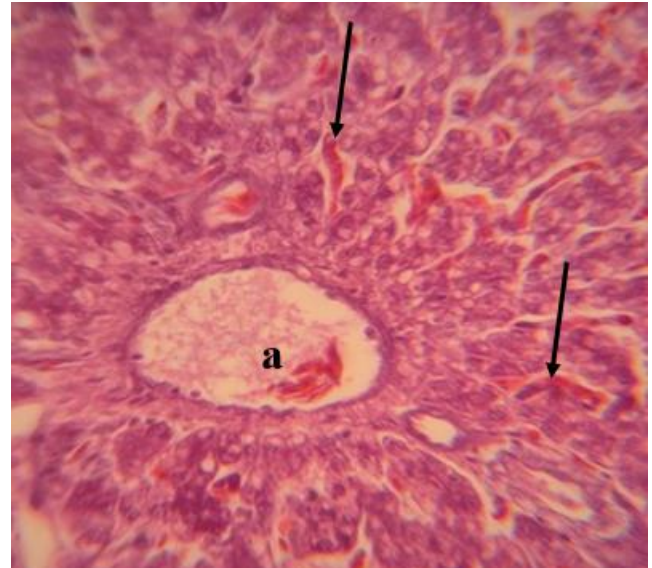


Figure 2: Histopathological examination of the liver in chicks treated with AF (70) ppm for 21 days revealed recent thrombus and proteinaceous deposition in the central vein (a) congestion and dilatation in the sinusoid (Black rows), 10 × 2.1X, H &E.

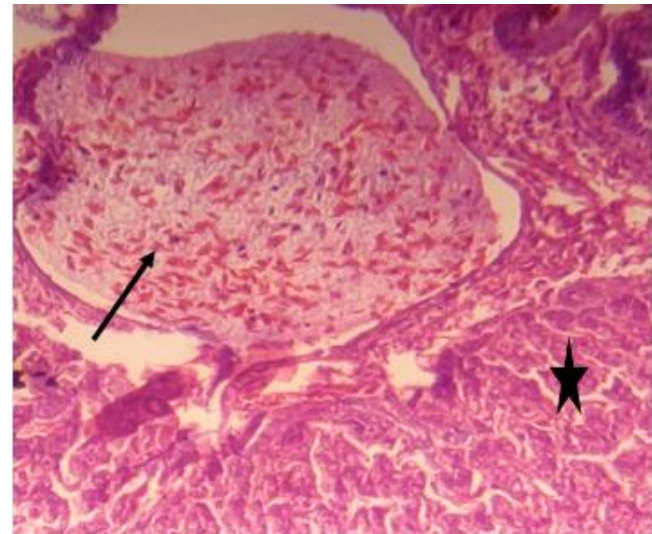


Figure 3: Histopathological examination of the liver in chicks treated with AF (70) ppm for 21 days revealed thrombus (Black row) and necrosis in the hepatic tissue (black star), 40 × 2.9X, H&E.

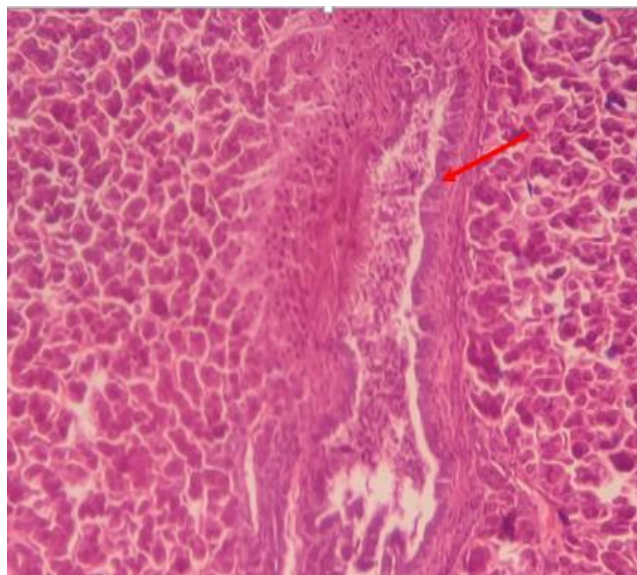


Figure 4: Histopathological examination of the liver in chicks treated with AF (70) ppm for 21 days revealed hyperplasia of epithelial cells lining the bile duct (red row);  $10 \times 5.1X$ , H&E.

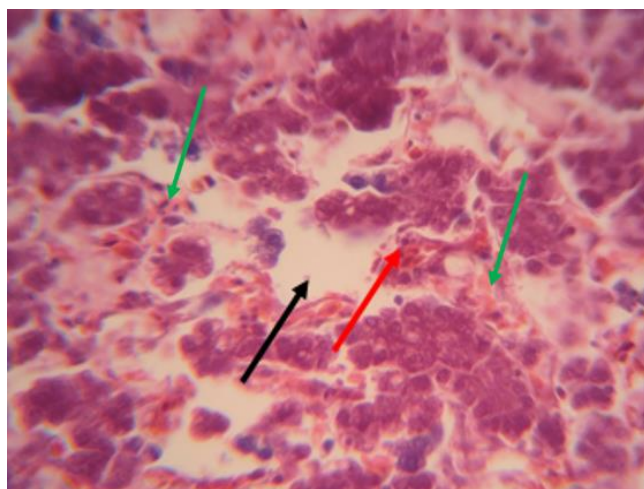


Figure 5: Histopathological examination of the liver in chicks treated with AF (70) ppm for 21 days revealed edema (Black row) and necrosis in the hepatocyte (red row) with hemorrhage (green row),  $40 \times 2.9X$ , H&E.

#### ISI Technique

The statistical analysis for the result of ISI of the liver section at variable groups revealed significantly elevated histopathological alteration in the liver of chicks treated with aflatoxin, but the role of silver nanoparticle was clearly in improvement in the lesions in group third in contrast to the second group but remain significantly elevated in comparison to the second group (Table 3).



Figure 6: Histopathological examination of the liver in chicks treated with AF (70) ppm + AgNPs for 21 days revealed central vein congestion (Black row),  $40 \times 2.7X$ , H&E.

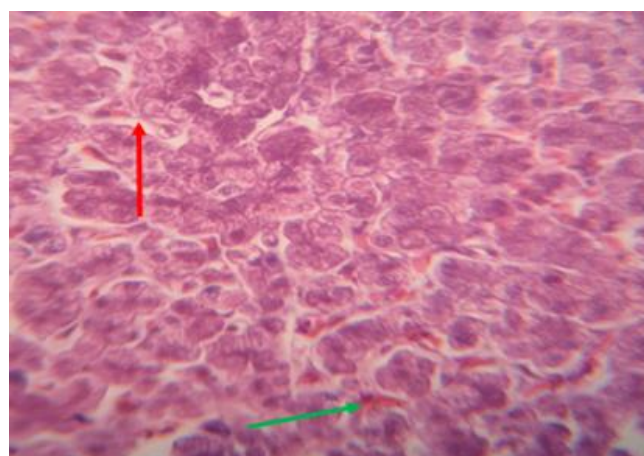


Figure 7: Histopathological examination of the liver in chicks treated with AF (70) ppm + AgNPs for 21 days revealed dilatation of sinusoids (green row) and edema (red row),  $40 \times 3.6X$ , H&E.

#### Discussion

Aflatoxin is one of the main mycotoxins that cause acute or chronic toxicity, mainly affecting the liver and leading to hepatitis and carcinogenic immunosuppression for humans and animals (26,27). There are several methods (chemical and physical) for removal and detoxification the aflatoxin from feedstuff, but the green synthesis nanomaterials depend on the herbal plant extract is the recent attention from researchers and future studies mainly silver nanoparticles because it is unique characteristic as an anti-microbial agent without causing deleterious effect for human also have the ability as antifungal *Candida* sp. and *Trichosporon* (28,29).

Table 3: ISI Score of liver histopathological analysis

Groups	Categories	IF	Score	ISI
Control	Congestion	1	1	1C
	Hemorrhage	3	0	
	Edema	2	2	
	Hyperplasia	1	0	
	Necrosis	3	0	
Aflatoxin	Congestion	1	3	30A
	Hemorrhage	3	3	
	Edema	2	3	
	Hyperplasia	1	3	
	Necrosis	3	3	
Aflatoxin with Silver	Congestion	1	3	9B
	Hemorrhage	0	0	
	Edema	2	2	
	Hyperplasia	1	2	
	Necrosis	0	0	

Different litters mean there was variable significantly at  $P \leq 0.05$ .

These studies revealed the activity of silver nanoparticles as an antifungal and a significant decline of the mean concentration of aflatoxin in the broiler ratio in contrast to the ratio with aflatoxin 1.86 and 1.91 ppm, respectively. These results come with the conclusion of the study of Mousavi and Pourtalebi (30), who reported that silver nanoparticles act as an antifungal and are useful to control aflatoxin contamination, Zhao *et al.* (31) referred to the ability of silver nanoparticles to inhibit the growth of *Aspergillus flavus* and decrease the production of aflatoxins.

Antifungal activity of silver nanoparticles depends on its ability to cause oxidative stress. The production of the active oxygen radical ( $\cdot O_2$ ) then changes the shape and function of the fungal hyphae, or it may be the active substances that in the manufacture of silver nanoparticles in the green industry method, it is important in the absorption of aflatoxins, low absorption and rapid excretion from the gastrointestinal tract (31), the previous study has indicated that nanoparticles consider cytotoxicity cause lipid peroxidation and interact with both nucleic acid synthesis and mitochondrial functions (32,33). Silver nanoparticles are synthesized by (pomegranate peel extract). These eco-friendly substances have bioactive compound as tannin at 9.33% and polyphenolic-containing and astringent agents, which are considered toxic for organisms or may relate to physical activity between the negative charge for these substances with a positive charge for fungal spores. These lead to the inhibition of fungal growth and a decline in mycotoxin production (34).

The liver is one of the main target organs for aflatoxicosis and causes microscopic alteration (35), the histopathological investigation of this study revealed variable alteration in the hepatic architecture as congestion, recent thrombus in the central vein with edema, dilatation of the sinusoid and sever

necrosis, these results come with the previous studies Magnoli *et al.* (36), Zabiulla *et al.* (37) and Ashry *et al.* (38), treated chick with 70 ppm of aflatoxin for 21 days lead to hyperplasia of epithelial cells lining bile ducts with sloughing in the lumen this lesion consider pathognomic lesion for aflatoxicosis, result from direct toxicity of mycotoxin or excessive production of prostaglandins (39), aflatoxin impaired lipid, protein and carbohydrate metabolism, increase oxidative stress, lipid peroxidation and disrupting cell permeability and loss ion osmoregulation all these lead to cell injury (40).

Recent studies indicated that biological and herbal plant extracts could be useful for the elimination of mycotoxin as probiotics, curcumin, eggplant shell, pomegranate extract, and sugar apple (41,42), nanomaterial as silver nanoparticles based on herbal plant extract is one of the adsorbents for aflatoxin, the improvement and protective roles of silver nanoparticles which are investigated in this study agreement with the result of Al-zubaidi *et al.* (43), Ismail *et al.* (44) and Śliżewska *et al.* (41) due to it is the activity for binding to the aflatoxin and increase stability it in the fluids of gastrointestinal, as it might decrease or not be absorbed via the digestive tract, another major advantage of silver nanoparticles based on green synthesis is act antifungal and inhibition fungal growth and then decrease mycotoxin production. The ISI technique has demonstrated the ability of aflatoxin to produce histopathological lesions in variable parts of the liver, which has been used by Domingues (25).

## Conclusion

This study concludes that silver nanoparticles can act as an adsorbent material for aflatoxin and protect and improve tissue architecture. This study is one of the first studies using the ISI technique for determining the significant variation in the liver tissue.

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## Conflict of interest

No conflict of interest.

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## تقييم دور التخليق الأخضر لجزيئات الفضة النانوية كعامل ادمصاص ووقاية لأنسجة الفروج اللاحم المعاملة بالأفلاتوكسين

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### الخلاصة

يعتبر الأفلاتوكسين أهم مشكلة في صناعة الدواجن، حيث يؤدي تلوث علف الدواجن إلى التسمم. هدفت هذه الدراسة إلى تقييم تأثيرات جسيمات الفضة النانوية والمصنعة بوساطة مستخلص نباتي في تحسين التغييرات المرضية النسجية في فروج اللحم والمعامل بالأفلاتوكسين. قسمت خمس وأربعون فرخة عشوائياً إلى ثلاث مجموعات، أولها مجموعة السيطرة وتم معاملة المجموعة الثانية بسموم الأفلا بتراكيز ٧٠ جزء بالمليون لمدة ٢١ يوماً، بينما عوملت أفراخ المجموعة الثالثة بسموم الأفلا ٧٠ جزء بالمليون وجزيئات الفضة النانوية ١٥٠ جزء بالمليون. انخفض معنوياً تركيز سموم الأفلا في عليقة المجموعة الثالثة ١,٨٦ جزء بالمليون مقارنة مع عليقة المجموعة الثانية ١,٩١ جزء بالمليون. أظهر الفحص المرضي النسجي في كبد الفروج اللاحم الإصابة بتسمم الأفلا اضطرابات الأوعية الدموية والتي تضمنت احتقان الوريد المركزي وتكون الخثرة وتوسع الجيبانيات مع اضطرابات تكيف الخلايا وبشكل رئيسي فرط تنسج الخلايا الظهارية المبطن للفتحة الصفراوية والتي تعتبر آفة واصمة للتسمم بالأفلا والنخر الكبدي. أظهرت جزيئات الفضة النانوية دوراً وقائياً لأنسجة ضد الأفلاتوكسين، وكان هنالك تحسن في البنية النسجية المرضية. تعد طريقة الرية في الداخل هي أداة جيدة للتحليل الإحصائي المرضي للنسيج والتي كشفت عن ارتفاع معنوي في درجة تغير الكبد في المجموعة الثانية على عكس المجموعة الثالثة والأولى، يستنتج من هذه الدراسة إلى أن جزيئات الفضة النانوية لها القدرة على ادمصاص سموم الأفلا وتقليل من تأثيراتها السمية الضارة وإن طريقة الروية في الداخل هي واحدة من التقنيات الحيوية للكشف عن التأثير السام في أجزاء مختلفة من الكبد.