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Determination the Effect of Silver Nanoparticles and *Bifidobacterium bifidum* on the Biological and Immunological Parameters of Male Rats

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Abstract

The study was aimed to determine the effect of oral dosage of AgNPs or *Bifidobacterium bifidum* on some biological and immunological parameters of male laboratory rats after fed with 2.5 mg of aflatoxin/ kg feed for 21 days. The results showed that there was a significant increased ($p<0.05$) in the relative weights of the liver, kidneys and spleen in the fed groups compared with the group of rats given aflatoxin alone. Also, the concentrations of liver enzymes AST and ALT and immunoglobulins of IgA, IgM, IgG and IgE were significantly increased compared with the Rats group that treatment with fed with aflatoxins. The oral dosage of AgNPs or *Bifidobacterium bifidum* were return the biological and immunological parameters of male laboratory rats significantly to the same values in male rats in control group.

Key words: AgNPs, *Bifidobacterium bifidum*, Aflatoxins, Male rats.

Introduction

Cereal crops, especially wheat grains, during agricultural production and after harvest are exposed to contamination by microorganisms, especially fungi, as the moisture content of these crops after harvest remains high or the storage is not typical, lack of ventilation and flipping in silos and grain stores all give the opportunity for different types of fungi to grow and multiply in them. And toxin production [(1)].

Among these fungi are the genera Aspergillus, Fusarium, and Penicillium, which are able to produce mycotoxins at high concentrations. Among the fungi species *A.flavus* and *A.Parasiticus* are the two most dangerous species among the species mentioned fungi. When a contaminant exists for wheat grains or other cereal crops, it is able to produce aflatoxin, especially the highly toxic type B1[(2),(3)].

Studies have confirmed that it is a carcinogenic factor in the liver and kidneys and a suppressor of the immune system and has significant effects on genetic factors through the induction defects in DNA of cells, and various other health effects in the cells and organs of the body [(4),(5)] Many methods have been used in attempts to remove or reduce mycotoxins in cereal crops when they are in the field or while they are consumed by animals, but all of these methods did not reach the level of total removal of those toxins, especially aflatoxins, and the levels of toxin reduction ranged in varying proportions. They are more than moderately efficient [(5)].

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Various species of lactic acid bacteria have been used in the process of reducing these toxins, but the *Bifidobacterium bifidum* is a few in its uses, which are characterized by its ability to produce various metabolites as well as its ability to be absorbed with the mycotoxin and its ability to compete with the fungus to grow in the environments with which it is present.

Nanotechnology, which is considered one of the modern technologies, can interfere in inhibiting fungi and reduce their level of growth as well as their production of toxins, and their ability to interfere to inhibit the effectiveness of the toxin or reduce its presence, especially the AgNPs, which are one of the most important types of nanoparticles in Microbial inhibition [(6)].

Therefore, the aim of the study was suggested to tring for use of probiotic *Bifidobacterium bifidum* or AgNPs silver nanoparticles in reducing the effects of aflatoxins on biological parameters were estimated in male laboratory rats.

Materials and methods

Preparation of *Bifidobacterium bifidum*: Bifidobacterium bifidium bacteria were obtained as a standard diagnostic strain from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) (Germany), where the bacteria were activated by cultivating them on liquid MRS medium and vaccinated under anaerobic conditions at 37 ° C for a period of 24 hrs. Then 0.1 of the liquid media was transferred and spread on the surface of the solid MRS medium. The dishes were incubated for 24 hours at 37 ° C. The isolates obtained were then preserved for use in subsequent experiments.

B. bifidium strain was grown in liquid MRS medium for 24-48 hrs and after its total numbers were determined at 1.5 x 10^8, it was placed in a centrifuge at 5000 r / min for 15 minutes. Then the sediment was separated and the supernatant discarded, then the sediment was kept in test tubes at 4 ° C until use.

Preparation of the concentration of AgNPs: The concentrations of AgNPs obtained from (Nanoshel, USA, with sizes of 30 nm) were prepared as suggested in the biological experiment after dissolving 25mg of the AgNPs in 25 ml of distilled water to obtain the stock concentration that was used to prepare the concentrations which required in the experiment.

Initialization of laboratory animals: Healthy and disease-free laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit with 24 male rats of Albino adult aged between 8-9 weeks and their weights ranged from 145-150 grams. The adult animals were divided randomly to 6 groups, each group included 5 animals as follows: (T1): a group of control animals, (T2): the group of animals fed with 2.5 mg/ kg of aflatoxin feed, (T3): the group of animals orally dosage 0.025 mg of AgNPs/ kg of body weight, (T4): group of animals orally dosage of 0.025 mg AgNPs/ kg body weight + 2.5 mg aflatoxin/ kg of feed, (T5): group of animals given orally 2 ml of *B. bifidum* bacterial suspension contains 1.5 x 10^8 cell/ ml, (T6): group of animals orally dosage of 2 ml of *B. bifidum* cell suspension / ml + 2.5 mg aflatoxin / kg of feed.

The animals were placed in plastic cages with sawdust that were replaced every two days. Animals were fed regularly using ready-made diet according to [(7)], aflatoxin was added with the feed, while the orally dosage of bacteria and AgNPs was given in a 2 ml for each one, one ml was orally in the morning and the other in the evening. The breeding period of male rats was continuous for 21 days.

The parameters assay: Immediately after the end of the experiment, the animals were left without food for 20 hours, then the animals were anesthetized with chloroform, after which the rats were dissected from the chest area and the internal organs of each of the liver, kidneys and spleen were extracted and their weight was taken. Also, blood was drawn directly from the heart with approximately 6 ml, which was centrifuged using a central centrifuge at 3000 rpm for 15 minutes to obtain the serum that was kept at -20 ºC until the analysis was conducted.

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Where the effectiveness of the liver enzymes AST and ALT was estimated by using the analysis kit provided by the British Randox Company. Also, IgG, IgA, and IgE IgM immunoglobulins were determined using ELISA Assay according to the manufacturer's instructions for the analysis crews and as in [8].

Statistical analysis: The data were analyzed statistically through the experiments system within the ready statistical program (SAS, 2012), and by using the complete random design system (CRD). The averages were chosen according to Duncan's test [9].

Results and discussion

The effect on the relative weight of the internal organs: The effect of oral dosage of AgNPs or *B. bifidum* on the relative weight rates of the liver, kidneys and spleen in rats given aflatoxin for a period of 21 days was shown in Table (1).

The results showed that the effect of aflatoxins caused a significant increase ($p<0.05$) in the relative weight of each of the liver, kidneys and spleen, as their weight averages were at 5.23, 0.84 and 0.88 g / 100 g of body weight compared with their relative weights in the control group. They were at 3.91, 0.69, and 0.62g / 100g of body weight. The oral administration of AgNPs or *B. bifidum* T3 and T5 did not cause a significant change in the relative weight of the internal organs above.

However, oral administration to animals fed on aflatoxin caused a significant reduction in the effect of these toxins compared with their relative weights in the control group. The relative weights of liver, kidney and spleen in the T4 treatment administered orally of AgNPs of laboratory rats fed with Aflatoxins were at 4.16 and 0.75. And 0.74 g / g body weight, respectively. Likewise, in the treatment T6 administered orally from B. bifidum, its relative weights were 4.04, 0.73, and 0.75 g / 100 g of body weight, respectively.

Table (1) Effect of oral dosage of AgNPs or *B. bifidum* on relative weight rates of liver, kidney and spleen in rats fed aflatoxin for 21 days.

Treatments	Spleen	Kidneys	Liver
	g / 100 g of body weight		
T1	0.05 ± 0.62 C	0.10 ± 0.69 B	0.30 ± 3.91 B
T2	0.06 ± 0.88 A	0.06 ± 0.84 A	0.20 ± 5.23 A
T3	0.12 ± 0.65 C	0.06 ± 0.71 B	0.37 ± 3.77 B
T4	0.06 ± 0.74 B	0.10 ± 0.75 B	0.26 ± 4.16 B
T5	0.03 ± 0.64 C	0.05 ± 0.68 B	0.13 ± 3.84 B
T6	0.14 ± 0.75 B	0.06 ± 0.73 B	0.34 ± 4.04 B

*** The different letters within one column indicate the presence of significant differences at the 0.05 probability level.**

T1: control, T2: aflatoxin at a concentration of 2.5 mg / kg, T3: AgNPs 0.025 mg / kg, T4: AgNPs + aflatoxins, T5: Bifidobacterium bifidum 1.5 × 810, and T6: Bifidobacterium bifidum + aflatoxins.

The results were in agreement with, who found that subcutaneous injection of AgNPs at concentrations of 5 and 50 μg / kg body weight in male rats led to a significant difference in the relative weights of the internal organs compared to the concentration of zero (control sample) [10].

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The main reason that caused the enlargement of the internal organs of the liver, kidneys and spleen could be due to the effectiveness of B1 aflatoxins in disrupting the work of enzymes in those organs by inhibiting their activity and thus causing the cells of those organs to turn into fat cells, which caused an increase in their size and weight. Comparison with same organ cells in control group animals [(11)].

Either the resulting improvement in the relative weight of internal members after oral administration both AGNPS or B.Bifidum, they can be caused by effectiveness effects each of the two cases. AGNPS silver nanoparticles can interfere with the B1 aflatoxin combination or link with some effective sites of toxin, As well as the possibility of Adsorbed it with the nanoparticle molecules caused to reducing its toxic effect on laboratory Animals [(12)], As for the oral administration of the B. bifidum bacterial suspension, it may be due to the activity of the metabolites resulting from the bacterial species.

In addition, its ability to produce organic acids from its fermentation of sugars present in the middle of the intestine caused a change in the periphery of the intestine to be acidic, which results in a change in the effectiveness of aflatoxin B1 and its decrease, and thus diminution its effect on the biological parameters in laboratory rats [(13)].

Effect on liver enzyme activity: Table (4-6) shows the effect of oral administration of AgNPs and B. bifidum on liver enzyme activity parameters of AST and ALT in the blood of laboratory rats fed aflatoxin for 21 days. The results showed that feeding the laboratory rats on a food containing aflatoxin (T2) caused a significant increase ($p < 0.05$) in the activity of liver enzymes for both AST and ALT after assessing them in the blood serum where their values were at 68.0 and 72.67 mg/L. Respectively, compared with the mean of its value in animals of the control group which were 43.33 and 41.0 mg / L, respectively.

The reason for oral administration of AgNPs or B. bifidum was to cause significant improvement in the values of liver enzyme activity rates, as the values of each of them were in the case of administration of AgNPs to laboratory rats fed with aflatoxin B1 at 51 and 49 mg /L, respectively. Administration of B. bifidum bacterium suspension, the mean values were 55.33 and 50.33 mg / L, respectively. Knowing that the results indicated that oral administration of AgNPs or B. bifidum did not cause a significant effect compared to the rates of their values in the control group animals.

Table (2). The effect of oral administration of AgNPs or B. bifidum on liver enzyme activity rates in rats fed aflatoxin for 21 days.

Treatments	ALT (mg / L)	AST (mg / L)
T1	C 5.56 ± 41.00	D 1.20 ± 43.33
T2	A 3.92 ± 72.67	A 1.15 ± 68.00
T3	C 3.21 ± 42.00	D 1.45 ± 43.67
T4	B 2.51 ± 49.00	C 2.08 ± 51.00
T5	C 3.05 ± 43.00	E 3.28 ± 37.33
T6	B 4.97 ± 50.33	B 0.67 ± 55.33

*** The different letters within one column indicate the presence of significant differences at the 0.05 probability level.**

T1: control, T2: aflatoxin at a concentration of 2.5 mg / kg, T3: AgNPs 0.025 mg / kg, T4: AgNPs + aflatoxin, T5: Bifidobacterium bifidum 1.5×10^8, and T6: Bifidobacterium bifidum + aflatoxin.

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The results agreed with [14]. Their study showed that dosing rats with aflatoxin B1 and T2 at a concentration of 1 mg / kg of body weight led to an increase in the level of ALT and AST enzymes at an increase rate of 6-12 compared to the control sample.

Effect on immunological parameters: The effect of oral administration of AgNPs and B. bifidum on the rates of immunological parameters of IgA, IgM, IgG and IgE in the blood of laboratory rats fed on aflatoxin for 21 days was shown in Table (4-7). The results showed that feeding laboratory rats with food containing aflatoxin caused a significant decrease ($p < 0.05$) in the values of IgA, IgM, IgG and IgE immunomodulators and they were at 169.67, 129.33, 1042.67 and 81.33 mg / L, respectively, compared with the values of The same parameters in animals of the control group which were 147.67, 100.17, 933.67 and 62.67 mg / L, respectively.

The oral administration of AgNPs or B.bifidum in laboratory rats (T3 and T5) did not cause significant difference in the values of IgA, IgM, IgG and IgE immunoassays from their values in control group animals(T1). Also, the oral administration of AgNPs or B. bifidum in laboratory animals fed food containing aflatoxin caused significant improvement in the values of IgA, IgM, IgG and IgE immune parameters.

Whereas, the effect of oral administration of AgNPs resulted in the values of the above immunoassays at 155.33, 115.63, 1007.67 and 72.0 mg / L, respectively. In the case of oral administration of B. bifidum bacterium suspension, it caused a significant improvement in the values of the immunomodulatory parameters. They were 154.0, 108.03, 982.0 and 73.0 mg / L, respectively, compared with their values in the control group.

Table (3) The effect of oral administration of AgNPs or B. bifidum on the rates of immunity parameters in rats fed aflatoxin for 21 days.

Treatments	IgE (mg / L)	IgG (mg / L)	IgM (mg / L)	IgA (mg / L)
T1	C 5.33 ± 62.67	16.30 ± 933.67 E	6.85 ± 100.17 D	12.73±147.67 C
T2	A 5.75 ± 81.33	14.18±1042.67 A	5.28 ± 129.33 A	5.71 ± 169.67 A
T3	C 6.42 ± 64.67	11.65 ± 935.00 E	13.13±101.03 D	9.81 ± 149.33 C
T4	4.65 ± 72.00 B	18.20±1007.67 B	8.72 ± 115.63 B	9.24 ± 155.33 B
T5	3.87 ± 63.67 C	14.51 ± 941.00 D	12.25 ± 98.60 E	7.13 ± 145.00 D
T6	B 2.56 ± 73.00	11.27 ± 982.00 C	8.22 ± 108.03 C	13.22±154.00 B

*** The different letters within one column indicate the presence of significant differences at the 0.05 probability level.**

T1: control, T2: aflatoxin at a concentration of 2.5 mg / kg, T3: AgNPs 0.025 mg / kg, T4: AgNPs + aflatoxin, T5: Bifidobacterium bifidum 1.5×10^8, and T6: Bifidobacterium bifidum + aflatoxin.

The results are in agreement with what was mentioned [15] who found that oral dosing of rats with zinc nanoparticles showed an increase in the level of immune proteins compared to the control sample. aflatoxin B1 significantly reduced levels of immune enzymes compared with the control sample, while the results were in agreement with mentioned by [16] who found that dosing male rats 1 ml of milk containing Bifidobacterium longum (108 CFU) per day led to a decrease in immune enzymes compared to the control group.

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The effect of feeding laboratory rats on aflatoxin B1 caused a significant increase in the values of the immunological parameters of all types of globulins, which indicates that the toxins above cause a defect in the functions of several organs of the body, which caused an increase in their values compared to their values in the control group, [17].

The positive effect of AgNPs or B. bifidum in causing significant improvement in immunoglobulin values in laboratory rats fed on Aflatoxins may be due to the properties of both of them in reducing the effectiveness of toxins.

As the AgNPs silver nanoparticles could have an effect on the interference with the toxin composition and thus the effect on the dissolution of the toxin composition and the loss of its effectiveness through changing its composition, which reduced its effect on liver enzymes and caused the return of their values to close to their normal values in the control group animals.

As for B. bifidum bacteria, Its effectiveness can be through its Adsorption with the toxin compounds and thus the effect on its efficacy and reduction as well as its ability to change the acidity of the medium in which the toxin is present, causing its lack of effectiveness and thus achieving a decrease in its effect on the effectiveness of liver enzymes and other health standards [18].

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