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Study of the Influence of the Biochemical Parameters of Laboratory Rats Treated with Aflatoxin and Black Seed Oil

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Abstract

The aim of this study was to determine the effect of Black seed oil on the reduction of the negative effects of Aflatoxin in white rat females and on the biochemical parameters The results showed that Aflatoxin B_1 (AFB_1) and Aflatoxin B_2 (AFB_2) had a significant effect on lowering blood cholesterol levels in the treated animals to (50 , 59) mg / 100 ml respectively compared to the control treatment of (89) mg / 100 ml , while the level of cholesterol to (67 ,70) mg / 100 ml when treated animals with aflatoxin (AFB_1+ 800) and (AFB_2 + 800)mg / kg oil. On the other hand , The level of TG triglyceride decreased to (78.5 , 79.9) mg / 100 ml when treated with Aflatoxin B_1 (AFB_1) and Aflatoxin B_2 (AFB_2) compared with the control treatment reached to (93) mg / 100 ml. while a significant increase in triglycerides was observed to (89 , 90) mg / 100 ml when treatment animals with aflatoxin (AFB_1+ 800) and (AFB_2 + 800)mg / kg oil .

Key words: *biochemical parameters, laboratory rats, aflatoxin*

Introduction

Cereals are important strategic crops at the local and global levels. their importance in terms of consumption is in the first place. They are infected with fungi that have the ability to produce metabolic substances, that carcinogenic to humans and animals called Mycotoxins. Studies indicate that most fungi found in food produce more of these Mycotoxins during their growth [1] and the secretion of these toxins in the grain makes them unsuitable for human consumption or animal feed [2] . *Aspergillus flavus* which affects the vitality of the seeds and prevented germination for seed [3]. The World Health Organization's Global Agency for Cancer Research has included aflatoxins as a cause of human cancer [4]. In animals when giving poultry, doses of aflatoxin in amounts ranging from 0.5-0.25 mg / kg in diets resulted in reduced immunity against bacterial and viral infections and cause of fetal loss after 21 days of administration [5]. In study Mushin and others, 2009 using *Aspergillus parasiticus* has been affected by liver toxins, where liver tissue decomposition occurred with the death of necrosis, either kidney. the other was affected by the toxins produced by the fungus, where the breakdown of renal tubules appeared either in the intestine and not affected by the fungus Produced [6]. *Bipolaris micropus* was also affected by toxicity in all studied organs, causing liver necrosis death with renal tubules. The intestines were also affected by the toxins produced by the fungus, where the palms appeared in the vesicles as they expanded. As a result of the increased content of these seeds from potassium and (0.789 mg / kg) is of great benefit to people who use diuretics to control high blood pressure and suffer from excess potassium loss from external body fluids. on the other hand, it is used against skin infections, fungi and bacteria [6]. Nigellon is the active ingredient in black seed oil that increases the killer cells by 70%, which protects the body against liver cancer, liver fibroid [7], rheumatic pains. Several experiments have shown that the Black seed oil inhibits the growth of bacteria and has proved to have a good effect against the cholera and that the effect of oil is similar to the effect of penicillin and its derivatives and found that the oil of the pond is effective against fungi, especially *Aspergillus* spp [8].

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Methodology

The isolation of *A.flavus* was obtained from the laboratories of the Faculty of Science / University of Kufa and its ability to produce aflatoxin was tested according to the method

Preparation of concentrations of the Black seed oil and study the effects in medium

In this experiment, tow concentration (200, 800) mg \ L were mixed with the sterile PDA after cooling. all dishes were vaccinated with 0.5 cm disc from the fungus at the center of the dish. Then incubated at 25 ° C for 7 days and by three replicates per treatment with a control contain *A.flavus* for each treatment. After the colonies reached the edge of the dish, the rate of inhibition of fungal growth was calculated. Their last rate fungi and calculate the amount of orthogonal inhibition according to equation, which is as follows:

$$\text{Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 Maximum Radial Growth of fungi Colony (control Treatment)

R_2 Maximum radial growth of fungi colony in oil-containing dishes.

Preparation and feeding of laboratory animals:

In this experiment used 16 animals divided into 7 groups:

The first group :

2 rats were injected orally with 0.5 ml of aflatoxin B_1 and Black seed oil at a concentration of 200 mg / kg of 0.5 ml daily for one week.

the second group :

2 rats were injected orally with 0.5 ml of aflatoxin B_1 and Black seed oil at a concentration of 800 mg / kg of 0.5 ml daily for one week.

Third group:

2 rats were injected orally with 0.5 ml of aflatoxin B_1 daily for one week.

Fourth group:

2 rats were injected orally with 0.5 ml of aflatoxin B_2 and Black seed oil at a concentration of 200 mg / kg of 0.5 ml daily for one week.

Fifth group:

2 rats were injected orally with 0.5 ml of aflatoxin B_2 and Black seed oil at a concentration of 800 mg / kg of 0.5 ml daily for 1 week.

Sixth group

Two rats were injected orally with 0.5 ml of aflatoxin B_2 per day for a week.

Seventh group

Included 4 rats without any dosage as control treatment.

Two days after the last dose, the animals were sacrificed after anesthesia with chloroform and explained by opening the ventral cavity. The blood was drawn by the heart puncture and the blood was drawn in tubes containing anticoagulant to calculate some biochemical blood parameters.

Measurement of biochemical parameters

Determination of total serum cholesterol

The enzymatic method described [10] was used to estimate total serum cholesterol and optical absorption was read along a wavelength of 500 nm. That the principle of the interaction depends on the conversion of cholesterol ester by cholesterol esterase to free cholesterol, which is oxidized by the enzyme (Cholesterol Oxidase) to the Cholest-4-en-3one and hydrogen peroxide, and the presence of a substance due to hydrogen and by the enzyme Peroxidase oxidation base material colorless to pigment The quinonein is pink, and the intensity of the color varies with serum cholesterol concentration.

Cholesterol esterase

Cholesterol ester + H_2o → Cholesterol free + Fatty acid

Cholesterol Oxidase

Cholesterol free + O_2 → Colest-4-en-3-one + H_2O_2

Peroxidase

2 H_2O_2+Phenol + 4–aminoantipyrine ——————› Quinoneimine+4H2O Pink

The screening process was carried out according to the following steps:

1- Put (2.5 ml) of the work solution in three tubes of choice.

2- Addition (0.025 ml) of the first tube sample serum and (0.025 mL) of the standard solution cholesterol for the second tube and (0.025 ml) of the distilled water of the third tube and the contents were mixed each tube is well.

3 - Then put the three tubes in the water bath for (5 minutes) has a temperature (37) ^{0}C.

4-The optical spectrometer was first calibrated with distilled water and then Blank solution on the wavelength (500 nm).

5- The absorption of the sample was read and the absorbance standard

was read.

6. Extract the concentration of cholesterol in the sample serum according to the following equation:

$$\text{Total Cholesterol (mg/dL)} = \frac{\text{A sample}}{\text{A Standard}} \times 200$$

Estimation of serum Triglycerides

The enzymatic method (Fossati and Prencipe, 1982) was used and the optical absorption on505 m.n

1- add 2.5 ml of work solution was added in three test tubes

2- Put 0.025 ml of the sample serum in the first tube and 0.025 ml of the standard solution in the second tube and 0.025 ml of distilled water in the third tube and then mix the tubes well.

3- The three tubes were transferred to the water bath for 5 minutes at a temperature of 37 °C.

4- The optical spectrometer was then calibrated with distilled water and then blank solution, at a wavelength 505 nm.

6- Absorbance sample and absorbance standard were read.

7- Extracting the value of Triglycerides in the sample serum according to the following

equation:

$$\text{Triglycerides Conce .(mg /Dl)} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

Determination of the efficiency of the two enzyme carriers of the amine group Alanine and Aspartate Transaminases (ALT, AST) in the serum

This method is based on the estimation of the amount of liberated pyruvate and Oxaloacetate by their interaction with dinyphenylhydrazine

Blank	**Test tube**	**Solutions**
-	0.1	Sample (serum)
0.5	0.5	ALT or AST solution

Mix the tubes well. It incubated at 37 ° C for 30 minutes		
0.5	0.5	Dinyphenylhydrazine solution
0.1	-	Sample (serum)
Mix the tubes well. and incubated at a temperature of 20-25 ° C for 20 minutes		
0.5	0.5	Sodium hydroxide solution

The concentration of the contents of the tubes was well, and the absorbance was measured at the wavelength (540nm). The standard curve was obtained for the determination of the pyruvate using different concentrations and as determined in the instructions for the use of the estimation kit. The correlation between the absorbance and the enzyme activity was plotted in U \ 1 units. Which causes the release of one macromolecule of pyruvate within one minute in reaction conditions.

Determination of activity of Alkaline phosphatase (ALP) in serum:

This method is based on an estimate of the amount of phenol released by its interaction with 4 –amino Antipyerine.

Flow Tube	Standard Tubes	Efficient Tube	Sample	Solution
2 ml	2 ml	2 ml	2 ml	Solution No. 1
incubation for 5 minutes at 37 ° C				
-	-	-	50 M	Serum
-	50 M	-	-	Solution No. 2
incubation for 15 minutes at 37 ° C				
0.5ml	0.5ml	0.5ml	0.5ml	Solution No. 3
incubation for 10 minutes at 37 ° C				
0.5ml	0.5ml	0.5ml	0.5ml	Solution No. 4
-	-	50 M	-	Serum
50 M	-	-	-	Distilled water

Mix the contents of the tubes well, then measured the absorption at the wavelength 540 nm)) and obtained the final value using the following equation:

Alkaline phosphatase = N * T \ ST

Where T is the sample for which the absorbance has been measured.

ST is the standard solution for which the absorbance is measured.

N is the concentration of the sample.

Statistical analysis.

All experiments were carried out according to (C.R.D) as single-factor experiments. The averages were compared with the least significant difference of L.S.D and below the level of significance (0.05) (Al-Rawii and Khalaf Allah, 1980).

Results and discussion

Biochemical parameters.

Estimate the level of cholesterol.

The results shown in Figure (1) showed that aflatoxin AFB_1 and AFB_2 had an effect on lowering the level of cholesterol in the treated animals, which

reached (50,59) mg / 100 ml respectively compared to the control treatment of (89) mg / 100 ml .

On the other hands , the cholesterol level increased to (67, 70) mg / 100 ml when treated with (aflatoxin AFB_1+ 800) and (AFB_2 + 800)mg / kg oil compared with the control treatment.

Determination of triglyceride (T.G).

The results indicated in Figure (2) that aflatoxin AFB_1 and AFB_2 resulted in lower triglyceride blood levels (78.5, 79.9) mg / 100 ml compared with the control treatment of (93) mg / 100 ml. On the other hand, a clear increase in triglyceride level was observed reached to (89 ,90) mg / 100 ml when treated animals with aflatoxin (AFB_1+ 800) and (AFB_2 + 800)mg / kg oil compared with the control treatment. These results were identical to that of Sakhare etal (2007), which showed that aflatoxin had an effect on cholesterol, TG, and total protein, leading to reduction in the treated animals.

Determination of the level of liver enzymes ALP, AST, ALT

The results indicate that aflatoxin AFB_1 and AFB_2 has a significant role in elevating the level of liver enzymes ALP, AST, ALT to (92, 250 ,126) IU / L for animals treated with AFB_1 and (107, 190 ,92) IU / L The treatment of AFB_2 compared to the control treatments (28.5, 24.23.5) IU / L respectively, and the oil of the black seed had a role in regulating the level of the enzymes studied and reduced its rates to (51.5, 72, 49) IU / L when using aflatoxin (AFB_1+ 800) mg / kg oil and (42,45,46.5) U / L has the use of (AFB_2 + 800) mg / kg oil. The liver enzymes ALP, AST, ALT are evidence of inflammation and necrosis in the liver. The effectiveness of enzymes is changed due to the destruction of hepato cellular damage, which causes the release of these enzymes and the effectiveness of the higher than in the natural situation and the more the liberation of these enzymes and effectiveness by the poison of the body. this case situation with what the results of the current study showed. The cause of the increased of these enzymes in the serum, but the mycotoxins has caused effects on the liver cells containing these enzymes, which leads to the liberation and then high level if affected the other members such as kidney and the endoplasmic membranes and other organ , increased thus enzymes effect on Bio-transportation system in the body

Conclusion

In addition, the results indicated that Aflatoxin B_1, Aflatoxin B_2, had a significant role in elevating liver enzymes Alanine (ALT) , Aspartate Transaminases (AST) and Alkaline phosphatase (ALP) to (91, 250 , 126) IU / L respectively for Aflatoxin B_1 and reached to (92, 190, 107) IU / L for Aflatoxin B_2 compared to control treatment its (28.5, 24, 23.5) IU / L respectively. The Black seed oil had a role in regulating the level of enzymes studied by reducing their rates this enzymes to (51.5, 72 , 49) IU / L when using aflatoxin (AFB_1+ 800) mg / kg and (42,45,46.5) IU / L when using aflatoxin (AFB_2+ 800) mg / kg.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing/University of Babylon/Iraq and all experiments were carried out in accordance with approved guidelines.

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