

## The Study Effect of Infected Wheat and Rice (With Aflatoxin and Benzoquinone) On Rat Liver

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### Abstract

The current study aimed to estimate the physiological and histological effect of rat liver that feeding of infected wheat and rice. Samples were collected in Kirkuk city, and included Kirkuk silo, Riyadh Al-Makhzani complex, and Taza Grain Center of the General Company for Grain Trade, one of the branches of the Iraqi Ministry of Trade. 10 samples of wheat and rice were collected from 2 kg for each sample of grains for the period from 1-9-2019 until 22-9-2019. The rats were randomly divided into 6 groups (6 rats in each treatment). It included the following: The first treatment (T1): a group of control rats for normal wheat, the second treatment (T2): a group of control rats for normal rice, the third treatment (T3): a group of rats given orally 50% of normal wheat and 50% of the infected wheat contains different concentrations of aflatoxin and benzoquinone. The fourth treatment (T4): the group of rats given orally 25% of normal wheat and 75% of the infected wheat contains different concentrations of aflatoxin and benzoquinone. The fifth treatment (T5): The group of rats given orally 100% to the infected wheat contains different concentrations of aflatoxin and benzoquinone. The sixth treatment (T6): 100% infected rice. The current results showed that there were significant ( $P \leq 0.05$ ) differences between the study groups. The current results group given infected 100% wheat showed a significant increase in AST, ALT and ALP levels compared with control group. On the other hand, it was observed that the group given infected 100% rice showed a significant increase in the levels of AST, ALT and ALP compared with control group given normal rice. Histological study, different histological lesions were diagnosed including degeneration of hepatocytes, fibrosis with lymphocytes infiltration and thickening wall of central veins.

Keyword: Aflatoxin; Benzoquinone; Liver

### Introduction

Damage to agricultural crops, whether in the field or during harvest and storage, due to fungi is also very large, amounting to billions of dollars around the world [1]. Insects, nematodes, fungi, and microorganisms are constantly competing with humans for these commodities. The interactions of these organisms may be more complex. The stored wheat flour is subject to not knowing the heat of the wet grain, and it is possible that the grains will sprout on the surface of the heap, rot and darken in color, acquire an unpleasant odor and increase the acidity in the resulting flour [2]. The

temperature of the grain in the center of the mass may rise due to an insect infestation, and heat is transferred very slowly to the outer layer of the grain [3]. Yellow corn, wheat, barley and soybeans are the main components of poultry feed and these grains are often attacked by many fungi, especially during the storage period [4]. Examples of this variation have been identified for the following: A: Aflatoxins biosynthesis genes from *Aspergillus flavus* and *Aspergillus parasiticus* B: Trichothecenes biosynthesis genes among *Fusarium* species; A: Fumonisins biosynthesis genes in plant species of the genus *Fusarium* [5]. Cereals are the crops most sensitive to the colonization of mycotoxin-causing species, which accumulate in cereals related mycotoxins both in the field, up to harvest, and in storage. According to a study by the Food and Agriculture Organization, nearly 25% of global food and feed production is contaminated with mycotoxins. Therefore, since a large proportion of the world's population consumes grains as a staple food, consumption of grains contaminated with mycotoxins is a major health risk problem worldwide. Furthermore, mycotoxin contamination can have a tremendous economic and social impact, especially when the occurrence of mycotoxins on food commodities [7-8].

## **Materials & Methods**

### **Sample collection sites**

Samples were collected in Kirkuk city, and included Kirkuk silo, Riyadh Al-Makhzani complex, and Taza Grain Center of the General Company for Grain Trade, one of the branches of the Iraqi Ministry of Trade. 10 samples of wheat and rice were collected from 2 kg for each sample of grains for the period from 1-9-2019 until 22-9-2019. Benzoquinone was extracted and determined by HPLC analysis [9]. Aflatoxin was detected by High Performance Liquid Chromatography (HPLC).

### **Laboratory animals**

This experiment was conducted in the animal house of College of Veterinary Medicine at Tikrit University, in which 24 albino male rats were used, their ages ranged between 8-9 weeks and weights between 145 to 150 g.

### **Experimental design**

The rats were randomly divided into 6 groups (6 rats in each treatment). It included the following: The first treatment (T1): a group of control rats for normal wheat, the second treatment (T2): a group of control rats for normal rice, the third treatment (T3): a group of rats given orally 50% of normal wheat and 50% of the infected wheat that contain different concentrations of aflatoxin 0.035 B1 and 0.15 NI and benzoquinone amounting to 1.250 MBQ and 0.8 EBQ and as indicated in the examination tables, the fourth treatment (T4): the group of rats given orally 25% of normal wheat and 75% of the infected wheat that contain different concentrations of aflatoxin 0.05 B1 and 0.0225 NI, benzoquinone The fifth treatment (T5): The group of rats given orally 100% to the infected wheat contains different concentrations of

aflatoxin 0.07 B1, 0.03NI and benzoquinone amounting to 2.5 MBQ and 1.6 EBQ and as for the sixth treatment (T6): 100% Uruguayan rice Infected containing different concentrations of aflatoxin g1 0.047, MBQ benzoquinone (2.00) and EBQ (0.650) PPM as indicated in the examination tables. Rats were dosed for 28 days by determining parameters of liver functions and tissue.

### Liver enzymes

The activity of liver enzymes was estimated using a kit supplied by the company ROCHE (Germany) and the results were read using the Swiss-origin Reflotron device according to the instructions of the processing companies as mentioned in [10].

### Statistical analysis

An experiment was conducted with a CRD design for wheat (0, 1, 2, 3, 4, 5, 6) months and two storage methods are laboratory. The comparison between the averages of transactions was done using the least significant difference test LSD at the level of significance 0.05 in addition to calculating the correlation coefficient ( SPSS, 2009).

## Results & Discussion

### Liver function

The results showed that there were significant ( $P \leq 0.05$ ) differences between the study groups compared with control groups. It was observed that the group given infected 100% wheat showed a significant increase in the activity of AST enzyme ( $98.55 \pm 6.42$ ) and ALT ( $92.1 \pm 5.1$ ) compared to the control group given normal wheat ( $24.12 \pm 5.15$ ,  $17 \pm 3.21$ , respectively). On the other hand, a significant increase in the activity of AST enzyme ( $79.4 \pm 3.42$ ) and ALT ( $65.4 \pm 5.19$ ) was found in the group given 100% infected rice compared to the control group given normal rice ( $18.4 \pm 4.13$ ,  $19 \pm 4.34$  respectively).the activity of ALP enzyme, it was observed that the group given infected 100% wheat showed a significant increase in the activity of ALP enzyme ( $108.4 \pm 4.11$ ) compared to the control group given normal wheat ( $49.4 \pm 8.53$ ). While there was a significant increase in the activity of ALP enzyme ( $94.4 \pm 5.13$ ) in the group given infected rice 100% compared to the control group given normalrice ( $45.3 \pm 6.54$ ), as shown in table (1).

**Table (1): Effect of Aflatoxin and Buzuquinone on liver functions**

Treatments	Liver function parameters in rats		
	AST U/L	ALT U/L	ALP U/L
T1	$24.12 \pm 5.15$ c	$17 \pm 3.21$ c	$49.4 \pm 8.53$ a

<b>T2</b>	18.4±4.13 c	19±4.34 c	45.3±6.54 a
<b>T3</b>	68.52±5.19 b	71.34±5.31 b	85.2±4.21 b
<b>T4</b>	79.61±8.32 b	78.11±3.25 b	65.9±6.31 b
<b>T5</b>	98.55±6.42 a	92.1±5.1 a	108.4±4.11 b
<b>T6</b>	79.4±3.42 b	65.4±5.19 b	94.4±5.13 b

### Liver tissue

Cross sections of control group that given normal wheat (T1) showed the normal shape of central vein, hepatocytes, sinusoids and kupffer cells, as shown in figure (1). The Cross sections of control group given normal rice (T2) show the normal shape of central vein, hepatocytes, sinusoids and kupffer cells, as shown in figure (2). Otherwise, the infected wheat (T3) 50%, showed degeneration of hepatocytes, lymphocytes infiltration and thickening wall of blood vessels as shown in figure (3). Also, the liver of the treated group 75% infected wheat (T4), showed thickening wall of blood vessels, congestion, lymphocytes infiltration and fibrosis shown in figure (4). The treated group (100% infected with wheat) (T5), showed degeneration of hepatocytes, thickening wall of blood vessels and congestion as shown in figure (5). Also, the kidneys of the group given 100% infected rice showed normal wall of blood vessels, lymphocytes infiltration and congestion as shown in Figure (6).

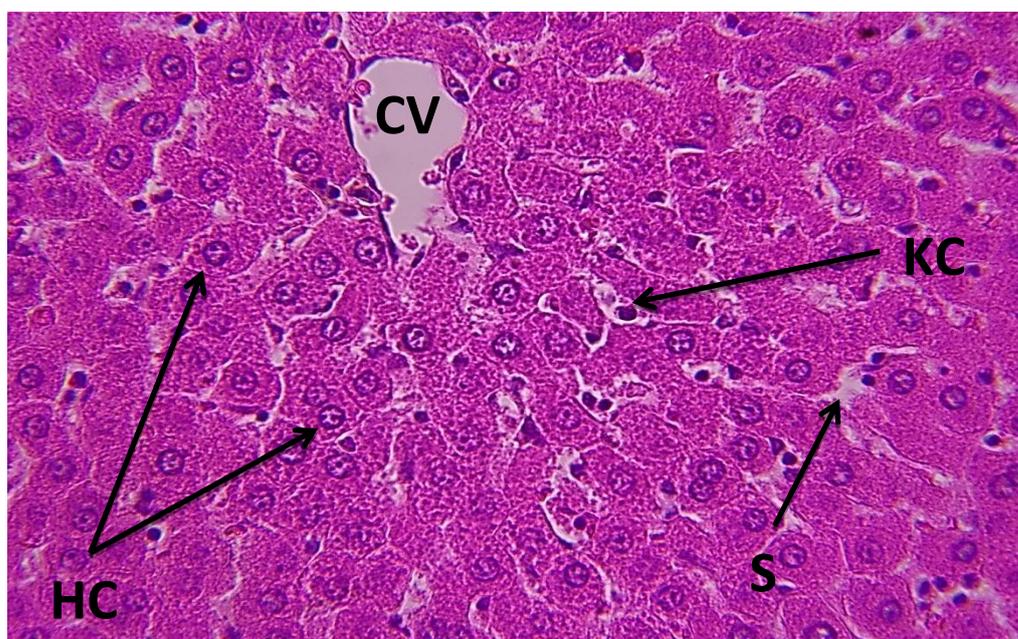


Figure (1): liver of control group fed on normal wheat showed normal central vein (CV), hepatocytes (HC), sinusoids (S) and kupffer cells (KC) H&E X400.



Figure (2): liver of control group fed on normal rice showed normal central vein (CV), hepatocytes (HC), sinusoids (S) and kupffer cells (KC) H&E X400.

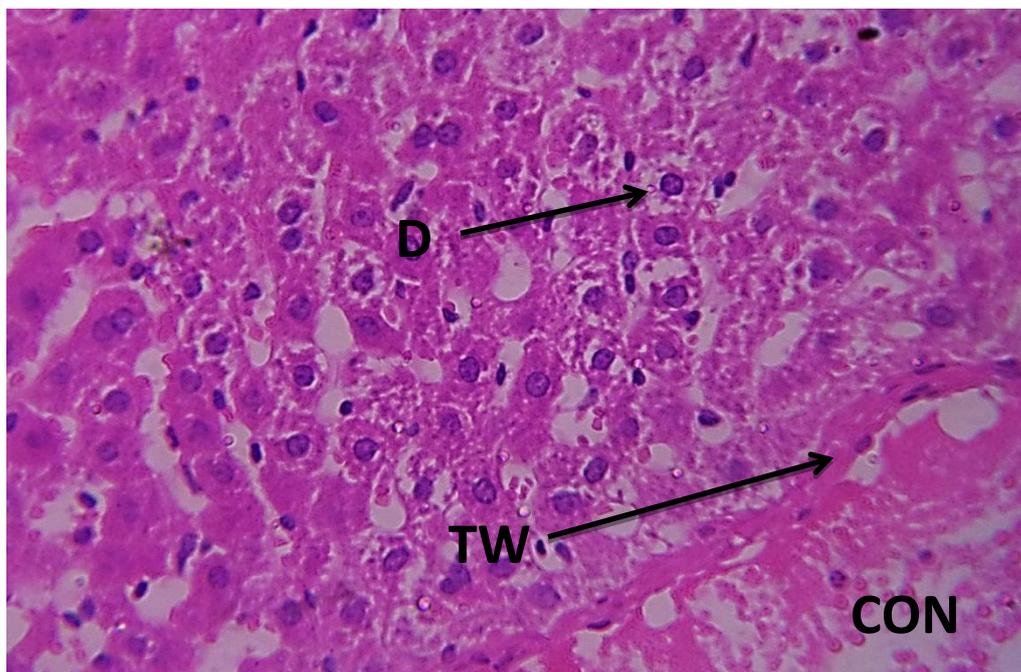


Figure (3): liver of group (T3) showed degeneration (D) of hepatocytes, thickening wall (TW) of central and hepatic vein with congestion (CON)H&E X400.

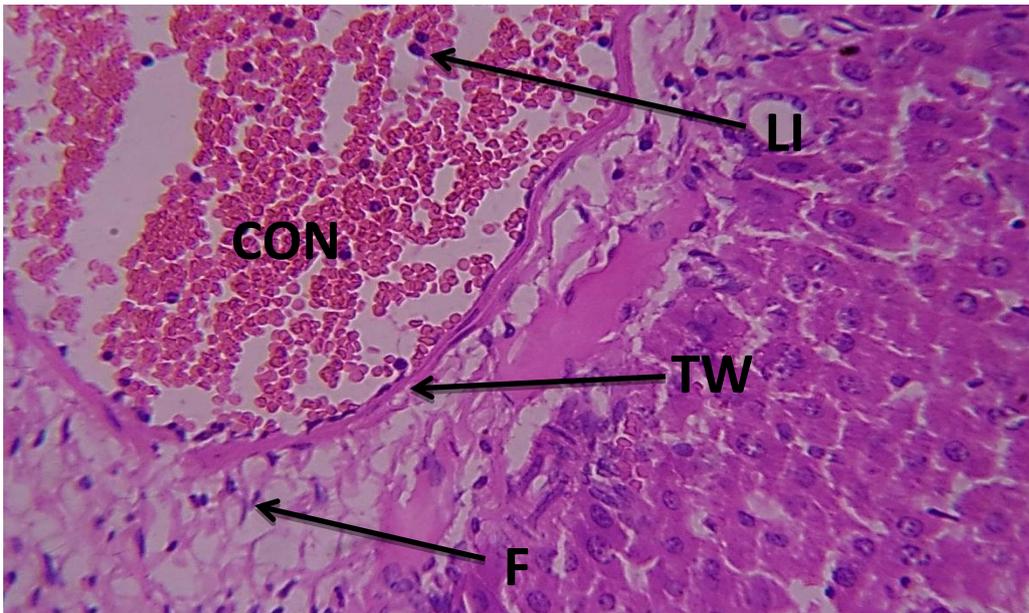


Figure (4): liver of group (T4) showed thickening wall (TW) of central and hepatic vein with congestion (CON), and infiltration of lymphocytes (LI) with fibrosis (F) H&E X400

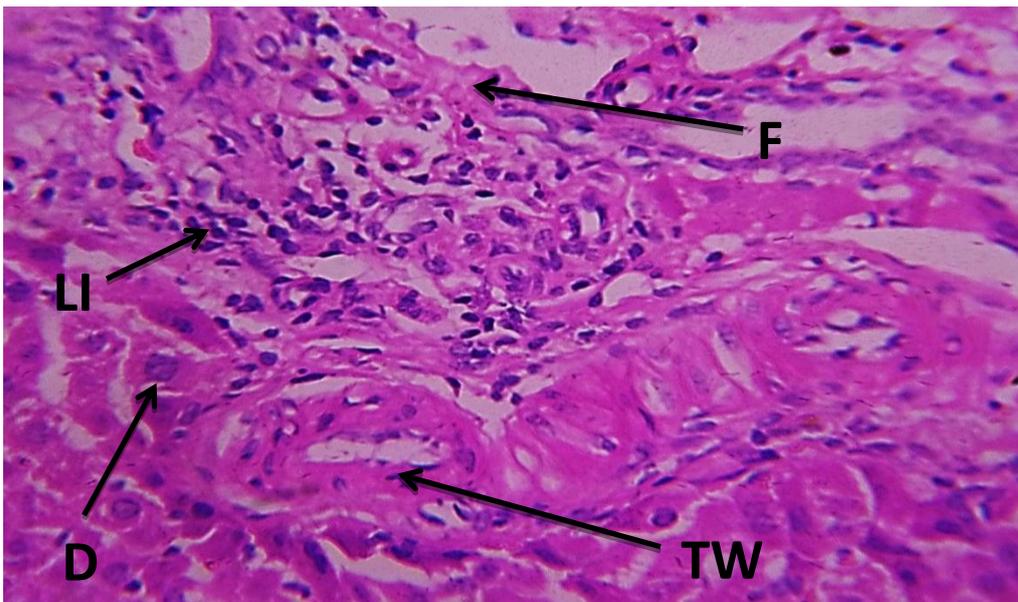


Figure (5): liver of group (T5) showed thickening wall (TW) of central and hepatic vein, degeneration (D) of hepatocytes and infiltration of lymphocytes (LI) with fibrosis (F) H&E X400

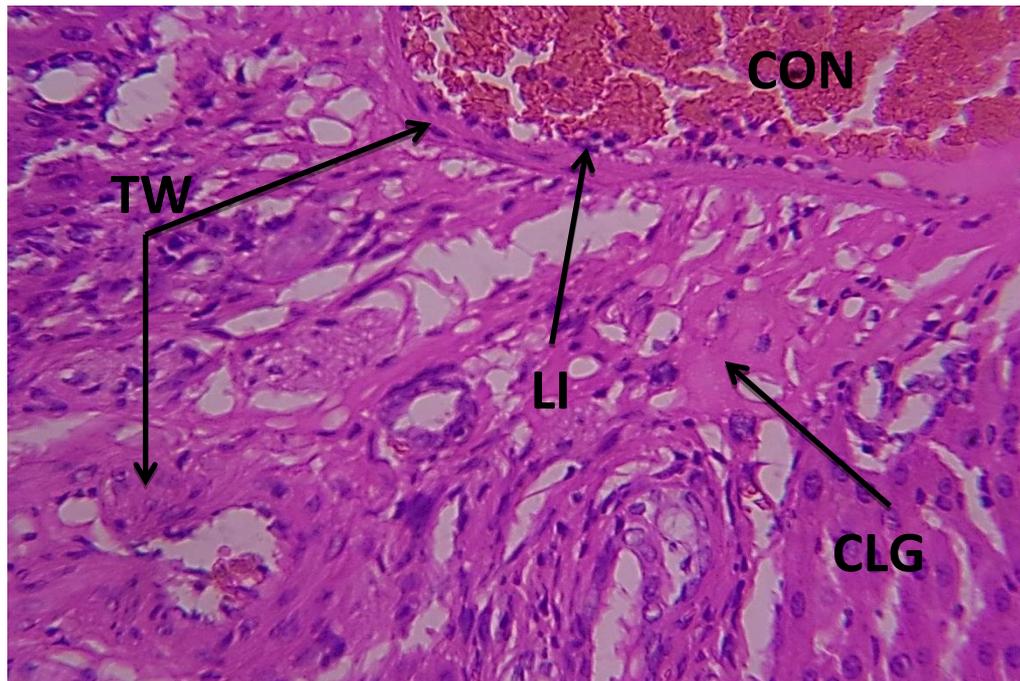


Figure (6): liver of group (T6) showed thickening wall (TW) of central and hepatic vein with congestion (CON), and infiltration of lymphocytes (LI) with collagen accumulation (CLG) H&E X400.

These results are in agreement with the findings of [11] who indicated that the toxins of aflatoxin after ingesting it to mice led to many histological changes in the liver, including degeneration of hepatocytes and congestion of blood vessels. The results of the current study also agreed with the study [12] who indicated that aflatoxin led to various histological changes in the liver tissues of mice, including degeneration and necrosis of hepatocytes arranged radially around the central veins. The researcher also referred to the infiltration of mononuclear cells between the hepatocytes as a result of cell breakdown. The results of the current study are in agreement with the findings of [13] It was observed that female mice dosed with 2 mg of AFB1/kg of body weight had negative changes due to the effect of aflatoxin, and when determining the activity of GPx and SOD in renal and hepatic tissues of rats, a significant increase in the activity of both enzymes was observed in the given groups of AFB1. Negative effects were also observed in hepatocytes. Mycotoxins are toxic substances that can infect many foods with carcinogens, genotoxic, teratogenic, nephrotoxic, and hepatotoxic. Mycotoxin contamination of foodstuffs causes diseases all over the world. Major classes of mycotoxins of greatest agricultural economic importance. The liver is the target organ for AFB1. Ingestion of this mycotoxin is known to be able to cause acute toxicity, aflatoxin, and is believed to contribute to the development of primary hepatocellular carcinoma [14-15].

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