Bioremediation of Zearalenone by using *Lactobacillus acidophilus* in albino rats bodies (*in vivo*)

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Objective This study aimed to evaluate the efficiency of *Lactobacillus acidophilus* to reduce zearalenone toxicity in vital systems of albino white rats.

Methods *Fusarium graminearum* isolate was tested to produce zearalenone toxin. *L. acidophilus* was used to reduce the toxin in rats. This ability was studied by many parameters such as biochemical and physiological parameters in addition to histological study.

Results The results showed that *L. acidophilus* had the ability to reduce zearalenone toxicity. *L. acidophilus* had the ability to raise Hb 11 g/dl in blood of animals that was treated with zearalenone only to 13.06 g/dl in animal's blood that was treated with zearalenone + *L. acidophilus*. As well as the number of red blood cells in animals blood treated with these treatment was 6.62×10^{12} cell/ L. *L. acidophilus* reduces the toxicity of zearalenone through its ability to reduce the number of platelets to normal levels.

Conclusion *L. acidophilus* had a role in repeated biochemical parameters to normal levels. Total protein rose to 6.6 g/dl in animal's blood treated with zearalenone + *L. acidophilus* compared with these levels in blood of control group. Also GPT and sugar levels in animal blood that was treated with zearalenone + *L. acidophilus* were at normal level. Histological study proved that *L. acidophilus* had the ability to protect liver, kidney, and uterus from the toxicity of zearalenone.

Keywords *Lactobacillus acidophilus, Fusarium graminearum*, zearalenone

Introduction

Zearalenone (ZEN) is a mycotoxin by virtue of its estrogenic effects, and produced by certain various species of *Fusarium* genus, including *F. culmorum, F. equiseti, F. graminearum* and *F. moniliforme* and is associated mainly with cereal crops, in particular, maize. Although the mycotoxin is probably most common in maize, very high level (11.15 µg/kg) can also be found in other cereals.[1]

The effective dose (ED_{50}) of ZEN is very low (0.2 µg/kg), and suspected to cause human diseases, including premature puberty syndrome as well as hyper estrogenic in farm animals.[2] Collins et al.[3] showed that ZEN caused diseases in heart, brain, kidney and ovary of rats when treated with ZEN.[4] It also found a correlation between ZEN excited in blood plasma of women and breast cancer.

There are many species of bacteria used in bioremediation of mycotoxins, as *Lactobacillus acidophilus* which reduced the toxicity of ochratoxin A in white rats.[5] Sezer et al. (2013)[6] found that *L. plantarum* has the ability to reduce the amounts of aflatoxin B1 (AFB1) with 46% while *L. lactis* with 27%.

There are methods to control mycotoxins in food and feeds including physical methods, chemical methods and biological controls. But there is no antitoxin to control ZEN toxin. So the aim of this study was to evaluate the efficiency of *L. acidophilus* to reduce ZEN toxicity in some vital systems of albino white rats.

Materials and Methods

This study was conducted in the clinical laboratory of College of Applied Medical Sciences, University of Karbala, Iraq.

1. **Preparation of potato dextrose agar:** This medium was prepared according to the procedures of the manufacturing company (HIMEDIA, India).
2. **Preparation of ZEN stander:** It was prepared by adding 4 ml of chloroforme to 1 mg of ZEN, the concentration of mycotoxin (ZEN) becomes 250 µg/ml.
3. **Testing the ability of *F. graminearum* to produce ZEN:** We obtained one *F. graminearum* isolate from toxins. This testing was conducted according to Ishii et al. (1974)[7] and Scott et al (1981).[8]
4. **Efficiency of *L. acidophilus* to reduce ZEN toxicity:** This study was conducted as follows:
   i. **Isolation and diagnosis of *L. acidophilus*:** *L. acidophilus* was isolated according to a study by Macfaddin (2000),[9] and *L. acidophilus* was diagnosed by VITEK2 compact.
   ii. **Preparation of bacterial inoculum:** Preparation of *L. acidophilus* inoculum was done according to the method by Williams et al. (1983).[10]
5. **Preparation of laboratory animals**
   A. Twelve white female rats with similar ages were divided into four groups, each group included three animals.
   B. Each group of animals were treated as follows:
      i. First group of animals were treated only with ZEN (6 mg/kg animal weight).
      ii. Second group of animals were treated with *L. acidophilus* inoculum (1 ml /kg). After 24 hours it was treated with zearalenone (6 mg/kg animal weight).
      iii. Third group of animals were treated only with *L. acidophilus* inoculum (1 ml/kg).
      iv. Fourth group of animals were treated only with distil water (control treatment).

Treatment of animals continued till 14 days. At the end of the prescribed period, animals were sacrificed after being

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(*Submitted: 01 December 2014 – Revised version received: 25 January 2015 – Accepted: 28 January 2015 – Published online: Winter 2015*)

treated with chloroform and anatomy was done by opening the abdominal cavity. Blood was drawn by a stab heart (heart puncture).

One part of the blood was sterilised without anti-clotting materials and the other part was poured into test tubes which contained anti-coagulant materials, and physiological and biochemical tests were conducted. Also samples taken from liver, kidney, spleen and ovaries were saved in formalin (10%).

Biochemical Parameters

The level of glucose, total protein and glutamate pyruvate transaminase (GPT) enzymes in the blood serum of animals were estimated and treated as instructed by manufacture's guidelines (spectrum; SAE, Egypt).

a. Estimate the level of glucose in the blood
b. Estimate the level of total protein in the blood
c. Estimate the level of GPT enzyme in the blood

Physiological Parameters

Physiological parametric study included to calculate the amount of haemoglobin (Hb), red blood cells (RBCs), monocytes and platelets by using of HumaCount 60TS, Germany.

Histological Study

The tissue sections were prepared in the Laboratory of Applied Medical Sciences and followed the method of Bancroft and Stevens (1892).[11]

Statistical Analysis

The design of experiment was carried out as complete random design (CRD) with single factor, and the mean was compared according to least signification difference (LSD) at 0.05 probability level.[12]

Results

1. Testing the ability of *F. graminearum* isolates to produce ZEN

The results of chemical analysis; thin layer chromatography (TLC); showed that *F. graminearum* isolates have ability to produce ZEN.[13]

2. Physiological parameter

a. Haemoglobin: ZEN caused to lower the Hb amount to 11 gm/dl in animal blood that was treated with ZEN whereas the level for this parameter in control group was 12.65 gm/dl. The amount of Hb rose to 13.06 gm/dl in animal blood that was treated with toxin and bacterium (ZEN + *L. acidophilus*). Also the amount of Hb increased to 13.2 gm/dl in animals that were treated with only bacterium *L. acidophilus*) (Table 1).

This result was in agreement with a study by AL-Khafaji (2014)[13] who found that ZEN decreased Hb amount to 10.8 g/dl while these amount in control treatment group was 12.3 g/dl. AL-Fatlawi (2014)[14] reported that when treated with *F. foetens* it produced toxic metabolites and produced less amount of Hb.

AFB1 and B2 decreases Hb amount in rats to 8.6 g/dl and 7.3 g/dl, respectively, whereas the amount of Hb in blood of control animals group was 13 g/dl.

Kubena et al. (1987)[15] found that secondary metabolic products of the fungi has the ability to bind with blood proteins which is responsible for the biosynthesis of the Hb causing the lowest number of RBCs.

b. RBCs: The results showed that toxin ZEN reduces the number of RBCs to 4.7×10^{12} cell/mm^3 in animals treated only with toxin compared with control group (6.29×10^{12} cell/l), while the number of RBC in the blood of experimental animal that was treated with ZEN + *L. acidophilus* was 6.62×10^{12} cell/l (Table 1).

AL-Khafaji (2014)[13] found that ZEN reduced the number of RBCs to 4.7×10^{12} cell/l while it was 5.8×10^{12} cell/l in the control group. Also Moura et al (2004)[16] reported that ochratoxin A decreased the count of RBC when treated with ZEN.

c. Monocytes: The results as shown in Table 1 revealed that ZEN toxin treatment caused an increase in monocytes number to 0.97×10^3 cell/mm^3 while the number of these cells were 0.41×10^3 cell/mm^3) in the control group. At the same time, ZEN + *L. acidophilus* treatment reduced the number of monocytes to normal level in animal blood (0.48×10^3 cell/mm^3). Also the numbers of monocytes in animal blood that was treated with only *L. acidophilus* were at normal level (Table 1). AL-Hashemi (2014)[17] showed that AFB1 caused an increase in monocytes numbers to 1.3×10^3 cell/mm^3 while it was 0.04×10^3 cell/mm^3 in animals that were treated with AFB1.

d. Platelets: ZEN toxin affected the number of platelets, this parameter raised to 1400×10^3 plat/mm^3 in animals that were treated with only ZEN compared with control group (883×10^3 plat/mm^3). ZEN + *L. acidophilus* treatment decreased the number of platelets to 860×10^3 plat/mm^3 (normal level), while it was 768×10^3 plat/mm^3 in animal that were treated with only bacterium (Table 1). AL-Hashemi (2014)[17] showed that AFB1 caused increase in number of platelets in the blood of animals. Also Samuel et al. (2009)[18] found that AFB1 increased the platelets in chicken blood to 135×10^3 plat/mm^3.

3. Biochemical parameters

a. Total protein: Biochemical blood parameters affected by ZEN caused decreased level of total proteins from 7.1 g/dl in animal blood of control treatment to 5.3 g/dl in animal blood of ZEN treatment, while *L. acidophilus* and ZEN treatment raised the level of protein in blood to 6.6 g/dl (Table 2). This result agreed with the study by AL-Khafaji (2014)[13] who found that total protein level decreased in blood of animals that were treated with ZEN. He also found that (ZEN + *Bacillus subtilis)* treatment raised total proteins in animals treated with these toxins.

b. GPT: ZEN caused raise of GPT in blood of animals that were treated with ZEN to 19 U/l compared with control treatment (15 U/l). ZEN + *L.*

Table 1. **Effect of *L. acidophilus* on some physiological parameters in the blood of albino white rats that were treated with zearalenone**

Treatment	Hb g/dl	RBC cell/l	MON cell/mm^3	PLT cell/mm^3
ZEN	11	4.7×10^{12}	0.97×10^3	1400×10^3
ZEN+LBA	13.06	6.62×10^{12}	0.48×10^3	860×10^3
LBA	13.2	5.76×10^{12}	0.67×10^3	768×10^3
Control	12.06	6.29×10^{12}	0.41×10^3	883×10^3
LSD (0.05)	2.2	0.6	0.5	164

LBA: *L. acidophilus;* LSD: least signification difference; MON: monocytes; PLT: platelet; RBC: red blood cell; ZEN: zearalenone.

Table 2. **Effect of *L. acidophilus* on some biochemical parameters of albino white rats treated with zearalenone**

Treatment	Total protein (g/dl)	GPT (U/L)	Glucose (mg/dl)
ZEN	5.3	19	165
ZEN + LBA	6.6	11.6	90
Bacteria	6	12.3	119
Control	7.1	15	97
LSD (0.05)	1.6	9.5	43

GPT: glutamic pyruvic transaminase; LBA: *L. acidophilus*; LSD: least signification difference; ZEN: zearalenone.

acidophilus treatment decreased the level of GPT to 11.6 U/l in blood of animals (Table 2).

This result agreed with Ali and Kazem (2012)[19] who found that compound produced by *B. holmii* caused an increase of GPT level to 33 U/l. Also AL-Khalidy (2012)[20] showed that *G. candidium* and *G. penicillatum* produced toxic phenolic compounds which raised the level of GPT to 44 U/l. AL-Kelkali (2005)[21] reported that *F. solani* filtrate raised the level of GPT in blood of rats when treated with these filtrates. *F. graminearum* filtrate raised the level of GPT in the blood of mice. These filtrates caused degradation of liver cells membrane and lysis of liver cells causing exit of these enzymes to blood.[22]

c. Sugar: On the other hand, ZEN raised sugar in animal to 165 mg/dl compared with control treatment (97 mg/dl), while the level of sugar in blood of animals when treated with ZEN + *L. acidophilus* was 90 mg/dl (Table 2). Increase in the level of sugar in animals blood belong to ZEN able to decrease of hexokinase and glycogen phosphatase level in blood of animals causing defect in sugar and glycogen metabolism.[23]

4. Histological study

The results of our a current study agree with AL-Khafaji (2014)[13] who found that ZEN caused pathogenic changes in liver, intestine, and spleen. Also he found that *B. subtilis* was able to reduce the toxicity of ZEN in vital systems of rats (Fig. 1–4).

This study indicated that *L. acidophilus* had the ability to protect the vital systems of animal from toxic effect of ZEN (Figs. 1C–4C).

Also *L. acidophilus* did not cause pathogenic changes in organs of the animals (Figs. 1D–4D). This result agrees with AL-Khafaji (2014)[13] who found that ZEN caused pathogenic changes in liver, intestinal and spleen. He also found that *B. subtilis* was able to reduce the toxicity of ZEN in vital systems of rats. Al-Khalidy (2010)[24] reported that the metabolics of *G. canidium* and *G. pencillium* caused abnormal changes in liver and kidney.

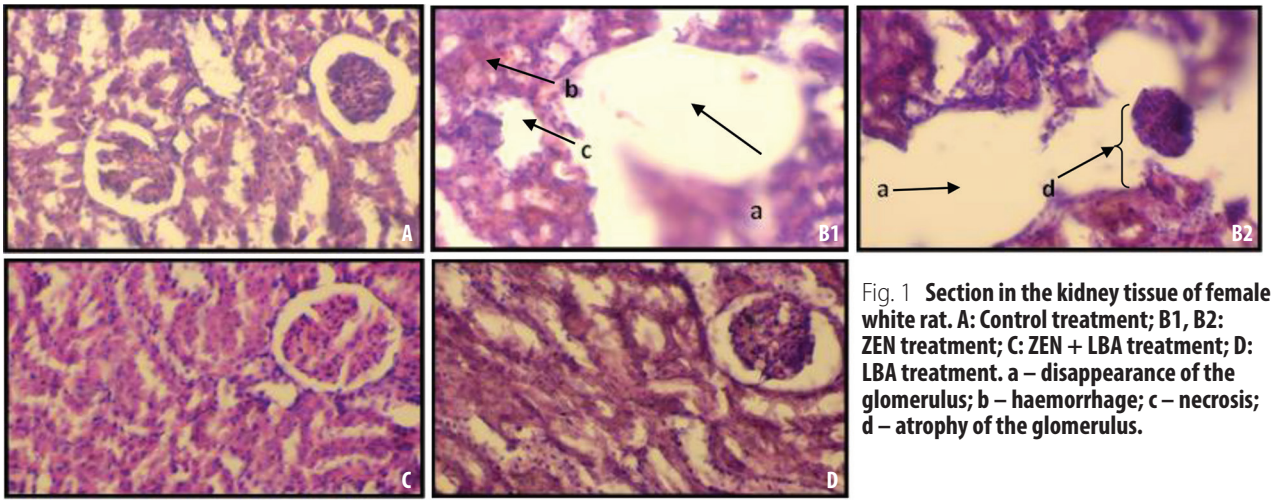

Fig. 1 **Section in the kidney tissue of female white rat. A: Control treatment; B1, B2: ZEN treatment; C: ZEN + LBA treatment; D: LBA treatment. a – disappearance of the glomerulus; b – haemorrhage; c – necrosis; d – atrophy of the glomerulus.**

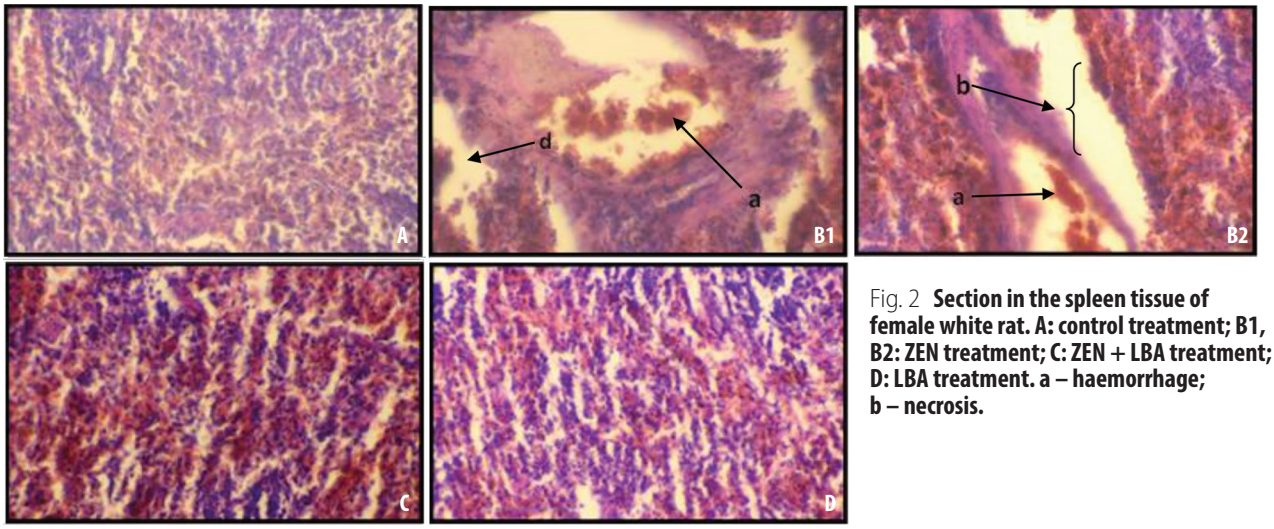

Fig. 2 **Section in the spleen tissue of female white rat. A: control treatment; B1, B2: ZEN treatment; C: ZEN + LBA treatment; D: LBA treatment. a – haemorrhage; b – necrosis.**

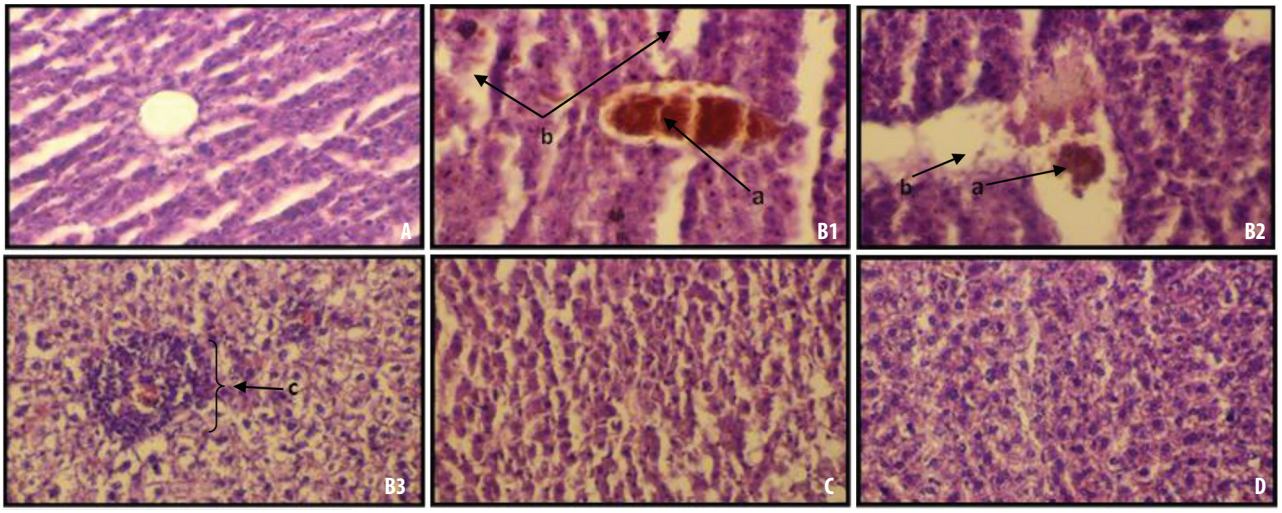

Fig. 3 **Section in the liver tissue of female white rat. A: control treatment; B1, B2, B3: ZEN treatment; C: ZEN + LBA treatment; D: LBA treatment. a – vascular dilation and congestion; b – multifocal hepatocytes necrosis; c – focal aggregate inflammatory cells.**

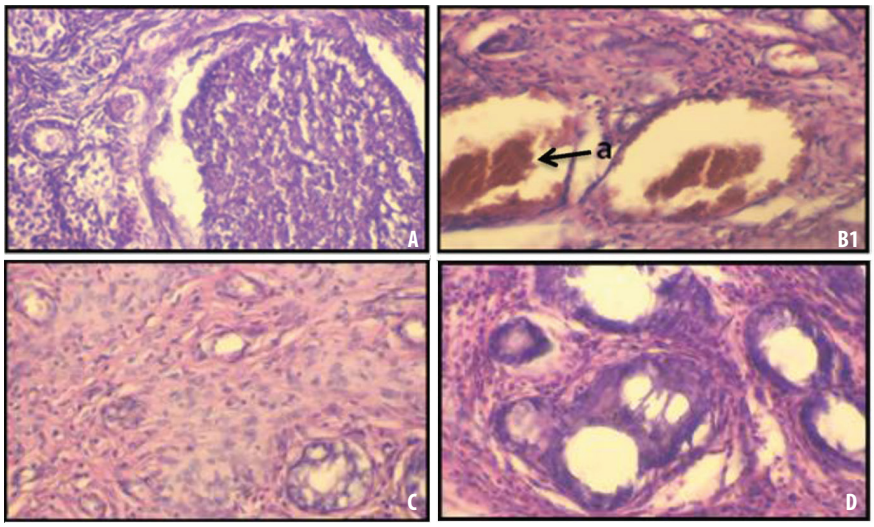

Fig. 4 **Section in the uterus tissue of female white rat. A: Control treatment; B1, B2: ZEN treatment; C: ZEN + LBA treatment; D: LBA treatment; a: congestion; b: hemorrhage.**

Verma (2004)[25] reported that *Aspergillus flavus* produced toxic metabolites that caused inflammation in renal glomeruli and bleeding.

Histological study showed that ochratoxin A caused pathological effects like thickening of vascular wall in kidney, while ochratoxin A caused severe micro abscess and necrosis in liver of animals.[26]

The mechanism of mycotoxin detoxification by bacteria includes:

a. Mycotoxin binding with bacterial cell wall.
b. Some bacteria produce enzymes like lysis of mycotoxin.[2]

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