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Original Article

Protective Role of Bromelain and *Lactobacillus* spp. in Ochratoxin A-Induced Hematological Toxicity in Guinea Pigs

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ABSTRACT

Background: Ochratoxin A (OTA), a mycotoxin commonly produced by *Aspergillus* and *Penicillium* species, is responsible for several toxicological effects, including hematotoxicity. The prophylactic efficacy of bromelain, *Lactobacillus spp*., and their combination against OTA-induced hematotoxicity in guinea pigs is discussed in this article.

Methods: Fifty male guinea pigs were randomly distributed into five groups: a negative control group, an OTA-alone group, an OTA + bromelain group, an OTA + probiotic group, and an OTA + combination therapy group. For 30 days, the following hematology parameters were observed: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), packed cell volume (PCV), and platelet count.

Results: Findings indicated profound hematotoxicity in the OTA-alone group, characterized by severe anemia, leukopenia, and thrombocytopenia. Bromelain or probiotics alone regained hematological values in part, whereas the combined treatment resulted in nearly complete recovery to values approximating controls.

Conclusions: These results establish the synergistic detoxifying activity of bromelain and *Lactobacillus* spp. and indicate their potential as natural detoxifiers for the mitigation of OTA-induced toxicity in animals.

Keywords: Ochratoxin A, bromelain, Lactobacillus spp., hematotoxicity.

1 Introduction

Mycotoxins, toxic metabolites of many fungi, represent a significant public health concern due to the risk of food and feed contamination. One of the most toxic mycotoxins, produced predominantly by Aspergillus and Penicillium species, is Ochratoxin A (OTA), which is primarily nephrotoxic, hepatotoxic, teratogenic, immunosuppressive, and genotoxic to humans and animals [1, 2, 3,4]. OTA likewise has a tendency to accumulate in cereal grains, coffee, dried fruits, and animal foods, thus resulting in widespread exposure to a majority of people. There are also a number of experimental studies that have unraveled OTA-induced toxicity, indicating its ability to induce oxidative stress, impair cellular antioxidant defense, and introduce DNA and reproductive organ damage [5, 6]. To mitigate such harmful impacts, researchers have tried other ways of achieving this, including the use of natural antioxidants, supplementation with probiotics, and detoxification using enzymes. Bromelain, a proteolytic enzyme derived from pineapple stem, has emerged as an area of interest because of its anti-inflammatory, antioxidant, and cytoprotective properties [7]. Probiotics, particularly Lactobacillus, have been successful in binding and degrading OTA in the gut and for enhancing host immunity [8-9]. A number of studies demonstrate that *Lactobacillus rhamnosus* GG was able to prevent oxidative and genotoxic stress induced by OTA in vivo [10], pointing towards a synergistic potential when given in combination with enzymatic or antioxidant therapy. Furthermore, the simultaneous treatment with natural substances might exert additive or even synergistic safeguarding effects [11-15]. Despite these hopeful alternatives, numerous facets about the optimal ways to integrate various measures against mycotoxin-induced health issues remain a mystery.

For the first time, restricted studies have systematically assessed the single and combined effects of bromelain and probiotics in antagonizing OTA toxicity in vivo. Bromelain [16] and *Lactobacillus* species [17, 11] have protective activities and interact

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with OTA; however, their combined effect in reversing OTA-induced damage is ill-defined. Secondly, there is a considerable dearth of knowledge about hematological parameters in existing OTA toxicity models. Although some research focuses on biochemical or reproductive toxicity [1,18], essential parameters such as red and white blood cell counts, hemoglobin, packed cell volume (PCV), and platelet counts are hardly explored. These indicators are crucial in the diagnosis of systemic toxicity, assessing oxidative changes, as well as immune response changes due to chronic exposure, such as anemia or immunosuppression. Ultimately, a better grasp must be gained in understanding interactions among detoxifying agents and some biomarkers of oxidative damage and blood composition changes. There is sparse current research to extensively review protective effects of natural remedies against OTA toxicity on these significant biomarkers. The present study aims to compare and evaluate the protective effects of bromelain, probiotics (*Lactobacillus spp.*), and their combination against OTA toxicity in a guinea pig model in terms of potential alleviation of hematological alterations.

2 Materials and Methods

2. 1 Ethical Acceptance

The research design was reviewed and approved by Local Research Ethics Committee of the College of Veterinary Medicine of the University of Baghdad (Approval Number 1132, dated May 20, 2022). And animals were monitored daily for clinical signs, behavior, feed intake, and potential distress throughout OTA exposure. Humane handling procedures were followed, and any observed discomfort was addressed according to institutional animal welfare guidelines to ensure minimal suffering.

2. 2 Animal Facility

The experiment was conducted at the animal house of the College of Veterinary Medicine, University of Baghdad. The research protocols were in line with the ethical guidelines for using animals.

2. 3 Experimental Animals and Design

Five hundred male healthy guinea pigs were randomly allocated into five groups (n = 10 in each group):

• Group 1 (Negative Control): Fed with plain pellets and water only.

• Group 2 (Toxic Control): Administered Ochratoxin A (140 µg/kg body weight) orally once a day for 30 days [15].

• Group 3 (OTA + Bromelain): Administered OTA (140 µg/kg b.w.) and bromelain enzyme (200 mg/kg b.w.) orally for 30 days [19].

• Group 4 (OTA + Probiotic): Administered OTA (140 µg/kg b.w.) orally and 1% *Lactobacillus* spp. probiotic in drinking water daily for 30 days [17].

• Group 5 (OTA + Bromelain + Probiotic): Administered OTA (140 µg/kg b.w.), bromelain (200 mg/kg b.w.), and 1% probiotic co-administered for 30 days.

2. 4 Blood Sampling and Hematology

Blood was collected by cardiac puncture under mild anesthesia on Day 0, 10, 20, and 30. The specimens were analyzed using an automated hematology analyzer to determine red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), packed cell volume (PCV), and the platelet count.

2. 5 Sample strain

The Fungal Unit of the Microbiology Department from the College of Veterinary Medicine, University of Baghdad, provided the fungal sample.

2. 6 Growth and Inactivation Method for Aspergillus ochraceus on PDA and Rice Seeds

A single fungal colony was first established by cultivating Aspergillus ochraceus on Potato Dextrose Agar (PDA) as figure 1 A. To make PDA medium, 39 g PDA powder was dissolved in 1 L of distilled water, autoclaved at 121°C for 15 minutes, and then dispensed onto sterile Petri plates. After being injected with A. Inoculation with mycelial plugs or ochraceus spores, the plates were incubated at 25 to 28°C for five to seven days. The bulk growth consisted of autoclaving 50–100 g of drained and water-soaked rice seeds for 24 hours. Cooled rice was inoculated with fungus cultivated in PDA and incubated at 25–28°C for 14–21 days. Shaking flasks every few days was performed to provide equal development. To ensure complete sterilization, the fungal culture was autoclaved at 121°C for 30 minutes following complete colonization as figure 1 B.

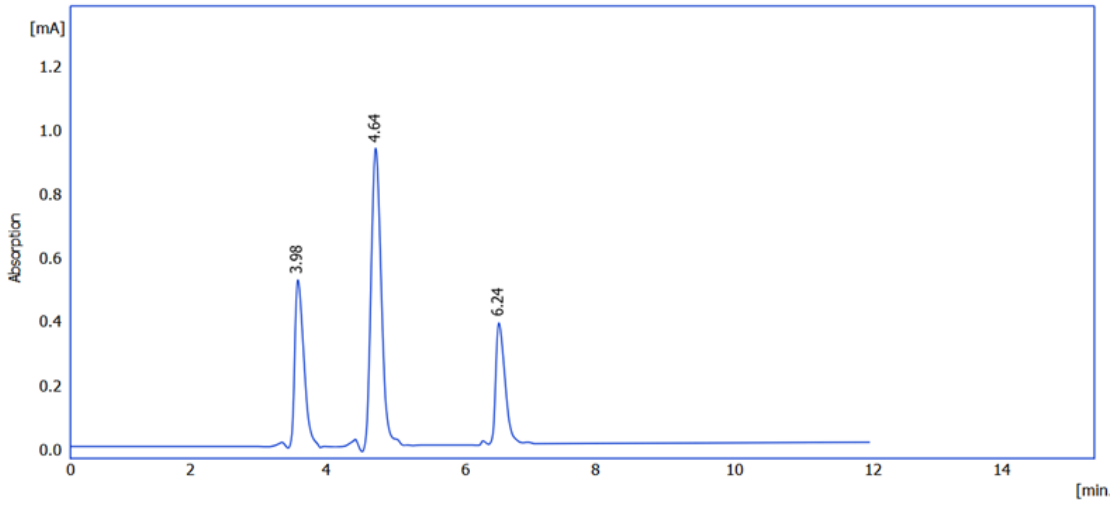
2.7 Quantification of Ochratoxin A produced by Aspergillus ochraceus using HPLC

Ochratoxin A, also known as the toxin of the mold species Aspergillus ochraceus, was measured after growth on rice substrate. The measurement process involved the application of high-performance liquid chromatography, or HPLC, with fluorescence detection as the analysis. After 14 to 21 days of accurate incubation at sustained temperatures ranging from 25 to 28 degrees Celsius, the infected rice was then dried and finely ground. A 25-gram aliquot was vigorously shake with 100 mL of 80:20 (v/v) methanol-water solvent along with the addition of 5 grams of sodium chloride for optimal extraction efficiency. The liquid was also shaken vigorously for 30 minutes and then filtered to eliminate any potential solid component. Ten milliliters of filtered extract were then separated and cleaned on an immunoaffinity column that was specifically made to capture OTA. The OTA was subsequently eluted from the column using 2 mL of methanol, which was evaporated to dryness to yield a concentrated residue that was afterwards reconstituted in 1 mL of a mobile phase made up of acetonitrile, water, and acetic acid in a volumetric ratio of 99:99:2 (v/v/v). The high-performance liquid chromatography analysis employed a C18 reversed-phase column and fluorescence detection with excitation and emission

at 333 nanometers and 460 nanometers, respectively. The mobile phase was operated at a constant flow rate of 1.0 mL/minute, and the OTA concentration was quantitated by comparing peak areas within the chromatogram with a standard curve obtained from OTA standards of 1-100 ng/mL (Figure 2). The research demonstrated that the processed rice holds 229.7 ppb of OTA micrograms per kilogram [20-21].

(A) **B**

Figure 1: (A) Cultivating *Aspergillus ochraceus* on Potato Dextrose Agar (PDA); **(B)** *Aspergillus ochraceus* growth on the rice seeds in a flask.



Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	3.98	17412.98	570.22	30.00	30.00	0.05	
2	4.64	62145.80	987.45	50.00	50.00	0.15	
3	6.24	9895.77	389.07	20.00	20.00	0.03	
	Total	89484.25	1946.08	100.00	100.00		

Figure 2: HPLC Chromatogram and Associated Data for Ochratoxin A Quantitation. The sample was from the culture of *Aspergillus ochraceus*, and the peak at 4.64 minutes indicative of Ochratoxin A represented 69% of the total peak area.

2. 8 Daily Feed Intake and Dilution Calculation

The HPLC test showed that the concentration of ochratoxin A (OTA) in the rice grains subject to examination was 229.7 parts per billion (ppb). For solid materials, ppb is the same as micrograms per kilogram (µg/kg), and the sample had 229.7 µg/kg of OTA. It is above the established safety level of 140 µg/kg, and it is the maximum acceptable concentration of OTA in animal feed. A. dilution to a concentration that will. bring the poison to a level considered safe for feeding animals must be performed.

To achieve this, the below standard dilution formula is employed:

$C_1 \times V_1 = C_2 \times V_2$

Where:

- C1 The initial concentration of the contaminated feed (229.7 µg/kg);
- V1 The initial quantity of the contaminated feed for dilution (1 kilogram);
- C2 The final intended concentration is 140 µg/kg.
- V2 Overall weight of the mix (tainted feed and untainted feed)

Rearranging to solve for V_2:

$V_2 = (C_1 \times V_1) / C_2 = (229.7\ \mu g/kg \times 1\ kg) / 140\ \mu g/kg = 1.640\ kg$

This calculation demonstrates that to reduce the OTA concentration in 1 kilogram of tainted feed to an acceptable level, the overall mixture weight has to be 1.640 kg. Therefore, blending is required to mix 0.640 kg of pure, non-spoiled feed for every 1 kg of spoiled feed. This adaptation will lower the OTA content in the end product to the desired limit of 140 µg/kg, hence eliminating the risk of toxin exposure for animals and maintaining standards in animal feed safety.

2. 9 Statistical Analysis

One-way ANOVA was used to compare groups at each time point. Data normality and homogeneity of variance were checked using Shapiro–Wilk and Levene's tests. When significant differences were detected ($p < 0.05$), Tukey's post hoc test was applied. Results are presented as mean ± SD, and differences between groups are indicated using letter annotations. Because hematological parameters were measured repeatedly in the same animals over time (Days 0, 10, 20, and 30), the study followed a longitudinal design. While one-way ANOVA allowed clear comparison between groups at each time point, it does not account for correlations within the same animals across time and may increase the risk of Type I error. Therefore, this was considered a limitation of the study, and future research should apply repeated-measures or mixed-effects statistical approaches to better evaluate time-dependent treatment effects. All analyses were performed using SPSS version 25.

3 Results

3. 1 Effects of Ochratoxin A and Protective Treatments on Hematological Parameters

Baseline measurements (Day 0) demonstrated that all experimental groups exhibited comparable hematological values, with no statistically significant differences among groups ($p > 0.05$). Red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), packed cell volume (PCV), and platelet counts were within normal physiological ranges, confirming uniform health status and appropriate randomization of animals at the start of the experiment. From Day 10 onward, the positive control group receiving ochratoxin A (OTA) alone showed a marked and statistically significant decline ($p \leq 0.01$) in all hematological parameters compared with the negative control group (Table 1). This downward trend continued progressively through Days 20 and 30. By Day 30, OTA-exposed animals exhibited severe reductions in RBC (5.95×10^6/µL), WBC (4.0×10^3/µL), Hb (10.3 g/dL), PCV (29.0%), and platelet count (310×10^3/µL), indicating pronounced anemia, leukopenia, and thrombocytopenia.

The animals treated with bromelain in combination with OTA showed significant improvement in all measured parameters compared with the OTA-only group ($p \leq 0.01$). Although values did not fully return to control levels, partial recovery was evident across all time points. At Day 30, bromelain-treated animals demonstrated higher RBC (7.15×10^6/µL), WBC (6.8×10^3/µL), Hb (12.6 g/dL), PCV (36.5%), and platelet count (410×10^3/µL) relative to the OTA group. Similarly, the OTA + probiotic group exhibited significant amelioration of hematological deterioration. Progressive improvements were observed across Days 10, 20, and 30, with hematological values consistently higher than those of the OTA-only group ($p \leq 0.01$). By Day 30, RBC, WBC, Hb, PCV, and platelet values approached those observed in the bromelain-treated group, reflecting moderate restoration of hematopoietic function. The combined treatment group (OTA + bromelain + probiotic) demonstrated the greatest recovery among all treated groups. Hematological parameters increased steadily over time and approached the values of the negative control group by Day 30. Several parameters were not statistically different from the control group, as indicated by shared letter notation (ab), reflecting near-complete normalization of blood indices.

Table 1: Hematological parameters in different treatment groups over time.

Day	Group	RBC ($\times 10^6$/µL)	WBC ($\times 10^3$/µL)	Hb (g/dL)	PCV (%)	Platelets ($\times 10^3$/µL)
0	Negative Control	8.1 A	9.2 A	14.6 A	42.5 A	490 A
	Toxic Control (OTA)	7.1 B	6.5 B	12.3 B	35.5 B	390 B
	OTA + Bromelain	7.5 B	7.5 B	13.2 B	38.5 B	430 B
	OTA + Probiotic	7.5 B	7.4 B	13.1 B	38.2 B	425 B
	OTA + Bromo + Probiotic	7.7 B	8.0 B	13.5 B	39.5 B	450 B
10	Negative Control	8.15 A	9.1 A	14.5 A	42.3 A	485 A
	Toxic Control (OTA)	6.5 C	5.3 C	11.3 C	32.5 C	350 C
	OTA + Bromelain	7.2 B	6.9 B	12.7 B	37.0 B	410 B
	OTA + Probiotic	7.25 B	6.9 B	12.5 B	36.9 B	407 B
	OTA + Bromo + Probiotic	7.55 ab	7.7 ab	13.2 ab	38.5 ab	440 ab

20	Negative Control	8.13 A	9.2 A	14.55 A	42.6 A	493 A
	Toxic Control (OTA)	5.95 C	4.3 C	10.5 C	30.0 C	320 C
	OTA + Bromelain	7.05 B	6.6 B	12.4 B	36.0 B	400 B
	OTA + Probiotic	7.15 B	6.7 B	12.3 B	36.1 B	399 B
	OTA + Bromo + Probiotic	7.5 ab	7.7 ab	13.2 ab	38.0 ab	435 ab
30	Negative Control	8.15 A	9.15 A	14.65 A	42.8 A	490 A
	Toxic Control (OTA)	5.95 C	4.0 C	10.3 C	29.0 C	310 C
	OTA + Bromelain	7.15 B	6.8 B	12.6 B	36.5 B	410 B
	OTA + Probiotic	7.25 B	7.0 B	12.6 B	36.8 B	411 B
	OTA + Bromo + Probiotic	7.6 ab	7.9 ab	13.5 ab	38.8 ab	450 ab

Note: The capital letters denote highly significant differences ($p \leq 0.01$) and small letters denote significant differences ($p \leq 0.05$). Identical letters across rows (i.e., same day) indicate no significant statistical difference, while different letters indicate statistically significant or highly significant differences among the groups. The letter notation (A, B, C, ab) qualitatively indicates the severity of OTA damage and the degree of protection offered by each treatment, where C = severe damage (OTA); B = moderate recovery (bromelain or probiotic); ab = near-full recovery (combined treatment); A = full health (no OTA).

4 Discussion

The present study clearly demonstrates the hematotoxic effects of ochratoxin A (OTA) and the protective efficacy of bromelain and *Lactobacillus* spp., administered individually or in combination. The consistent decline in erythrocytic, leukocytic, and platelet indices in OTA-exposed animals confirms systemic toxicity affecting hematopoiesis, immune competence, and blood homeostasis. Similar hematological impairments following OTA exposure have been reported in multiple animal models and are largely attributed to oxidative stress, inhibition of protein synthesis, and mitochondrial dysfunction [22-24]. The marked reduction in RBC count, hemoglobin concentration, and PCV observed in OTA-treated animals indicates the development of anemia, likely resulting from impaired erythropoiesis and oxidative injury to erythrocyte membranes. OTA has been shown to induce DNA damage, lipid peroxidation, and disruption of cellular redox balance, leading to premature erythrocyte destruction and reduced bone marrow productivity [25-26]. Concurrent leukopenia suggests compromised immune cell turnover, while thrombocytopenia reflects suppressed megakaryocyte activity and platelet formation, consistent with OTA-induced bone marrow toxicity [18, 5]. Bromelain administration partially restored hematological parameters, indicating its capacity to attenuate OTA-mediated cellular damage. Bromelain possesses well-established antioxidant, anti-inflammatory, and immunomodulatory properties that stabilize cell membranes, reduce lipid peroxidation, and promote tissue repair [27-29]. Experimental studies have demonstrated that bromelain enhances microcirculation, modulates cytokine release, and protects erythrocytes from oxidative injury, which explains the observed improvement in RBC indices, hemoglobin levels, and platelet counts in the present study [16].

Similarly, probiotic supplementation significantly improved hematological outcomes compared with OTA exposure alone. *Lactobacillus* spp. are capable of binding and degrading mycotoxins within the gastrointestinal tract, thereby reducing systemic toxin absorption and bioavailability [8-9]. In addition, probiotics enhance gut barrier integrity, regulate immune signaling, and reduce intestinal inflammation, indirectly supporting hematopoietic recovery [10-11]. Previous studies have shown that *Lactobacillus rhamnosus* mitigates OTA-induced oxidative stress and genotoxicity in vivo, supporting the protective trends observed in this experiment [12].

The most pronounced protective effect was observed in the combined bromelain–probiotic group, where hematological values approached those of the negative control group. This outcome suggests a synergistic interaction between systemic antioxidant protection and intestinal detoxification mechanisms. While bromelain mitigates oxidative and inflammatory damage at the tissue level, probiotics reduce OTA uptake and modulate immune homeostasis, resulting in amplified physiological recovery. Similar synergistic benefits of combined natural agents have been proposed in toxin mitigation strategies and nutritional interventions [13-14, 30-31]

Collectively, these findings reinforce the concept that multi-target nutritional strategies are more effective than single-agent interventions in combating chronic mycotoxicosis. The integration of enzymatic antioxidants with probiotic detoxifiers represents a practical and biologically rational approach for reducing OTA-induced health risks in veterinary medicine and animal production systems. Further investigations are warranted to elucidate molecular pathways, optimize dosage regimens, and validate field-level applications across different species and exposure scenarios.

5 Conclusion

This study provides evidence that ochratoxin A induces severe hematological toxicity in guinea pigs, as reflected by reductions in RBCs, WBCs, hemoglobin, PCV, and platelets. Bromelain and *Lactobacillus* spp. administered individually produced moderate protective effects, whereas their combined administration demonstrated a synergistic action and resulted in near restoration of hematological parameters. Co-administration of these agents appears to be a promising strategy for mitigating mycotoxin-induced health risks in veterinary settings. Further studies are required to clarify the underlying mechanisms and to evaluate their efficacy in other experimental models of chronic mycotoxicosis before any broader application can be considered.

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Authors' Contributions

Mahmood Ahmed Hussein contributed to the conceptualization, study design, supervision, and data interpretation. Bushra I. Al-Kaisie carried out the experimental work, animal handling, laboratory analyses, and data collection. Both authors participated in data analysis, manuscript writing, critical revision, and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or personal interests that could have influenced the work reported in this paper.

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