Histopathological Changes in the Small Intestine of Broiler Chickens Exposed to Aspergillus niger Mycotoxins and the Protective Effects of Clove (Syzygium aromaticum) Supplementation

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Abstract

The study aimed to determine the effect of *A. niger* mycotoxins on the small intestine. Pathological histological changes were observed as a result of administering the fungal toxin to the poultry, leading to histopathological changes in the small intestine; the intestinal villi appeared clearly atrophied (thinner) with a reduction in lumen diameter. In contrast, the control group exhibited a normal histological structure. Nystatin helped mitigate the severity of fungal toxicity by promoting the widening of small intestinal villi and stimulating mucus secretion in the intestinal villi of the treated poultry. Furthermore, administering cloves to poultry at a concentration of 5 g/kg, following prior exposure to mycotoxins, resulted in a significant increase in intestinal cells accompanied by an increase in infiltrating cells, with heightened activity of intestinal epithelial cells observed in the small intestinal villi. It has been demonstrated that the combination of nystatin at a dose of (1 mL/L water) and cloves improves the histological structure of the toxin-damaged intestine, which was reflected in a marked improvement in intestinal absorption efficiency and hormonal activity, manifested by an increase in intestinal cell length and an expansion of villus area and crypt diameters.

Keywords: *A.* niger, poultry, mycotoxins, nystatin, pathological histological changes, cloves.

Introduction

"Inevitable contaminants," "natural toxins," "hidden thieves," and "silent killers"—these are common names for a group of fungal metabolites known as mycotoxins, and the disease caused by these toxins is known as mycotoxicosis (mycotoxicosis). In poultry, this disease typically occurs when fungi that produce mycotoxins grow on grains and feed. Although more than 500 different types of mycotoxins have been identified, scientists have focused on those of medical and agricultural importance whose toxicity has been established. The pathogenesis and treatment of mycotoxicosis in the poultry industry remain significant challenges. Oxidative stress is the driving force behind the pathogenesis of mycotoxicosis [1]. "Contamination with mycotoxins resulting from feed spoilage poses a threat to the sustainability of the poultry sector [2]. Poor storage of feed crops according to proper storage standards makes them more susceptible to fungal growth and the formation of mycotoxins; as temperate and tropical regions of the

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world are among the most susceptible to fungi or mycotoxins, depending on their types [6].

Studies have shown that mycotoxins can contaminate food and feed ingredients, and that animals exposed to these toxins through the consumption of contaminated feed may suffer acute toxicity due to high doses, or chronic toxicity due to long-term exposure to low doses. Side effects on animals consuming feed contaminated with mycotoxins include reduced feed intake, effects on the reproductive and productive systems, and reduced weight gain and feed efficiency [12]. The most important classes of mycotoxins of public health and agricultural economic significance include aflatoxins (AF), ochratoxin A (OTA), zearalenone (ZEN), fumonisin (F), and patulin [7]. The genus *Aspergillus* is one of the most widespread and highly dangerous fungal species in warm and tropical countries, where it can lead to crop contamination before the harvest season or, more commonly, during the storage period (EFSA, 2006). Scientific studies have demonstrated that these fungi have carcinogenic, nephrotoxic, and teratogenic effects, as well as other toxic effects on various living organisms [4،10]. Approximately 25% of food fit for human consumption and animal feed is exposed to fungal contamination, causing massive economic losses in the animal production and agricultural sectors [9]. Contamination of animal feed with mycotoxins, particularly those produced by *A.* niger, poses a serious global threat to agricultural sustainability and food safety. Given its profound impact on animal nutrition, mitigating the risks posed by *A. niger* requires a multifaceted approach that integrates strict prevention, advanced detection, and immediate remedial interventions. These strategies are essential not only for safeguarding feed safety and livestock productivity but also for protecting the entire food chain and ensuring human consumer health. This study aimed to evaluate the pathological impact of Aspergillus niger mycotoxins on intestinal tissues and to investigate the protective efficacy of Clove (*Syzygium aromaticum*) in mitigating these effects, while comparing its performance against the standard antifungal treatment (Nystatin)

Materials and Methods

Isolation of *A. niger*:

The fungal isolate was obtained from Prof. Dr. Zainab Alawi Mohammed, who had previously isolated it from poultry feed pellets at the Physiology Laboratory, Department of Animal Production, College of Agriculture, University of Karbala. The fungal isolates were inoculated onto sterile Petri dishes with a diameter of 9 cm containing potato dextrose agar (PDA). The sterile medium was prepared by dissolving 42 grams of it in one liter of distilled water; chloramphenicol (250 mg/L) was added before freezing, and the medium was then sterilized in an autoclave at 121°C and 15 psi for 20 minutes. The plates were then incubated for 5–7 days at 25 ± 2 °C until fungal growth appeared.

Potato dextrose broth (PDB) was prepared and chloramphenicol (250 mg/L) was added to prevent bacterial

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contamination. The medium was then poured into 250-mL flasks, sterilized, and allowed to cool to room temperature. Each flask was then aseptically inoculated in triplicate with 5-mm fungal discs from the target fungal isolate and incubated for 14 days at 25 ± 2 °C. To extract metabolites, the cultures were vortexed for three minutes using an electric mixer, then filtered by vacuum through a Buchner funnel. To ensure a cell-free filtrate, the liquid was passed through a 0.45-micrometer pore-sized membrane filter, The mycotoxin was produced through a controlled incubation process of A. niger for 14 days under optimal laboratory conditions. The resulting toxin was extracted and purified to a 100% purity level. This pure stock. The nutrient medium (PDB) was prepared according to the standard procedures described by Samson *et al*. (2010) to ensure optimal fungal growth and secondary metabolite production.

. The resulting crude extract was placed in sterile glass containers and stored at 4°C for a maximum of 24 hours prior to use. A total of one-day-old white broiler chicks of the same breed were obtained from a private hatchery in Karbala Governorate. The birds were housed in a specialized poultry facility equipped with standard rearing systems, following a strict management and prevention program to ensure optimal health conditions. The experimental groups consisted of 5 birds per treatment, in three replicates, and were orally dosed with *Aspergillus niger* fungal toxin at a dose of 1 mL per 50 g of body weight, while the control group received only sterilized distilled water. Throughout the 45-day experimental period, feed and water were provided ad libitum. At the end of the experiment, sections of the small intestine were excised and immediately fixed in 10% neutral formalin for histological processing and examination.

Experimental Groups and Treatment:

Group 1 (Control group): Chicks were fed a standard basal diet without any additives.

Group 2: Chicks were fed a standard diet contaminated with Aspergillus niger mycotoxins.

Group 3 (Nystatin group): The chicks received feed contaminated with mycotoxins to which nystatin (1 mL/L) was added via drinking water.

Group 4 (Clove Group): The chicks were fed standard feed containing both mycotoxins and clove powder.

Group 5 (Combination Group): The chicks received feed contaminated with mycotoxins, with clove powder added to the feed and nystatin (1 mL/L) in their drinking water.

Results

Effect of *A. niger* mycotoxins on the pathological histological structure of the small intestine in poultry.

Pathological histological examination of the small intestine in poultry exposed to the fungal toxins under study revealed clear pathological changes. These changes were primarily manifested by marked lymphocytic infiltration within the lamina propria, indicating an inflammatory response to the fungal

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toxins. Furthermore, the intestinal villi appeared markedly atrophic (thinner) with a reduction in lumen diameter (Figure 1). In contrast, the control group exhibited a normal histological structure that maintained its structural integrity without any lesions caused by mycotoxins (Figure 2). Therapeutic intervention with nystatin (1 mL/L) in poultry challenged with mycotoxins resulted in a marked widening of the small intestinal villi and enhanced villus activity within the intestinal crypts (Figure 3). Similarly, the addition of clove powder (5 g/kg) to the diet demonstrated a corrective effect on the mucosal structure, characterized by the widening of the villi and increased secretory activity of the crypt cells (Figure 4). The synergistic combination of nystatin and clove in the groups of poultry exposed to mycotoxins showed the greatest improvement in terms of histomorphological structure; this was evident in increased intestinal cell height, widened villus area, and a marked increase in crypt height coupled with strong secretory activation.

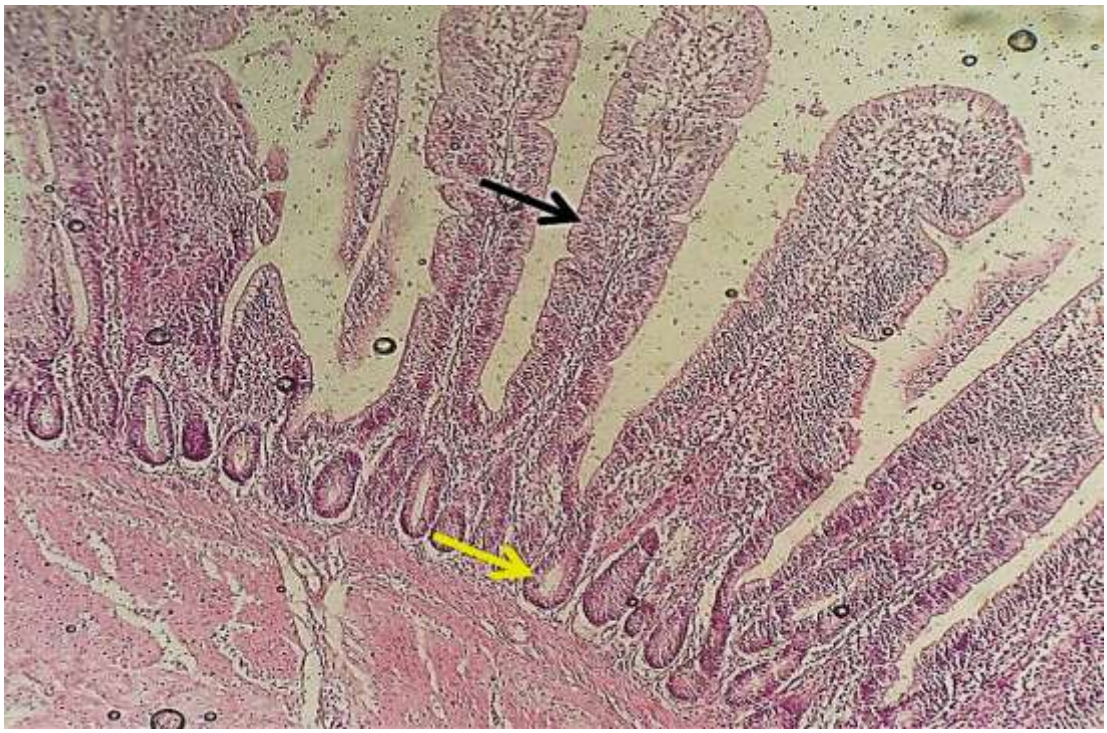

Figure 1. Microscopic image showing lymphocyte infiltration in the lamina propria of the poultry intestine (yellow arrow). The intestinal villi are clearly visible within the lumen (black arrow). H&E stain.

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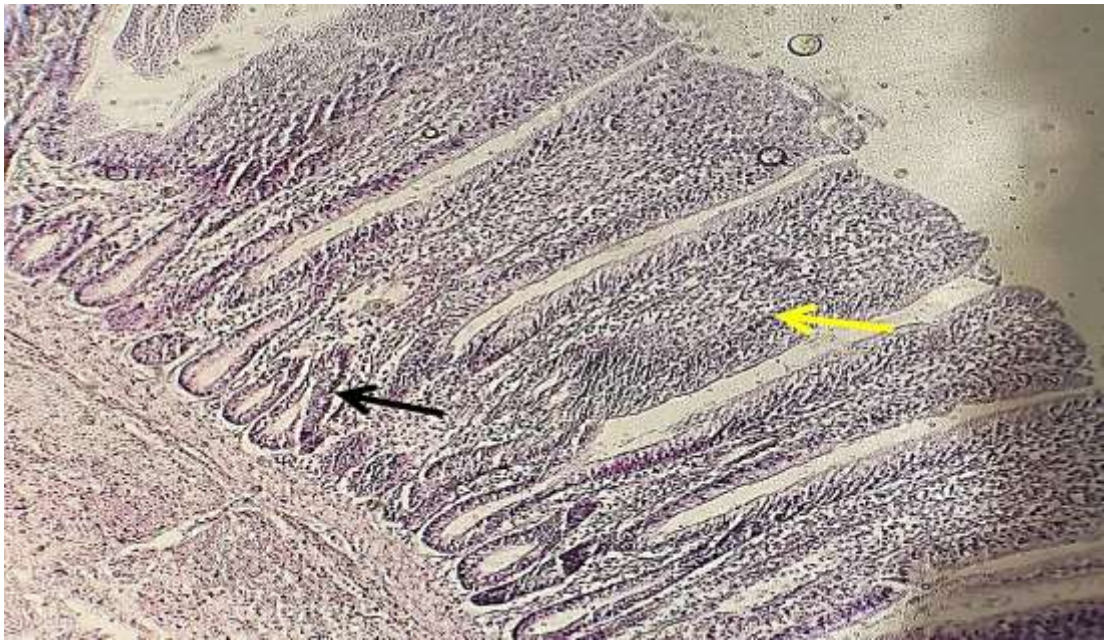

Figure 2. Microscopic image showing the normal condition of intestinal cells in the small intestine with a very small number of goblet cells (yellow arrow). It demonstrates a decrease in the number of mucous cells present in the intestinal glands. H&E stain. Control group.

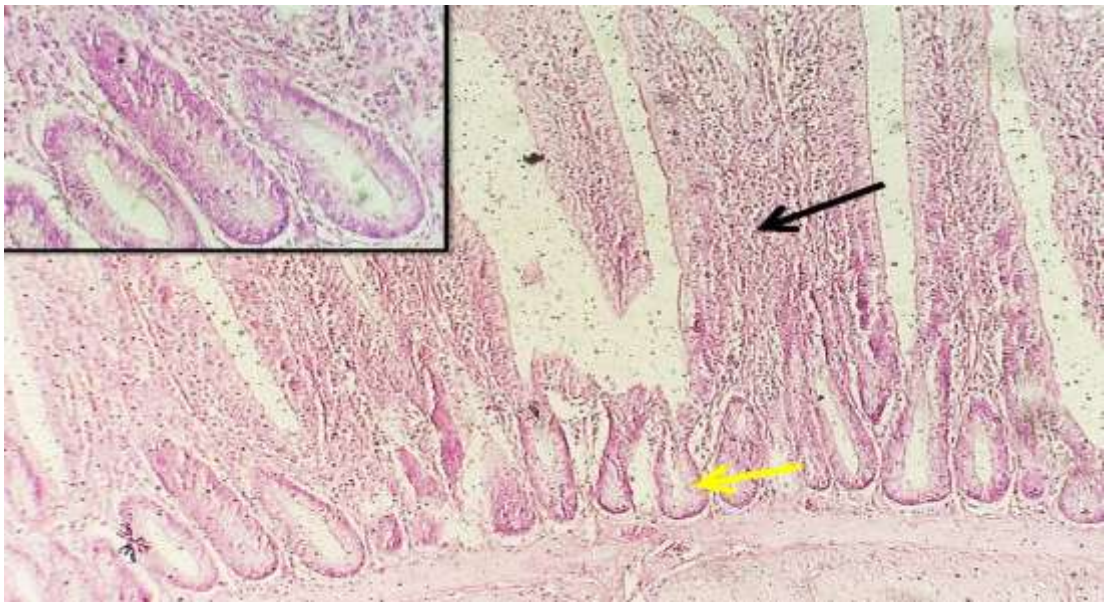

Figure 3. Microscopic image showing widening of the villi in the small intestine (black arrow; the yellow arrow indicates increased goblet cell activity in the intestinal folds). H&E stain.

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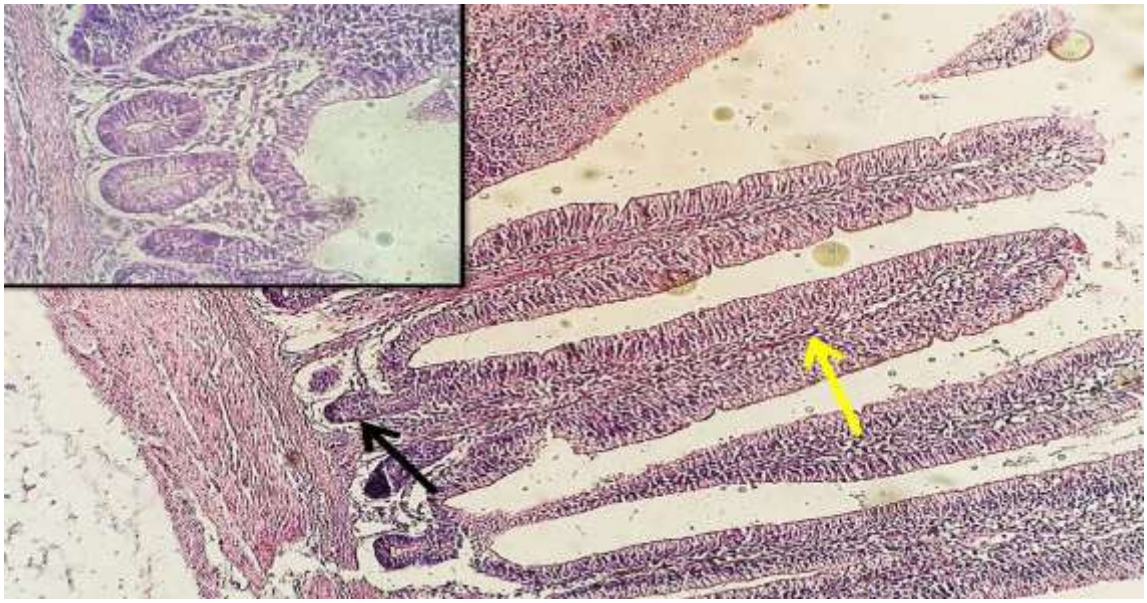

Figure 4. Microscopic image of the small intestine showing elevated intestinal folds with an increase in infiltrating cells (yellow arrow). The intestinal folds demonstrate increased activity in the intestinal epithelial cells and an increase in the number of mucous glands (black arrow). H&E stain.

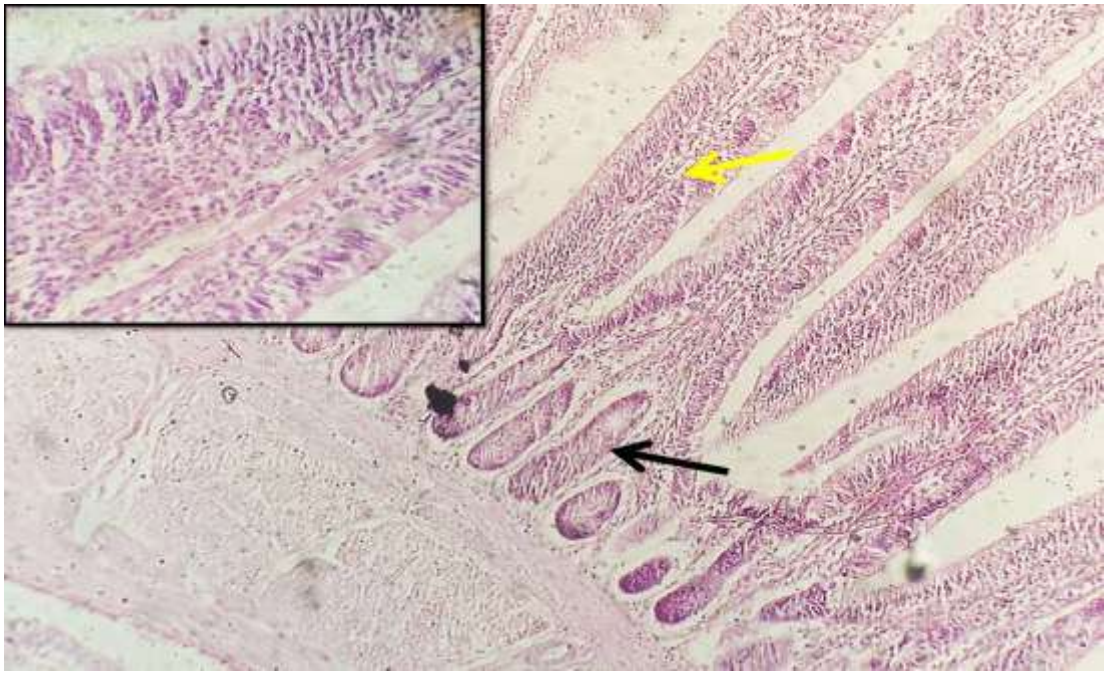

Figure 5. Image showing elevated intestinal cells and widened space in the intestinal folds of the small intestine (yellow arrow). Increased crypt diameters in the intestine and elevated villus activity (black arrow). H&E stain.

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Discussion

Mycotoxins caused severe histological damage to the small intestine in poultry, leading to impaired nutrient absorption and a decline in the birds' overall health. The results confirm that mycotoxins damage the intestine through marked lymphocytic infiltration within the villi, indicating an inflammatory response to mycotoxins. Mycotoxins exert severe deleterious effects on intestinal integrity, leading to profound physiological disturbances. These intestinal disturbances not only trigger acute poisoning but also constitute a major factor in the pathogenesis of chronic inflammatory bowel diseases [5]. Administration of mycotoxins to chicks has led to clear immunological alterations and disruption of intestinal mucosal activity due to an inflammatory response or hormonal imbalance. The observed improvement in intestinal villi structure is a key indicator of recovery. While mycotoxins typically cause villus atrophy and impair nutrient absorption, the observed increase in villus width and cryptal hormonal activity suggests that the treatment effectively alleviated mycotoxin toxicity and repaired damaged tissue, thereby restoring intestinal absorptive capacity [8،11]. In contrast, the addition of cloves demonstrated a positive effect in mitigating these effects, attributed to their content of active compounds (eugenol) that act as natural antioxidants, anti-inflammatory agents, and antimicrobials. The improvement in the intestinal cell profile may indicate that cloves contributed to initiating the repair process of damaged intestinal tissue. Furthermore, the combination of nystatin and clove powder resulted in a marked restoration of intestinal morphology in poultry groups challenged with the mycotoxin , characterized by increased villus height and width, enhanced crypt activity, and activation of the enteric nervous system. These results are consistent with research indicating that antifungal treatments stimulate the body's natural defense mechanisms [3]. The synergy between the drug and clove powder resulted in a marked improvement in gut health, suggesting that clove not only acted as a potent antioxidant but also enhanced the therapeutic efficacy of the treatment.

Conclusions

The study confirmed the harmful effect of *A. niger* mycotoxins in causing severe pathological histological changes in the liver and intestine, and the effective role of clove powder and nystatin in reducing this damage and improving the overall health of poultry.

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