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Histological Change on mice Spleen treated with Aflatoxin B1

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Abstract

The results appeared that in this study, histopathological examination of spleen of mouse treated with 12, 25, 50 µgmL^{-1} of Aflatoxin B1. gross-examination showed clear pathological changes in examined spleen of showed deposition of amyloid like substance in congested red pulp in other section ,the main lesions characterized by inflammatory cells particularly mononuclear cells infiltration in congested red pulp fatty changes were seen in spleen in addition to extensive apoptosis of lymphocytes in white pulp that characterized by cellular debris in multiple irregular spaces the spleen revealed mononuclear cells in congested blood vessels with depletion of white pulp with apoptosis of lymphocytes in white pulp.

Key word *Aspergillus flavus*; Aflatoxin B1; Spleen; Necrosis; Mice.

1. Introduction

Aspergillus flavus is the name now used to describe a species as well as a group of closely related species (1). It is a saprotrophic and pathogenic fungus with a cosmopolitan distribution (2). This fungus develops in a wide range of temperatures in substrates with high hydrocarbon content, with humidity being the most important variable, and grows better with water activity between 0.86 and 0.96 (3). In addition, it is the main *Aspergillus* species infecting insects, and it is also able to cause diseases in economically important crops, such as maize and peanuts, and able to produce potent mycotoxins (4). Primarily, infection results from inhalation of the airborne spores, which are small enough to reach the alveoli of the respiratory system. Most patients suffering from aspergillosis have an impaired immune system that is often evoked by leukemia, neutropenia or after prolonged treatment with steroids, such as solid organ transplantation patients (5). The mortality rate of aspergillosis among these patients lies between 30 to 90% (6).The primary route of human infection is *via* the inhalation of these airborne spores,, followed by conidial deposition in the bronchioles or alveolar spaces. In healthy individuals, conidia that are not removed by mucociliary clearance encounter epithelial cells or alveolar macrophages, the primary resident phagocytes of the lung. Alveolar macrophages are primarily responsible for the phagocytosis and killing of *A. flavus* conidia as well as the initiation of a proinflammatory response that recruits neutrophils (one type of polymorphonuclear cell (PMN) to the site of infection. Conidia that evade macrophage killing and germinate become the target of infiltrating neutrophils that are able to destroy hyphae (3).

2. Materials and Method

2.1. Preparation of spore suspension of *A. flavus* isolates

After the isolates were cultured on SDA at 28 °C for 7 days, fungal colonies were removed by adding 7 ml of distilled water and a drop of tween 80 per slant. Then the suspensions were prepared by gently agitation of the surface with a

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loop. The slant was shaken to give a uniform suspension of spores. The spore suspension was filtered through sterile gauze, and then the filtrate was transferred to a sterile test tube. Inoculums quantification was made by counting the spores using haemocytometer by added one drop of the suspension to haemocytometer by Pasteur pipette, spores were calculated

2.2. Aflatoxins production (in rice medium)

Selective isolates of *A. flavus* (were cultured initially on SDA medium) were grown in 250ml Erlenmeyer flask (80 flasks) containing 25g of sterile rice medium (7). Then a discs of 5 mm diameter which were cut with a sterile corkborer and punctured in the culture media to prepare the inoculums for *A. flavus.* Then the flasks were incubated for 21 days at 28°C (4).

2.3. Extraction of aflatoxins

After incubation time, the moldy rice was soaked overnight with 75 ml of chloroform 99.45% in dark place. Then, the soaked medium was homogenized with electric homogenizer for 15 min. The extracted solution was filtered through gauze then, through a Whatman No.1 filter paper. The residue of moldy rice was washed with 50 ml of chloroform, and filtered. Chloroform fractions were pooled and evaporated to dryness at 50°C. The extract (sticky paste) stored at 4°C until use (8,9).

2.4. Experimental animals

Seventy eight of Swiss albino mice (male) were purchased from the National Centre for Drug Control and Research /Baghdad were used, their ages were ranged (10-12) weeks (10). The mice were acclimatized for two weeks before treatment. They housed in plastic cages containing hard wood chips for bedding, in controlled animal house at 25± 2Cº, 4/10 hour's light / dark cycle. The animals were given water and fed with suitable quantity of complete diet. They were housed at the animal house in Biotechnology Research Center/ Al- Nahrain University. Detail of groups explained below:

- Group I: negative control (without any treatment)
- Group II: mice treated with 12 μgmL^{-1} of Aflatoxin B1
- Group III: mice treated with 25, μgmL^{-1} of Aflatoxin B1
- Group IV : mice treated with 50 μgmL^{-1} of Aflatoxin B1

3. Results and Discussion

Histological examination of the control spleen of mice revealed normal structure of splenic parenchymal tissue fig (1), consisting of lymphoid tissue, white and red pulp as showed .While histological sections in spleen treated with 10% methanol showed no lesions or other histopathological changes of organic tissue. While, the section of spleen treated with Aflatoxin B1, the results as explained in (fig 2,3 and 4), showed necrosis in addition to hemorrhage. Previous study agreed with us by revealing histological examination of AFs concentration treated group showing degeneration and necrosis of splenic in hepatocytes, lung, spleen and bone marrow cells, or human bronchial epithelial cells (13,14). scintific reported that an increased apoptotic splenocytes was observed in AFB1 treated animals, which revealed one mechanism of AFB1-induced immunosuppression, that were closely related to oxidative stress in the aflatoxicosis. Furthermore, previous studies have clarified that oxidative stress would induce mitochondrial dysfunction, nuclear translocation, DNA binding, and transcriptional activity of p53, and then activate the course of cell-cycle arrest and cell apoptosis (15). The results of present study is in accompanied with (16) how showed that feeding of albino rats on diet containing aflatoxins at concentration of 0.1 ppm for 2,4,6 weeks, lead to histological changes of spleen including lymphocytic degeneration, fatty changes with numerous hemorrhagic areas. Also agreed with (12) showed that mice treated with 2ppb aflatoxin B1daily for 28 weeks have a histological changes of spleen including diffusion of cancer cells in the red pulp with accumulation of mononuclear cell, also free hemosiderin accumulation in the red pulp, also there is a lymphoid hyperplasia which look like nodules elucidation for accumulation of hemosiderin was that sinusoid and pulp filled with blood with marked hyperplasia of endothelium of sinusoids.

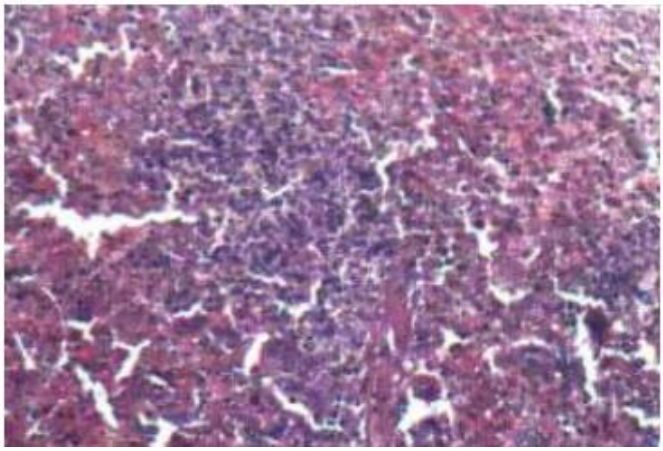

Figure 1 Control mice spleen tissue

Figure 2 Spleenic tissue treated with 12 μgmL^{-1} of Aflatoxin B1 showing light widening of mice white pulp

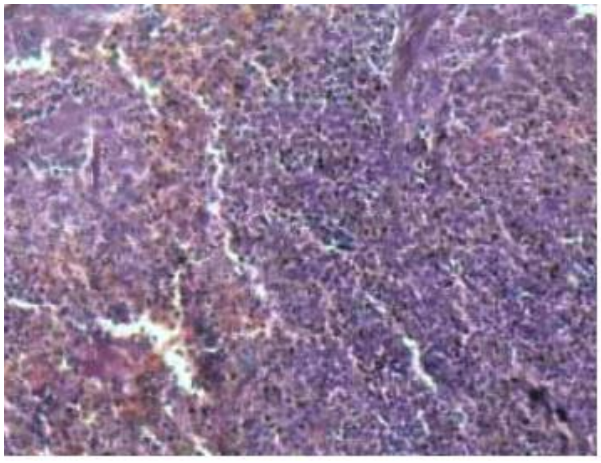

Figure 3 Spleen of mice treated with 25 μgmL^{-1} of Aflatoxin B1showed mild degenerative with hemorrhage

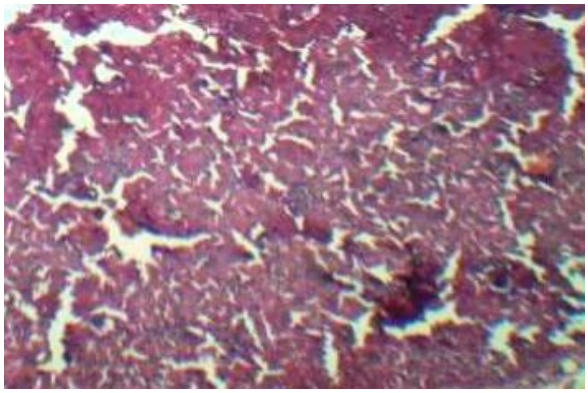

Figure 4 The section of spleen showing necrosis of pulp with hemorrhage after treated with 50 μgmL^{-1} of Aflatoxin B1

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to disclosed.

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