International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Histological Changes on Rat Gastrointestinal Tract after Exposure to Aflatoxins (AFs)

Rana Jalal Shaker*

Department of Biology, College of Education, Tikrit University

Abstract

Aflatoxins (AFs) are a mycotoxin produced mainly by the fungus Aspergillus flavus and A. parasiticus in food and feed . It is considered as a carcinogenic toxin for human and animals. The current study was designed to investigate the Histological Changes on Rat Gastrointestinal tract after exposure to Aflatoxins (AFs) Aflatoxins poisons are the most dangerous toxins for human and animal health. The biological studies, showed that, giving afltoxin with the food of the experimental animals , There are different pathogenic effects take place . These effects include; some disorder with immune system, mutation, carcinogenic effects with different organs of the body. Aflatoxin poisons were produced with gradient concentrations (0.312 ,0.625, 1.250 , 2.5 mg/kg of feed) . These concentrations mixed with food and feed to the animals for 21 days in different treatments. Then, the animals were killed, and histopathology of the tissue slices were made from Gastrointestinal tract. Physiological changes in the Gastrointestinal tract tissues were recorded .These changes ,represented a Alintation in Epitheliol cells lining the intestinal villi and infiltration in Lymphoma cell in the lamina basic and congestion in the bloody submucosal layer . However, the Gastrointestinal tract tissues, hese changes are increased and become pathogenic especially with high levels of aflatoxin . Also, Degeneration in the nuclei of cells and thickening. Also, Hemorrhage of blood vessels under the mucous layer and Swelling of the cells lining.

Keywords: Aflatoxin; Aspergillus parasiticus; histopathological changes.

1. Introduction

Aflatoxins are a group of fungal metabolites produced primarily by the fungi Aspergillus flavus and Aspergillus

parasiticus. The four major naturally produced aflatoxins are known as B1, B2, G1, and G2. B and G refer to the blue and green fluorescent colors produced under UV light, while the Subscript numbers 1 and 2 indicate major and minor compounds, respectively. Specific P450 enzymes in the liver metabolize aflatoxin into a reactive oxygen species (aflatoxin-8, 9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer [1,2].

Exposure to aflatoxins can lead to several health-related conditions including acute and chronic aflatoxicosis, aflatoxin-related immune suppression, liver cancer, liver cirrhosis, as well as nutrition-related problems in children such as stunted growth in many areas, due to widespread high-level consumption, aflatoxin contamination through food and feed is unavoidable due to the absence of alternative food and feed resources. When ingested, aflatoxin binds to liver proteins. The metabolic products may persist for 2 to 3 months or longer and can be detected through blood tests [3].

Aflatoxins exposure can be measured in two ways: (1) an analysis of prepared foods or (2) through biological markers of exposure from blood or urine samples that are obtained and analyzed for the presence of aflatoxin derivatives. Possibilities to minimize biological exposure include (1) chemoprotection through the use of drugs and dietary supplements that detoxify aflatoxin and (2) enterosorptive food additives that bind to the toxin and render the aflatoxin biologically unavailable to the body [4].

Aims of the study: The main objective of this study was to to investigate Histological Changes on Rat Gastrointestinal tract after exposure to Aflatoxins (AFs).

2. Material and Methods

2.1 Preparation of Spore Suspension for Aspergillus parasiticus NRRL2999

After the isolate were cultured on malt extract agar at37 °C for 7 days, fungal colonies were covered with 10 ml of sterile saline solution for spore suspension preparing, and the suspensions were prepared by gently agitation of the surface with the sterile tip of special glass rod. The spore suspension was filtered through sterile gauze, and then the filtration was transferred to a sterile test tube. Inoculums quantification was made by counting the spores using haemocytometer slide, spores were calculated under high power 40X of light microscope using the following equation :

Concentration of spores = (Z ×4 × 106) /n spores/ml

Where n: total No. of small squares Z: total No. of spores [5].

3. Aflatoxins Production and Analysis

Aflatoxins production was conducted through wetting of100g of rice grains in 500 ml conical flask size and sterilized in autoclave at 121ºC, 15Psi for 15 min with three serious times, after cooling then, inoculation with 5 ml of A. parasiticus suspension, then incubation for ten days at 27 ºC and 6% humidity, according to (

Bhatnagar et al., 2003).Then dry the rice and grind to a fine powder form, Was estimated rice powder from aflatoxin total by extracted with chloroform according to [7]. Detection of aflatoxins was obtains by High performance liquid chrmatografy HPLC technique after extraction of aflatoxins used the chloroform solvents.

4. Experimental Animals

4.1 Laboratory Animal's Initialization

Twenty Albino.-Sprague Dawley Rats (110-115 g) males at 45 day age have been obtained from the house livestock in the Faculty of Veterinary Medicine, University of Salah aldeen. Were used to determine the toxic effects of AFs reared at an optimal room temperature ranged between 22–25°C. Animals were fed on which formulated from natural ingredients suitable for growing maintenance according to [6] contain 35% weight, 25% soya bean 10% dairy protein 1% poder milk , and 1% vitamin and minerals. The experimental design consisted of five groups dietary treatments

Distributed designed of Rats groups according to the feed treatments with AFs .

First group Control (only diet without treatment).

Second group : Aflatoxins 0.312mg/kg diet.

Third group: . Aflatoxins 0.625 mg/kg diet.

Fourth group: Aflatoxins 1.250 mg/kg diet.

Fifth group: Aflatoxins . 2.5 mg/kg diet.

At the end of the experiment All rat were sacrificed after 28 day of the treatment. Gastrointestinal tract dissected out and were fixed in plastic containers containing 100 ml of formalin 10 %. After that parts samples were dehydrated in progressively , then embedded in paraffin and cut into section of 4-5 μm thickness and stained with haematoxylin and eosin (H & E) [7].

5. Results and Discussion

The results of pathhological examinations of tissue in the gastrointestinal tract totals animals that add to their diet concentration of 0.312 mg / kg diet of aflatoxins shows changes have represented the existence of a sharp and significant alienation of the number of vertical epithelial cells Statistics with the connective tissue of the villi intestinal sections and there are numbers of these associated with villi cells in a state of degeneration and thickening or break the nuclei as show in (Figure 1 A) and the possible distinction of large numbers of cellular inflammatory and lymphocytes near the surface of the villi in the heart of the villi, and spread this leaching cellular inflammatory to the rules of the villi of the cells lining the glands intestinal also the degeneration of a large and atrophy was observed and contained cavities glands meant to be isolated epithelial cells. In the third group with a focus 0.625 mg / kg of diet observed cellular widely cells vertical epithelial Statistics lining the

villi intestinal degeneration with the fall of large numbers of mentioned cells in the intestinal lumen (Figure 1 B) and notes that there are also eating away at the core of the villi with to contain the numbers of cells inflammatory especially lymphocytes, also observed that the mucous glands basic plate contained a swollen and overgrown and cavity cells amid all the small gland and circular in shape (Figure 1 C) and the mucous glands there are numbers of inflammatory cells and the presence of congestion bloody lymphoma small of these plate vascular and submucosa . But in the fabric of the gastrointestinal tract of animals fourth group was observed that most of the intestinal villi contained the tops of her degeneration and cell necrosis and crash the preparation of simple vertical cells and Fall cavity near the villi sites (Figure 1 D) There is also a quotient damage to the core of the villi at the site degeneration villi tops and most of the parties and sides villi contained a simple columnar cells intact and noted the presence of micro-villi on the surface, was also observed that most of the mucous glands contained swollen adenocarcinoma cells with scarcity Note holes It was noted the presence of focal cell lymphoma under the villi and the mucous glands (Figure 1 D), as well as the class submucosal contained a large congestion of blood vessels and observation of red blood cells sometimes

extravascular bleeding..

But in the fifth set of laboratory animals, it was observed that the vertical epithelial cells Statistics lining Statistics intestinal villi that line the intestinal villi was crashed and puffy surface villi So observed a strip most of the surfaces of the villi of these cells and the emergence of the core of the villi substitute, in the outskirts of the villi there is a simple columnar cells overgrown the nuclei (Figure 1 E) as there are inflation in the neighboring cells of the epithelial cells, was also noted that the core of the villi contained large numbers of lymphocytes and other inflammatory cells, and there is hemorrhage in most of the blood vessels in the class submucosal at the core plate when the rules of the villi (Figure 1 F).

Gut contained villi where severe atrophy and most of the cells in which flayed inside the gut cavity and there infiltration cell lymphoma in the core plate and the back of atrophy in the cells lining the glands of the mucous of the channel when the rules of the villi and the rest of the core plate surrounded by numbers of

lymphocytes, and vascular congestion (Figure 1 G).

Mucous membrane of the digestive tract contains the villi disjointed and lined vertical cells simple where atrophy and other puffy and disjointed from each other and surrounded by numbers of lymphocytes (Figure 1 G) and extended leaching lymphatic to sweat villi down to the basic near the mucous with a degenerative and atrophic cells glands also (Figure 1 G) there are bleeding red blood cells of the

blood vessels in the mucous layer underneath .

Significant damage to the villi and mucous lost a large number of epithelial cells vertical lining where diverged within the intestinal lumen and near the villi noon (Figure 1 H) pulp villi contains a large number of connective tissue cells and lymphocytes and other inflammatory intestinal mucosal glands where inflation to cells lining the glands with a large mucous droplets in Its cavities appeared hemorrhage submucosal glands and sharpening the infiltration of lymphatic glands between the mucous cells.

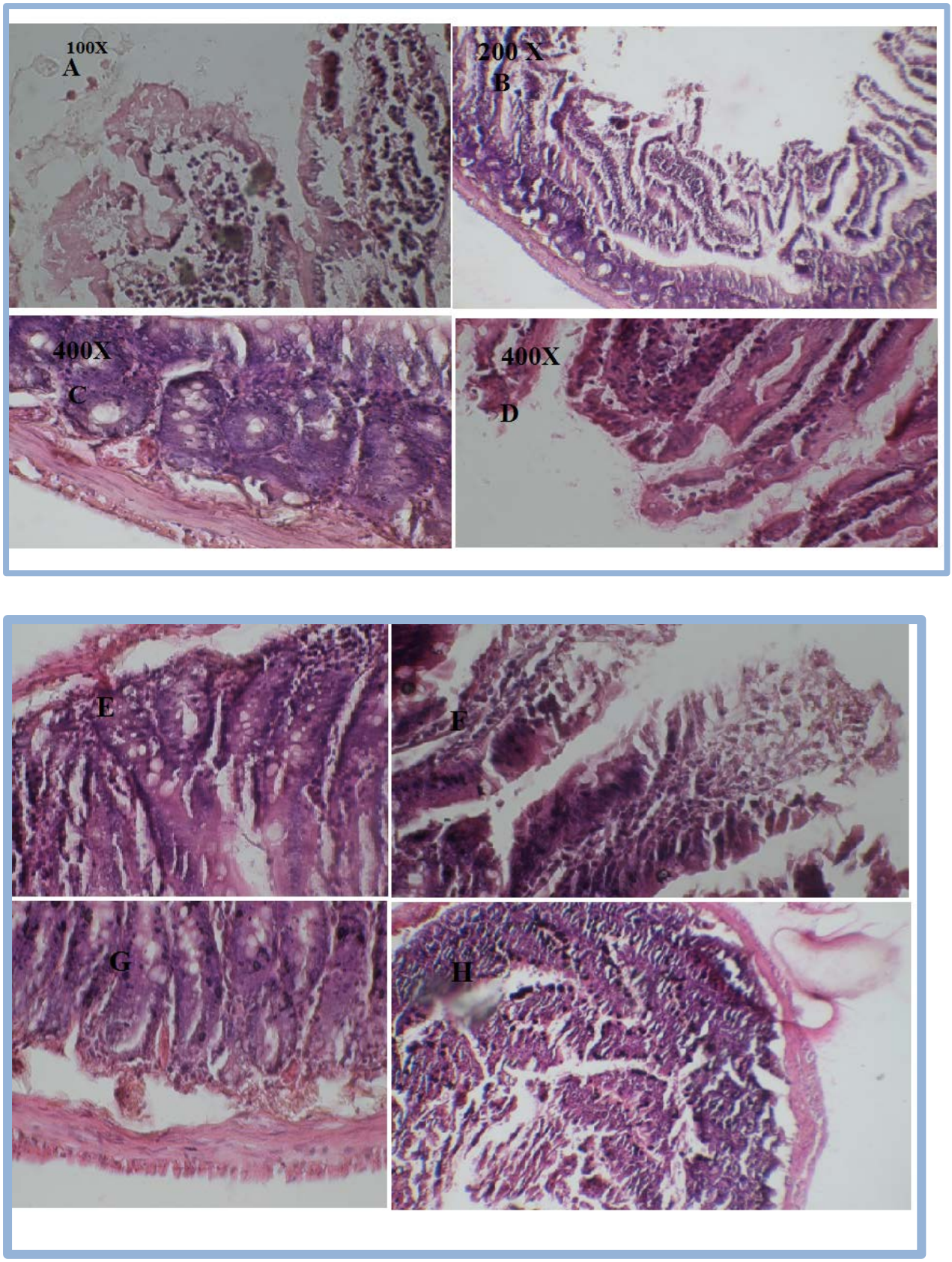

Figure 1 (A,B,C,D,E,F,G,H) : Histological Section in the Gastrointistinal tract of RAT treated with Aflatoxins 0.312mg/kg diet, Aflatoxins 0.625 mg/kg diet, Aflatoxins 1.250 mg/kg diet, Aflatoxins . 2.5 mg/kg diet. (H&E stain, 400X).

The results obtained by Fareed and Inaam [8] showed that female albino rats treated with 80 µg/kg aflatoxin B1 caused Significant damage to the villi and mucous accompanied by lymphocyte infiltration , while [9]showed that 1807 µg/kg aflatoxin B1 fed to pig's caused changes including moderate to extensive swollen, presence of enlarged nucleus and vacuolation of periportal parenchyma cells. Results were reached by [11], reference [10] revealed that treated of Westar rats with 1.5 mg/kg aflatoxin B1 orally cause broad infiltration of lymphocytes and villi cells, disturbed lobular architecture, fatty degenerative changes and focal necrosis, Devendran and Balasubramanian, [11] showed that many histological changes of gastrointestinal tract include degenerative reversible lesion, mild parenchymatous degeneration characterized by granular appearance of villi cytoplasm, severe hydrophilic and vacuolar degeneration. The vast majority of mucous cell had significant cytoplasmic visualization with disseminated necrotic cells on rats treated with 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm aflatoxin B1 for 8 days.

Acknowledgements

The authors wish to thank all researchers helps in arrange the paper and made the work possible.

References

[1]. Wild, C. P and Gong, Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. Carcinogenesis. 31(1): 71–82.

[2]. Wu, F and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies 61 in Africa: Case studies in biocontrol and postharvest interventions. Food. Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 27(4): 496–509.

[3]. Wagacha, J.M and Muthomi, J.W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. Into. J. Food Microbiol .124:1 –12.

[4]. Probst, C.; Schulthess, F and Cotty, P. J. (2010). Impact of Aspergillus section Flavi community structure on the development of lethal levels of aflatoxins in Kenyan maize (Zea mays). J. Appli. Microbiol. 108: 600–610.

[5]. Faraj, M. K. (1990). Regulation of mycotoxin formation in zea mays. Ph.D. Thesis, Department of bioscience and biotechnology. University of Strathelyde, Glascow, U.K .

[6]. Bhatnagar, K., Kumar, H. and R. Kuttan. (2003). Inhibition of drug metabolizingenzymes (Cytochrome P450) in vitro as well as in vivo by PhyllanthusamarusSchum&Thonn. Biol. Pharm. Bull. 29:1310-1313 .

[7]. Nabila, Salah. H., Khaled G. A., Yasser, A. K., Aziza, A. E., Fathia, A.M and Mosaad A. A.(2013) . Evaluation of radical scavenging properties and the protective role of papayafruits extracts against oxidative stress in rats fed aflatoxin-contaminated diet. Comunicata Scientiae. 4(1): 43-57

[8]. National Research Council Recommended (NAS-NRC). (2002). Dietary Allowance .15th ed. Washington. D.C. National Academy. Press

[9]. Fareed, H.A .A and Inaam, A. M.A. (2006). Histological study of the effect of aflatoxin on liver and kidney of female Wistar rats. Tikrit J. Pure Sci. 11 (1).

[10]. Guylaine, M. M.; Joelle L.; Nicolas, L.; Etienne, B.; Isabelle, R.; Philippe, P.; Anne-Marie, C.;

Ge´rard, B.; Isabelle, P.O and Pierre, G.(2007). Selective impairment of drug-metabolizing enzymes

[11]. Shyamal, S.; Latha, P. G.; Suja, S .R.; Shine, V. J.; Anuja, G. I.; Sini. S.; Pradeep, S.; Shikha, P and Rajasekharan, S. (2010). Hepatoprotective effect of three herbal extracts on aflatoxin B1-intoxicated rat liver. Singapore Med .J. 51(4): 326-331.

[12]. Devendran, G and Balasubramanian, U. (2011). Biochemical and histopathological analysis of aflatoxin induced toxicity in liver and kidney of rat. Asian J. Pla. Sci. Res.1 (4):61-69.