

The Protective Effects of *Lactobacillus Casei* and *Lactobacillus Acidophilus* Against Liver and Bursae of Fabricius Pathological Changes Induced by Aflatoxin B1 or Fumonsin B1 Contaminated Feed in Broilers

A.A. Shlej¹; J.M. Saaid¹ and K.M. Thlij²

¹Animal Production Department- College of Agriculture -Tikrit University

²Food Science Department- College of Agriculture -Tikrit University

ABSTRACT

Key words:

Aflatoxin B1, Fumonsin B1, Broiler, *Lactobacillus casei*, *Lactobacillus acidophilus*, pathological changes.

Correspondence:

Akeel A. Shlej

E-mail:

akeelabd79@yahoo.com

The aim of this study was to evaluate the ability of *Lactobacillus casei* and *Lactobacillus acidophilus* in counteracting the deleterious effects of aflatoxin B1 (AFB1) or Fumonsin B1 (FB1) in broiler chickens. One hundred and five Ross 308, one-day-old broiler chicks were assigned 7 treatments, 3 replicates with 5 birds cages each for 42 reared day. The experimental treatments were labeled as follows:-T1. BD no other addition (Control). T2. BD containing a 3 mg AFB1/kg diet. T3. BD containing a 3 mg FB1/kg diet + *Lactobacillus casei* CFU (1.5×10^8 cell /ml). T4. BD containing a 3 mg AFB1/kg diet + *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml). T5. BD containing a 300 mg FB1/kg. T6. BD containing a 300 mg FB1/kg diet + *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml). T7. BD containing a 300 mg FB1/kg diet + *Lactobacillus casei* CFU (1.5×10^8 cell /ml). Results of histological analysis showed that there was significant damage in the Liver and Fabricius tissues receiving AFB1 or FB1 alone. Feeding Aflatoxin caused liver fatty changes, necrosis, bile duct hyperplasia and aggregation of lymphocyte. The fumonsin treatment caused moderate to severe hydropic/fatty degeneration in the hepatocytes of the liver and the tubular epithelium of the liver and follicular depletion in the bursa of Fabricius. The supplementation of *L. casei* or *L. acidophilus* to AFB1 or FB1 treated birds not significantly diminished the negative effects of dietary AFB1 or FB1 ($p < 0.05$) on the compared to the control diet. In conclusion our results showed that addition of *L. casei* and *L. acidophilus* cannot reduce the adverse effects produced by the presence of AFB1 or FB1 in broiler chickens diet.

قدرة بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* على حماية الكبد وجراب فابريشيا من التغييرات النسيجية المرضية الناتجة عن تلوث عليقة فروج اللحم بـأفلاتوكسين B1 أو الفيومونسين B1

عقيل عبد شليج المجبل¹ و جميل محمد سعيد¹ و كركز محمد ثلج²

¹قسم الإنتاج الحيواني-كلية الزراعة- جامعة تكريت ²قسم علوم الاغذية - كلية الزراعة- جامعة تكريت

الخلاصة

هدفت هذه الدراسة لتقييم قابلية بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* في التقليل من التأثيرات الضارة للأفلاتوكسين أو الفيومونسين على التغييرات النسيجية للكبد وجراب فابريشيا ، وقد أستخدم في هذه التجربة 105 فرخ لحم تجاري من نوع Ross 308 وبعمر يوم واحد وزعت الطيور الى عشرة معاملات تضمنت كل معاملة ثلاث مكررات بواقع خمسة طيور للمكرر في القفس الواحد ولمدة 42 يوم . وكانت معاملات التجربة على النحو التالي:- المعاملة الاولى عليقة السيطرة بدون إضافات ، المعاملة الثانية عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف ، المعاملة الثالثة عليقة السيطرة 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 *Lactobacillus casei* خلية /مل، المعاملة الرابعة عليقة السيطرة 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 *Lactobacillus acidophilus* خلية /مل، المعاملة الخامسة 300 ملغم فيومونسين /كغم علف ، المعاملة السادسة عليقة السيطرة + 300 ملغم فيومونسين /كغم علف + 1.5×10^8 *Lactobacillus acidophilus* خلية /مل، المعاملة السابعة عليقة السيطرة 300 ملغم فيومونسين /كغم علف + 1.5×10^8 *Lactobacillus casei* خلية /مل . تسبب تلوث العليقة بالأفلاتوكسين B1 والفيومونسين B1 ضررا معنويا ($p \leq 0.05$) في كلا من نسيج الكبد وجراب فابريشيا حيث ظهرت

كلمات مفتاحية :

أفلاتوكسين B1 ، فيومونسين B1 ، فروج اللحم ، *Lactobacillus casei* ، *Lactobacillus acidophilus*، تغييرات نسيجية مرضية .

للمراسلة :

عقيل عبد شليج المجبل

البريد الالكتروني:

akeelabd79@yahoo.com

علامات تتخر الخلايا وتليفها وظهور البقع الدموية وتجمع الدهن حول الكبد . أن أعطاء كلا النوعين بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* الى الطيور المغذاة على العليقة الملوثة لم تقلل من التأثيرات العكسية الناتجة من وجود الافلاتوكسين B1 والفيومنين في علائق فروج اللحم .

Introduction :

Mycotoxins are secondary metabolites of some filamentous fungi, mainly those belonging to *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and the ergot alkaloid groups. They are can be found in raw materials and products of food industry as well as in feedstuffs (Pleadin *et al.*, 2012; Santos *et al.*, 2012). Toxigenic fungi are a major problem in cereal crops as they produce a multitude of toxic metabolites contaminating plants and food products (Clark *et al.*, 2012) The primary classes of mycotoxins are Aflatoxin (AFB), fumonisins (FB) (Var & Kabak, 2004).

AFB1 is classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen (IARC, 1993). This mycotoxin is also mutagenic, teratogenic and immunosuppressive in farm and laboratory animals (IPCS-WHO, 1998). AFB1 mainly affects the cell-mediated immunity (Williams *et al.*, 2004). This mycotoxin decreased total lymphocytes, T-cells sub-types (CD4 (+) and CD8 (+)), NK, and TNF- α and IL-1 β release in rats (Abbès *et al.*, 2010).

Fumonisins are fungal secondary metabolites produced by species of *Fusarium*, mainly *Fusarium verticillioides* and *Fusarium proliferatum* (CAST, 2003; Krska *et al.*, 2007). There are several identified fumonisins, but fumonisin B1 (FB1) and fumonisin B2 (FB2) are the most important and constitute up to 70% of the fumonisins found in naturally contaminated foods (Niderkorn *et al.*, 2009).

The most toxic and abundant of these is FB1, which causes esophageal and hepatic cancer in humans, and liver and kidney cancer in rodents (van der Westhuizen *et al.*, 2010). Consumption of foods containing high levels of AFs could even lead to death as it was experienced in Kenya (Probst, Njapau, & Cotty, 2007). The effect of aflatoxin and fumonisin on histological change was observed in the liver from birds fed mycotoxin contaminated ducts in the liver and hydropic degeneration in renal tubules in the kidneys (Tessari *et al.*, 2005 ; Zhengquan *et al.* ,2011 ; Magnoli *et al.* ,2011).

Lactic acid bacteria (LAB) as biopreservative organisms have been the focus of numerous studies. Generally, LAB are accepted as safe for use in food by the Food and Agricultural Organization of the United States (FAO) and by the European Food Safety Authority (EFSA) who have granted many species with Generally Regarded as Safe (GRAS) and Qualified Presumption of Safety (QPS) status, respectively (Franz *et al.*, 2010). Specific strains of LAB have the ability to bind aflatoxins and other mycotoxins to their surface (Fuchs *et al.*, 2008; Hernandez-Mendoza *et al.*, 2009; Pizzolitto *et al.*, 2012). Most of the strains were able to remove both toxins, but considerable differences were observed among these strains. The inclusion of appropriate microorganisms in the contaminated diet could prevent the absorption of mycotoxins during their passage in the gastrointestinal tract and eliminated in the faeces (Bueno *et al.*, 2007; Gratz *et al.*, 2004, 2007; Shlej *et al.*, 2015).

A focus in our research group is the development of probiotic or prebiotic strains of *Lactobacillus casei* and *Lactobacillus acidophilus* ability to bind AFB1 or FB1 could decrease the bioavailability of these compounds and limit their toxic effects on human and animals.

Materials and Methods:

This study was carried out at Poultry Farm of the Animal Production Department College of Agriculture, University of Tikrit for the period (2/4/2013) to (14/5/2013) Bacterial strains, *Lactobacillus casei* and *Lactobacillus acidophilus* were obtained from Microbiological Resources Center. Ain Shams University (ASU), Faculty of Agriculture, Cairo MIRCEN.

One hundred and five -one-day-old Ross 308 broiler chicks un-sexed that used in this study were purchased from commercial hatchery were divided into 7 treatments 3 replicate for each with 5 birds in each replicate. Birds were kept in identical wired cages, from day 1 to 42.

The experimental birds received a corn-soybean basal diet which was formulated to meet the standard nutritional requirements (NRC,1994) and was provided *ad libitum* and did not contain antibiotics, coccidiostat or growth promoters.

The parental and the transformed *Lactobacillus* feed supplements were prepared separately by inoculating MRS broth with the respective *Lactobacillus* strains at specific concentrations, under microaerophilic conditions for 24 h at 37 °C without shaking, using inoculum at 0.1% (v/v) from an overnight culture at 37 °. The cells were harvested by centrifugation at 1800g for 10 min at 4°C, and stored at -20° C before they daily use.

The LAB isolates suspensions was prepared by taking 10 ml from isolates suspensions and centerifugate at 2500 rpm for 10 mints. the cells precipitates were collected and adding the phosphates buffers gradually with shaking, until to obtained the turbidity equal the McFarland solution 0.5 concentration. Bacterial strains count was performed by the spectrophotometer instrument at the wave length of 600 nm so that an optical density of 1.5×10^8 CFU/mL from each bacterial strain was reached. The MRS medium was distributed at 5ml in test tubes after moderate to serious pH from 2 to 9 through adding 0.1 N HCl or 0.1 N NaOH, each tubes then inoculates with 0.1% from each LAB isolates, and incubated at 35° C for 24 hours. The aflatoxin was produced from *Aspergillus parasiticus* NRRL 2999 culture (College of Agricultural Tikrit Uni., Food science department Lab) via fermentation of rice by the method of Shotwell *et al.* (1966). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. Fumonisin B1 was produced at the Food microbial Laboratory/ College of Agricultural Tikrit Uni. whole shelled maize (100 g) and 100 ml distilled water were added to 0,946-l jars, autoclaved for 30 min at 121°C (Weibking *et al* ,1993). *Fusarium verticillioides* M-1325(College of Agricultural Baghdad Uni. plant protection science department Lap) The Aflatoxin and Fumoinisin content in powder was measured on an ELISA (ELX800; Bio-Tek Instruments, Winooski, VT) were determined by a monoclonal antibody-based ic-ELISA using Fumonisin FB1 ELISA test kits (Shenzhen Lvshiyuan Biotechnology Co., Ltd. Guangdong, China) sensitivity: 10 ppb and as the product protocol procedure. The powder was incorporated into the basal diet to provide the required 3 mg Aflatoxin/kg and Fumonisin 300 mg/kg feed.

Scarifying birds done at the end of the experiment, pieces of bursa of fabricius and liver were put in 10% buffered formalin, fixed in paraffin, and then sectioned at thickness of 5µ for microscopical examination. Staining sectioned organs was by hematoxylin and eosin.

Results and Discussion :

The histopathology of livers of broiler chicks showed multifocal and varied cytoplasmic vaculation with perilobular location. Some hepatocytes have small pyknotic nuclei, very mild infiltration of polymophonuclear leukocytes is also present.

Microscopic changes induced by feeding AFB1 or FB1 in liver of our experiment reflect the sensitivity of broiler to dietary aflatoxin or fumonisin. Fatty changes focal hemorrhages, Centrolobular fatty cytoplasmic vacuolation and necrosis, severe nodular lymphoid infiltration in poultry species reflect the chronic effect of AFB1. The toxic metabolites of AFB1 bind to nucleic acids and nucleoproteins, essentials to cellular activity, and result in build –up of hepatic lipids with enlargement of the liver. Fat accumulate as a clear vacuoles in the cytoplasm of hepatocytes in a dose – dependent and time faison.

There are hepatocellular degeneration and swelling due to hydropic degeneration and fatty changes. Bile duct proliferation and mononuclear infiltration in the portal triad with mild hydropic degeneration of hepatocytes. There is also hemorrhage and centrilobular to massive hepatocellular necrosis. There is a proliferation of fibroblast with fibrous tissue formation around blood vessels which extend to hepatic tissue.

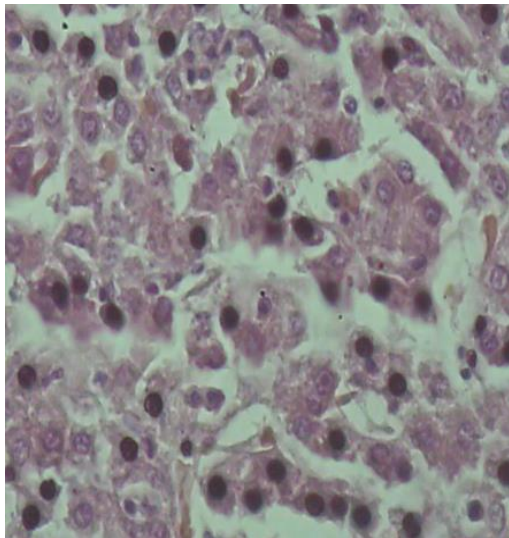


Figure 1. Normal histological structure of a control broiler show, There is no hydropic degeneration, but some hepatocytes are arranged in acinar pattern.

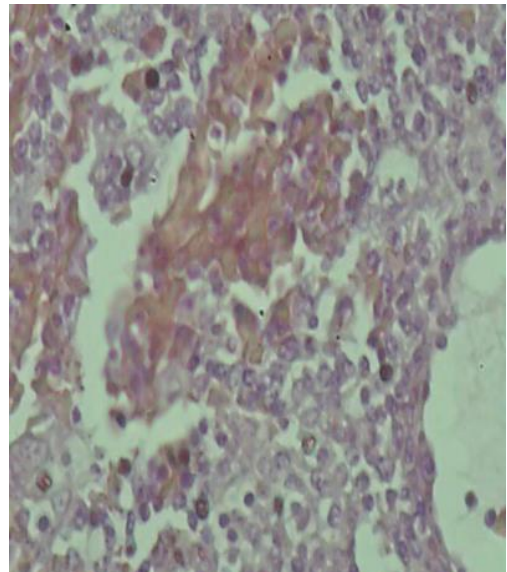


Figure 2. Comparative micrographs of livers AFB1-treated group. Severe hydropic degeneration in centrilobular hepatocytes. Macrophages containing ceroid pigment, severe vacuolar degeneration in hepatocytes, and hydropic degeneration. H E 400.

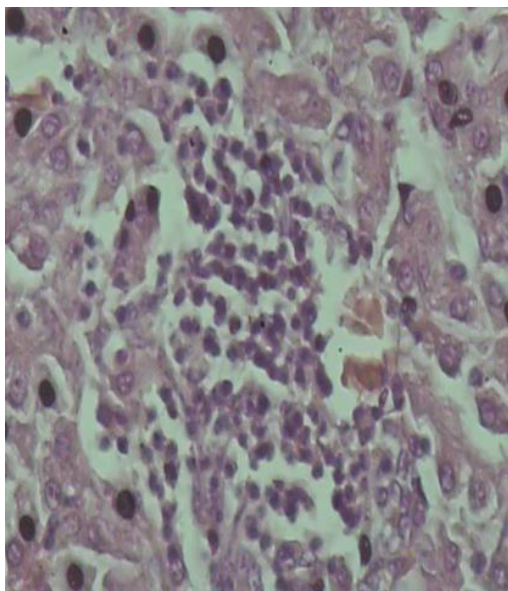


Figure 3. Morphology of liver from treatment 3, cytoplasm is homogeneous and less connective tissue presents in portal areas bile duct epithelium present in some portal areas. H E 400.

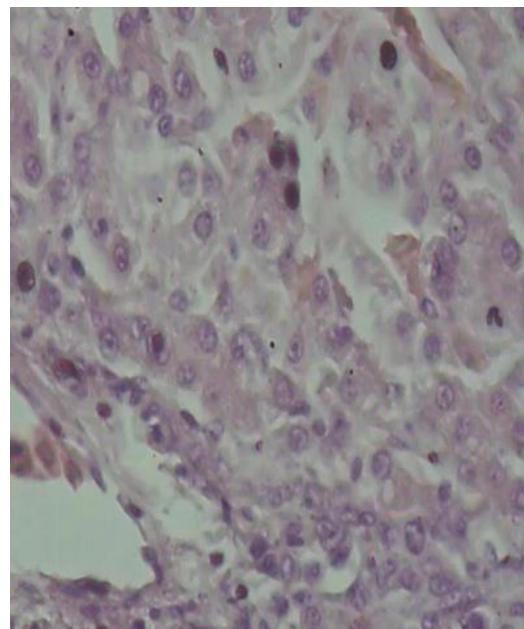


Figure 4. Morphology of liver from treatment 4 Liver of AFB1 treated plus bacteria group. Bile-duct proliferation in portal triad. Normal histological appearance of the liver compare to the control group. H E 400.

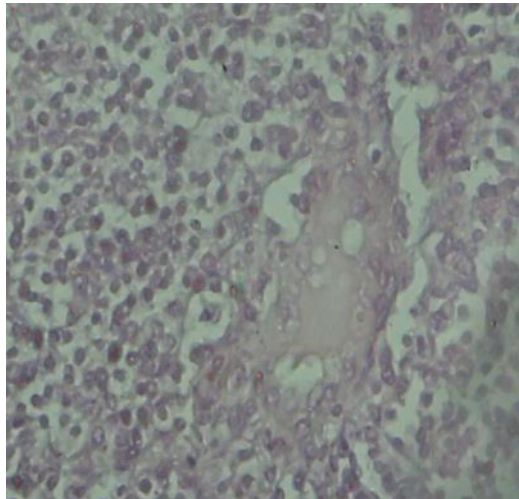


Figure 5. Morphology of liver from treatment 5. Liver from AF-treated group show in parenchymatous degeneration characterized by granular appearance of hepatocyte cytoplasm, vacuolar

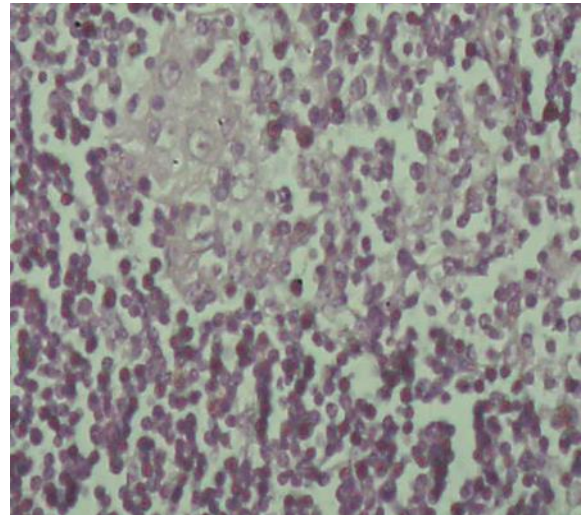


Figure 6. Morphology of liver from treatment 6 the liver cells had little fibrous tissue, and small amounts of lipid droplets can be seen in the cytoplasm of hepatocytes. All the livers had a normal histological structure. Liver cells showing fatty change of hepatocytes congestion of sinusoids Liver from AF-treated group

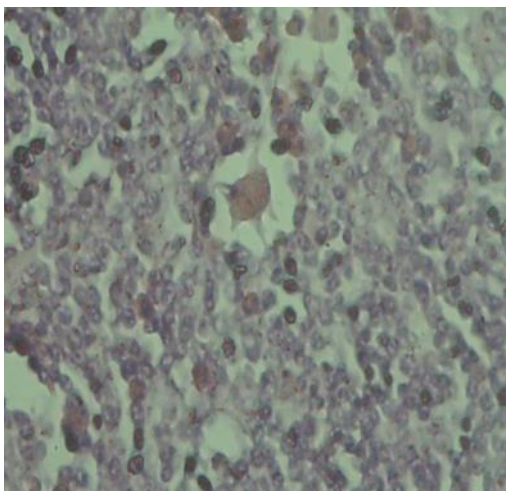


Figure 7. Photomicrographs of liver treatment 7 sections from fed with fumonsin B1 mold-contaminated diet The parenchyma of the liver were formed by the hepatocyte and these sinusoid were connected with the central vein which RBC present inside it. The portal was were containing the branches of the portal vein, hepatic artery and the branch of the bile duct. H E 400.

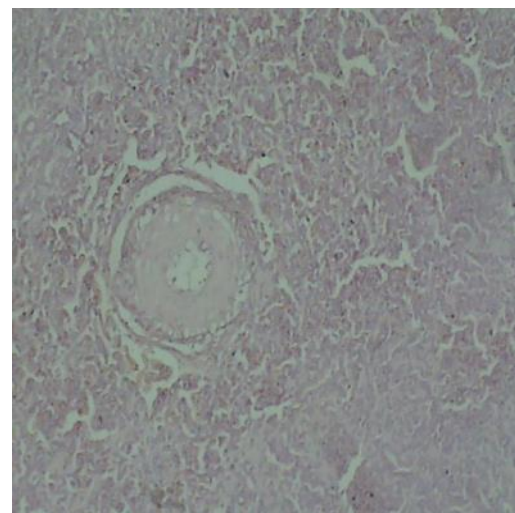


Figure 8. Bursa of Fabricius treatment 8 control group. Note: Severe lymphoid depletion is visible in the centre of follicles in the group. The gland was covered by a thick capsule of collagen bundle and a strand of bundle of these collagen extend to the parenchyma to

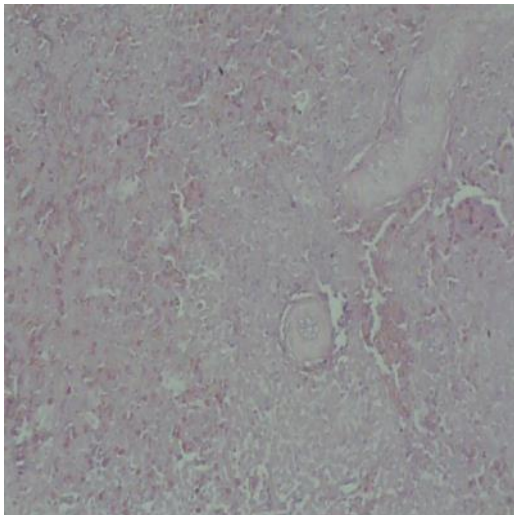


Figure 9. Basal diet plus AFB1 treatment 9. The capsules of the gland was thick and there was loosening of its collagen bundle the lymphatic nodule of the gland was containing a degenerated area of the cell, mainly in the center of the nodule H E 400.



Figure 10. Bursae of Fabricius from treatment 10. The capsule of the gland was thick which formed by a dense collagen bundle and from the capsule a trabecular extend to the interior of the gland carrying the blood vessels. H E

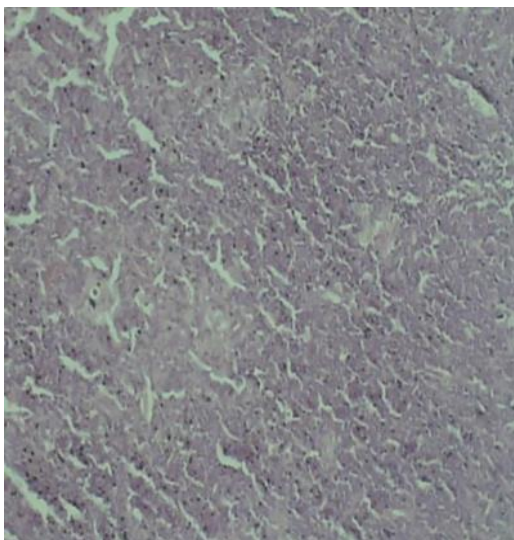


Figure 11. Bursae of Fabricius from treatment 11. Severe lymphoid depletion is visible in the center of follicles in the AF-treated group. The epithelium of the lobule of the gland was lined by simple columnar epithelium. The lymphocytes nodule were occupied by a great number of

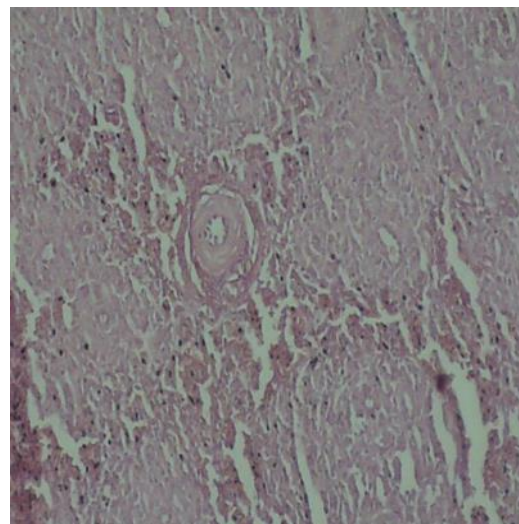


Figure 12. Bursae of Fabricius from treatment 12. The lobule of the fabricia were covered by simple columnar epithelium which mostly appeared degenerated and had a large spherical masses of homogenized structure reflected by a greenish color. The core of each nodule of these lobules were consist of scattered lymphocytes and degenerated

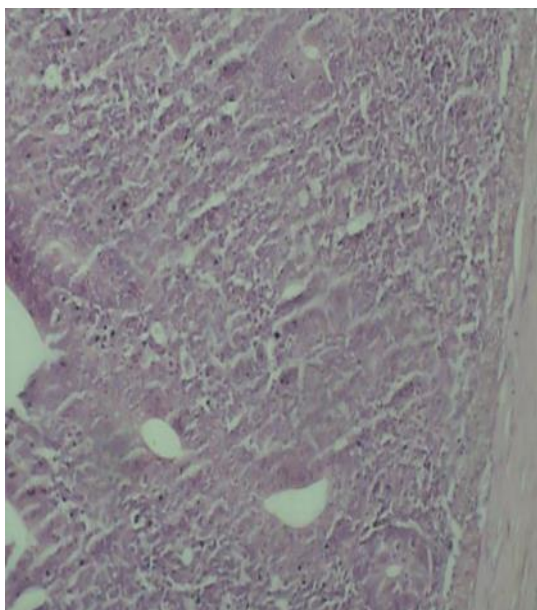


Figure 13. Treatment 13 the lobule of the gland covered by simple columnar epithelium associated with degenerated cells in the form of spherical masses in the epithelium the nodules were also infiltrated by lymphocytes and certain Number of the cells with reticular cells especially in the center of each nodule. H E 400.

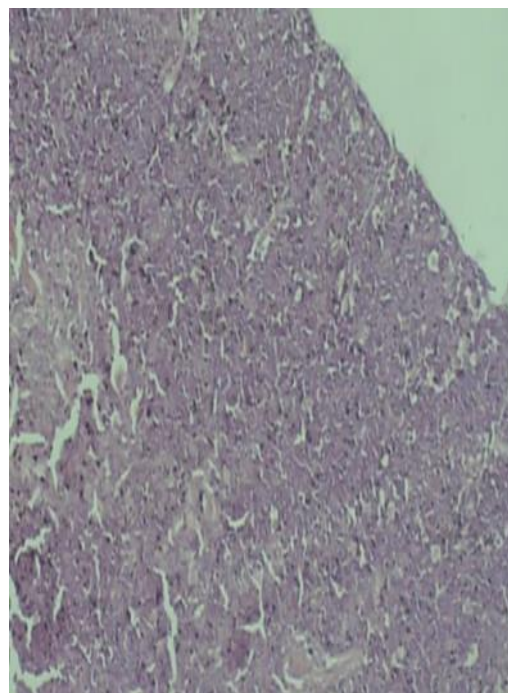


Figure 14. Bursae of Fabricius treatment 14. The gland were containing degenerate epithelium of its lobule, the degenerate was associated with the presence of spherical necrosis masses of epithelial cells the nodule of the gland also had degenerated and reticular cells most of the blood vessels in the capsular area. H E 400.

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