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## **Comparative study between Iraqi and imported probiotics to improve the histopathological changes in the internal organs that experimentally infected broiler with *Salmonella* spp.**

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**Abstract**---The aims of this study was conducted in the poultry farm of the College of Veterinary Medicine, University of Baghdad for the period from 01/05/2021 to 01/10/2021 to compare the effect of adding proportions (full dose and half dose) of the Iraqi Probiotic with the foreign (Imported) Biomin Probiotic to reducing the experimental infection of broiler chicks with *Salmonella* spp. and studying the histopathological changes in liver, kidney and intestine at 35 days. A total of 240 one day old broiler chicks were distributed into 6 groups; G1 group: Control –ve which the chicks fed normal diet without any addition or experimentally infection by salmonella. G2 group: Control +ve which the chicks fed normal diet with orally infected by salmonella. G3 group: Chicks fed normal diet with half dose of Biomin probiotic and orally infected by salmonella. G4 group: Chicks fed normal diet with half dose of Iraqi probiotic and orally infected by salmonella. G5 group: Chicks fed normal diet with full dose of Biomin and orally infected by salmonella. G6 group: Chicks fed normal diet with full dose of Iraqi probiotic and orally infected by salmonella. Results revealed that liver sections of control –ve chicks (G1) revealed no important pathological changes present, hepatocyte showed normal cells, liver cell, with normal sinusoid and ventral vein also, kidney showed normal kidney tissue composed of normal glomeruli tuft and normal proximal tubules in cortex, consist normal epithelial tissue and intestine showed normal villi with normal sub-mucosa layer and normal muscle layer. Sections in liver of control +ve chicks (G2) showed complete damage characterized by hepatocyte necrosis with sever necrosis and sever infiltration of inflammatory cells mostly

neutrophils, lymphocytes with areas of granulation tissue fibroblast, angioblasts, mutable areas idoular necrosis. Sections in kidney showed cystic dilation of proximal tubules, filled with acidophilic proteins material and sever interstitial inflammatory cells mostly lymphocyte. Sections in intestine showed very sever inflammatory cells infiltrated mucosa; sub-mucosa layers mostly lymphocytes and villus in mucosa necrotic. Sections in liver, kidney and intestine of chicks treated with half dose of Biomin and half dose of Iraqi probiotic showed no to slightly enhancing compared with full dose of Biomin (G3 and G4). Sections in liver, kidney and intestine of chicks treated with full dose of Biomin and full dose of Iraqi probiotic showed Hepatocyte of multinucleated nuclei, hepatocyte complete regulation and reorganization to normal hepatic cells, no inflammatory cells present, all tubules of kidney low degenerated with atrophied glomeruli, edema present in the interstitial layers, normal cortex consists normal hyper cellular glomeruli normal proximal epithelial tubules all tissue without any abnormal pathological changes, all villi elongated in intestine with increased goblet cells in size number with heavy basophilic mucin secretion, all layers appear as normal layers (G5 and G6). In conclusion full dose of Iraqi probiotic was a good treatment for salmonella infection in broiler chicks compared with Biomin.

**Keywords**---broiler, probiotic, Iraqi probiotic, histopathological changes, internal organs.

## Introduction

The indiscriminate and intensive use of antibiotics, and the accompanying misuse on both the human and veterinary levels, led to the emergence of many serious problems represented in the emergence of resistance to some strains of pathogenic microorganisms such as *E. coli* and *Salmonella* against some types of these antibiotics, which is expressed by the term drug resistance (WHO, 1997) In addition to posing a threat to public health as a result of its increased accumulation in animal food products such as meat and eggs, and consequently its transmission to the human body, and antibiotics have become a cause for the emergence of allergic diseases (Jin et al., 1997), all of these problems led to a trend towards finding ways an alternative to antibiotics that can be used for therapeutic or prophylactic purposes or as growth stimulants. Probiotics are one of the most important effective alternatives that have taken the place of antibiotic use, and they are defined as cultures of one type or a mixture of several types of beneficial microorganisms that are given to the organism (human or animal) at an early date or at any time. An age stage for the purpose of promoting general health and growth, as well as its preventive or curative uses for most health problems (Pena and Karimi, 2003).

Recent studies have demonstrated the important role of using the nutritional probiotic to improve production by improving the productive performance of poultry. It has been proven that treating broiler chicks with the probiotic since

the age of one day improves live body weight, weight gain and food conversion efficiency (Jin et al., 1997), and improves egg production. In addition to improving the quality characteristics of eggs and lowering the cholesterol concentration in eggs produced from laying hens fed on a feed containing the live fortified, it has led to the improvement of most of the productive traits and the increase in the immune response of broilers fed with proportions of it. It was noted that the local probiotic called Iraq probiotic has a significant role in improving the productive performance of fed broilers as well as lowering the concentration of cholesterol in the blood serum (Al-Tamimi, 2004).

Contaminations with microscopic organisms of *Salmonella* spp. are liable for an assortment of intense and interminable ailments in poultry. Infected poultry rushes are likewise among the most habitually ensnared repositories of *Salmonellae* that can be transmitted through the natural pecking order to people, *Salmonella* is viewed as significant medical issue worldwide in circulation and have been distinguished as a major nourishment and waterborne pathogen (5). *Salmonella* remains genuinely high as nourishment borne pathogens contrasted with others and has not being debilitated for over a period. *Salmonella* acts like the main source of foodborne ailments in scarcely any republics, here and there adding to the most elevated dismalness and death rates among foodborne pathogens (6). Sometimes *Salmonella* can also cause diseases in chickens. It will ultimately depend on the serotype and the condition of the environment that the birds are subjected to in the broiler farms (7). The increase in the number of adult breeding flocks found positive for *Salmonella* in 2018, compared to previous years, is largely attributable to an increase in the number of flocks testing positive for the partially type able serovar *Salmonella* (8).

The aim of the study was to compare the effect of adding proportions (full dose and half dose) of the Iraqi Probiotic with the foreign (Imported) Biomin Probiotic in reducing the experimental infection of broiler chicks with *Salmonella* spp. and studying the histopathological changes in liver, kidney and intestine at 35 days.

## **Materials and Methods**

### **Broiler experimental groups**

As summarized in Table (1), a total of 240 one day old broiler chicks were distributed into 6 groups:

G1 group: Control –ve which the chicks fed normal diet without any addition or experimentally infection by salmonella.

G2 group: Control +ve which the chicks fed normal diet with orally infected by salmonella.

G3 group: Chicks fed normal diet with half dose of Biomin probiotic and orally infected by salmonella.

G4 group: Chicks fed normal diet with half dose of Iraqi probiotic and orally infected by salmonella.

G5 group: Chicks fed normal diet with full dose of Biomin and orally infected by salmonella.

G6 group: Chicks fed normal diet with full dose of Iraqi probiotic and orally infected by salmonella.

## Salmonella used for experimentally infection

### Preparing infectious dose

To prepare the different infectious doses of salmonella according to Pivnick et al. (1981), where I took 5 colonies of pure germ of salmonella refrigerated at a temperature 4°C on the medium of BHIA (Brain Heart Infusion Agar), and placed each colony in a standard bottle sterile (Universal Bottle) containing 5 ml of nutrient broth NB (nutrient broth), and mix the contents well and incubated at a temperature 37°C for 24 hours and then mix the contents of the five bottles in the flask sterile, taking 0.1 ml of them and planted in a standard bottle of sterile containing 9 ml of nutrient broth (NB) degree 37°C for two hours and then was counting its contents germ way Miles and Misra (1938) so that the concentration became germs in each 1 ml of about  $9 \times 10^8$  bacteria, and then reduced the dose by take 1 ml of this cultivar was added to 100 ml of sterilized physiological buffer saline (pH=7.2), bringing the concentration of bacteria in each 1 ml of about  $9 \times 10^6$  bacteria, then 1 ml were orally administrated for each chick at day 1 of age.

### Probiotics

Two type of probiotics were used in this study, first locally prepared probiotic obtained from Prof. Dr. Saad Abdul-Hussein Nagy (Iraqi probiotic), and its microbial contents consisted of  $10^7$  *Lactobacillus acidophilus* cells,  $10^7$  *Lactobacilli* cells, and  $10^7$  cells *Bacillus subtilis* and  $10^7$  *Saccharomyces cerevisiae* yeast cells per kg (Table 2). The second imported probiotic (Biomin IMB52), which obtained from locally commercial market in Baghdad and it consisted of more than  $10^{12}$  cells of *Enterococcus faecium* in addition to Fructo-Oligosaccharide as prebiotic.

Table (2): Microbial contents of Iraqi probiotic

| Microorganism                    | Microbial contents cells |
|----------------------------------|--------------------------|
| <i>Lactobacillus acidophilus</i> | $10^7$                   |
| <i>Bacillus subtilis</i>         | $10^7$                   |
| <i>Bifidobacterium</i>           | $10^7$                   |
| <i>Saccharomyces cerevisiae</i>  | $10^7$                   |
| <i>Lactobacillus acidophilus</i> | $10^7$                   |

### Chicks feeding program

Diets were formulated to meet or exceed requirements by the National Research Council (1994).

Table (3): Ingredients of starter and finisher diets used in this study

| Ingredient (%)             | Starter (1-21) day | Finisher (22-42) day |
|----------------------------|--------------------|----------------------|
| Yellow corn                | 58.0               | 64.0                 |
| Soybean meal (45% protein) | 38.0               | 32.0                 |

|                    |     |     |
|--------------------|-----|-----|
| *Premix            | 3.0 | 3.0 |
| Oil (8900 kcal/kg) | 0.5 | 0.5 |
| Salt (NaCl)        | 0.3 | 0.3 |
| Methionine         | 0.1 | 0.1 |
| Lysine             | 0.1 | 0.1 |
| Total              | 100 | 100 |

### Calculated chemical analysis

|                           |      |      |
|---------------------------|------|------|
| ME (Kcal/kg)              | 2850 | 2900 |
| Crude protein (%)         | 22.4 | 20.2 |
| Calcium (%)               | 0.13 | 0.23 |
| Available Phosphorus (%)  | 0.13 | 0.16 |
| Methionine + Cysteine (%) | 0.80 | 0.75 |
| Lysine (%)                | 1.22 | 1.15 |

### Histopathological examination

Specimens with dimensions 1 cm<sup>3</sup> were taken from liver, intestine and bursa of Fabricius, the tissues were fixed in 10% buffer formaldehyde solution immediately after remove. After 72 hrs. of fixation, the specimens were washed with tap water and then processing was routinely done with a set of upgrading alcoholic concentration from 70% to absolute 100% for 2 hrs. in each concentration to remove water from the tissues, then clearance was done by xylol, then the specimens were infiltrated with semi-liquid paraffin wax at 58 °C on two stages, then blocks of specimens were made with paraffin wax and sectioned by rotary microtome at 5µm for all tissues. All tissues were stained with Hematoxylin and Eosin (H & E) stain and the histopathological changes were observed under light microscope (Luna, 1968).

### Results and Discussion

#### Liver

Treatment of control -ve chicks (not infected group) revealed no important pathological changes present, hepatocyte showed normal cells (a) liver cell, with normal (b) sinusoid and (c) ventral vein. Section in liver of control +ve chicks (infected group) showed complete damage characterized by hepatocyte necrosis with sever necrosis and sever infiltration of (a) inflammatory cells mostly neutrophils, (b) lymphocytes with areas of granulation tissue (c) fibroblast, (d) angioblasts (e) mutable areas idoular necrosis. Section in liver of chicks treated with half dose of Biomin showed (a) polymorphic cells (neutrophils), (b) lymphocytes and macrophages (c) sever hepatic cells damage, Sever infiltration of polymorphic cells mostly macrophages and lymphocytes. Most areas of liver damaged. Section in liver of chicks treated with half dose of Iraqi Probiotics showed (a) diffuse hepatocyte damage, (b) Central area of inflammatory cells infiltration mostly neutrophils and lymphocytes. Section in liver of chicks treated with full dose of Biomin showed (a) Hepatocyte of multinucleated nuclei with dark eosinophilic cytoplasm, (b) granulation tissue consist of fibrous connective tissue.

Section in liver of chicks treated with full dose of Iraqi probiotic showed (a), hepatocyte complete regulation and reorganization to normal hepatic cells, (b) no inflammatory cells present.

### **Kidney**

Section in kidney of control -ve group showed (a) normal kidney tissue composed of normal glomeruli tuft and normal proximal tubules in cortex, (b) consist normal epithelial tissue. Section in kidney of control +ve group showed (a) cystic dilation of proximal tubules, and (b) filled with acidophilic proteins material and (c) sever interstitial inflammatory cells mostly lymphocyte. Section in kidney of chicks treated with half dose of Biomin showed (a) sever acute cellular swelling (hydropic degeneration) of proximal tubules most are complete closed with (b) sever glomeruli enlargement and hyper cellular. Section in kidney of chicks treated with half dose of Iraqi probiotic showed (a) cellular swelling of epithelial cells of proximal tubules with (b) normal glomeruli. Section in kidney of chicks treated with full dose of Biomin showed (a) all tubules degenerated & necrosis with atrophied glomeruli, (b) edema present in the interstitial layers. Section in kidney of animal treated with full dose of Iraqi probiotic showed (a) normal cortex consists normal hyper cellular glomeruli normal proximal epithelial tubules (b) all tissue without any abnormal pathological changes.

### **Intestine**

Section in intestine of control -ve group showed (a) normal villi (mucosa) with (b) normal sub-mucosa layer and (c) normal muscle layer. Section in intestine of control +ve group showed (a) very sever inflammatory cells infiltrated mucosa and sub-mucosa layers mostly lymphocytes, (b) villus in mucosa necrotic. Section in intestine of chicks treated with half dose of Biomin showed (a) fibrin and inflammatory cells present in mucosa layer (b) infiltrated with neutrophils causing thickening of mucosal layer (c) congestion and hemorrhagic (c) in mucosa and sub-mucosa layers and (d) diffuse lymphocytic infiltration in sub-mucosal layer. Section in intestine of chicks treated with half dose of Iraqi probiotic showed (a) sloughing of mucosal layer (b) increase in goblet cells size and number with present of mucin (c) granulation tissue present in sub mucosa layer fibroblast (d) some lymphocyte cell infiltration. Section in Intestine of chicks treated with full dose of Biomin showed (a) Sever lymphocytic infiltration in mucosal layer some are eosinophilic cells (b) with damage in villi. Section in Intestine of chicks treated with full dose of Iraqi probiotic showed all villi elongated (a) with increased goblet cells in size number (b) with heavy basophilic mucin secretion, all layers appear as normal layers.

Probiotics are one of the most important effective alternatives that have taken the place of antibiotic use, and they are defined as cultures of one type or a mixture of several types of beneficial microorganisms that are given to the organism (human or animal) at an early date or at any time. An age stage for the purpose of promoting general health and growth, as well as its preventive or curative uses for most health problems (Pena and Karimi, 2003).

Al-Tamimi (2004) noticed that the Iraqi probiotic containing this bacterium as well as the lactobacilli would significantly reduce the number of colon bacteria in the intestines of broilers. Each type of microorganism in probiotic used as a bioenergetic enhancer has its own mechanism of action, although there are common mechanisms of action, and the mechanisms of action can be revealed as competitive crowding with pathogenic microorganisms on the adhesion sites in the intestine, where these bacilli are implanted in the outer tissue of the intestinal lining at special sites. Production of broad-spectrum antibiotics such as Acidophilin. Production of hydrogen peroxide  $H_2O_2$ , which is known for its high toxicity to pathogenic bacteria. Reducing the pH through the production of lactic acid. Working to increase the numbers of IgG- and antibody-secreting cells in the intestinal mucosa. Increasing the numbers of white blood cells and raising the level of the immune response (Audisio et al., 2000; Cuttng and Casula, 2002; Wysong, 2003).

Biomin®IMBO may be recommended for inclusion into broiler feed based on the potential to improve performance as reported in this and other studies. Moreover, in overall, synbiotic Biomin®IMBO may be considered as a valuable alternative to antibiotics, especially during the starter period because of most studies including this synbiotic reported improved bird performance. Also, according to this study feeding broiler with 0.075% synbiotic Biomin®IMBO seems to be more cost effective level (Mohnl, 2006; Aziz Mousavi et al., 2015). In our study Biomin have a good potential effect against salmonella infection in broiler but not as Iraqi probiotic tis may due to high levels and many genera of benefit microorganisms in Iraqi probiotic, it treatment of full dose enhanced health and reduced histopathological changes in liver, kidney and intestine sections. Hes results are agreed with the finding of Mcfarland et al. (1994), Wysong (2003), Al-Tamimi (2004).

## Conclusion

Full dose of Iraqi probiotic was a good treatment for salmonella spp. infection in broiler chicks compared with Biomin and half dose.

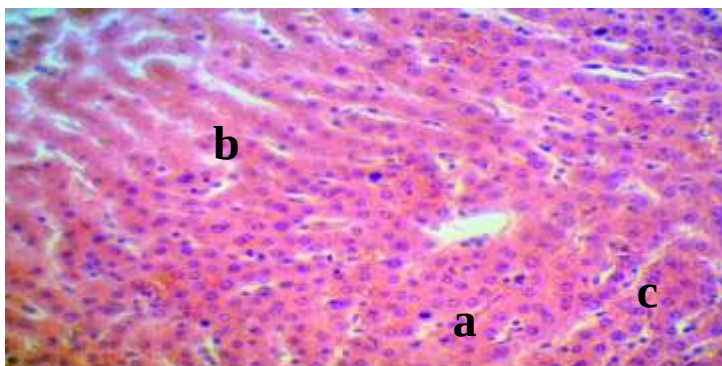


Figure (1) H&E Stain X400, Section in liver of control -ve group showed: a: normal hepatocyte, b: normal sinusoid, c: normal central vein, no important pathological changes present



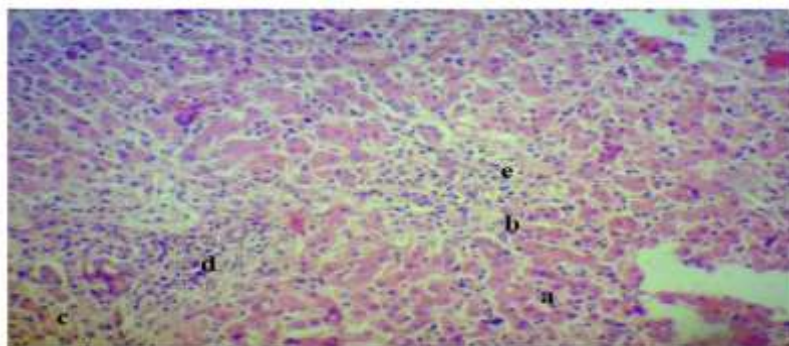


Figure (2) H&E Stain X200, Section in liver of control +ve group (infected) showed complete damage characterized by hepatocyte necrosis with sever necrosis and sever infiltration of (a) inflammatory cells mostly neutrophils& (b)lymphocytes with areas of granulation tissue (c)fibroblast & (d)angioblasts (e) mutable areas idoular necrosis

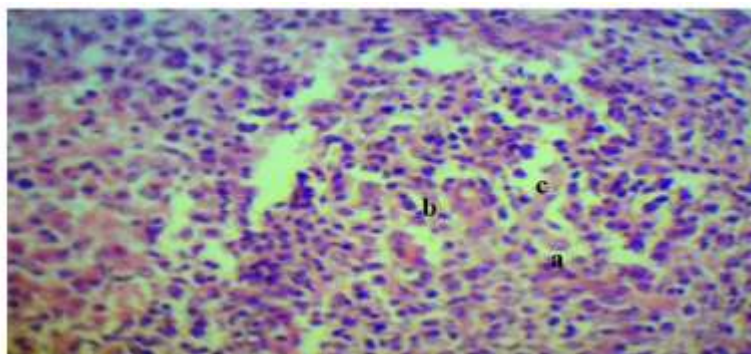


Figure (3) H&E Stain X200, Section in liver of animal treated with half dose of Biomin showed: (a)polymorphic cells (neutrophils) (b)lymphocytes & macrophages (c)sever hepatic cells damage, Sever infiltration of polymorphic cells mostly macrophages & lymphocytes, Most areas of liver damaged

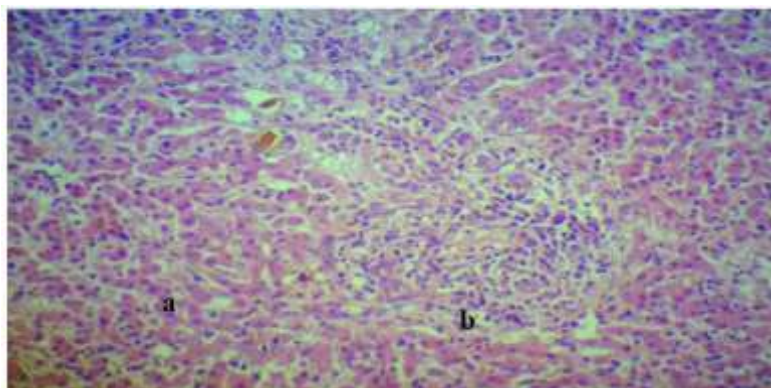


Figure (4) H&E Stain X200, Section in liver of animal treated with half dose of Iraqi Probiotics showed: Diffuse hepatocyte damage (a) Central area of inflammatory cells infiltration mostly neutrophils &lymphocytes (b)



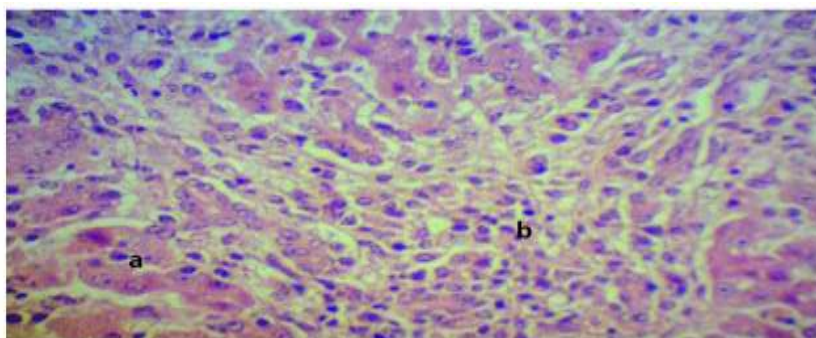


Figure (5) H&E Stain X200, Section in liver of animal treated with full dose of Biomin showed: Hepatocyte showed multinucleated nuclei with dark eosinophilic cytoplasm (a) Granulation tissue consist of fibrous connective tissue

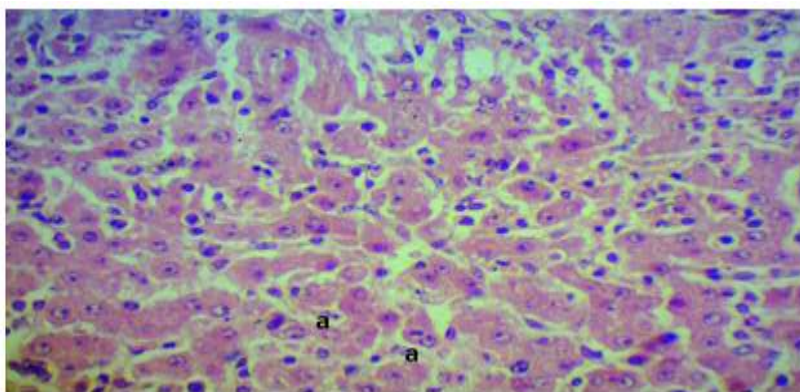


Figure (6) H&E Stain X200, Section in liver of animal treated with full dose of Iraqi probiotic showed: Hepatocyte complete regulation and reorganization to normal hepatic cells (a) No inflammatory cells present (b)

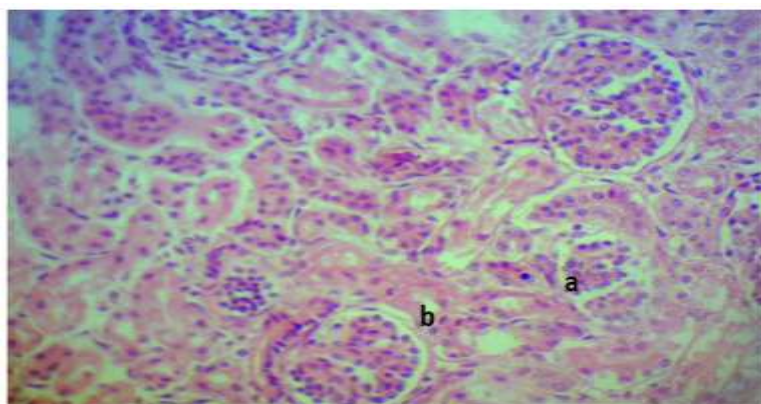


Figure (7) H&E Stain X400, Section in kidney of control negative group showed: Normal kidney tissue composed of normal glomeruli tuft & normal proximal tubules in cortex (a), Consist normal epithelial tissue (b)

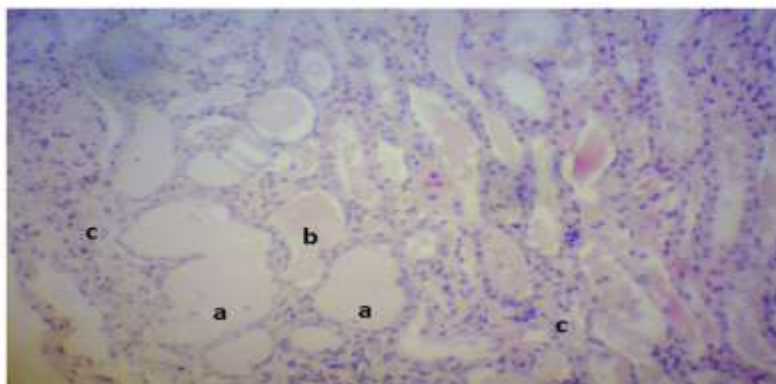


Figure (8) H&E Stain X400, Section in kidney of control positive group showed: Cystic dilation of proximal tubules (a) and filled with acidophilic proteins material(b) sever interstitial inflammatory cells(c)mostly lymphocyte

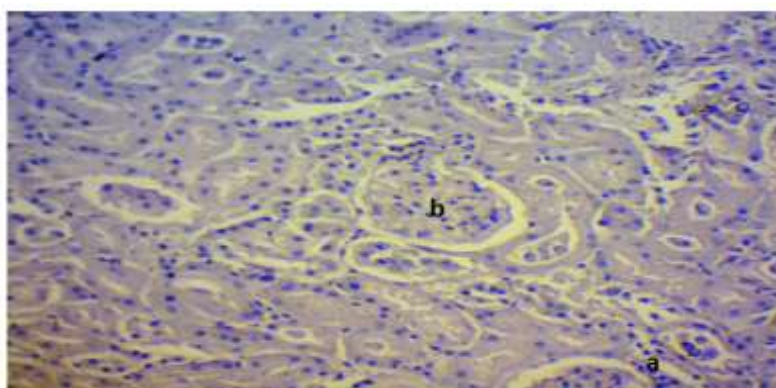


Figure (9) H&E Stain X200, Section in kidney of animal treated with half dose of Biomin showed: Sever acute cellular swelling (hydropic degeneration) of proximal tubules(a) most are complete closed with sever glomeruli enlargement and hyper cellular (b)

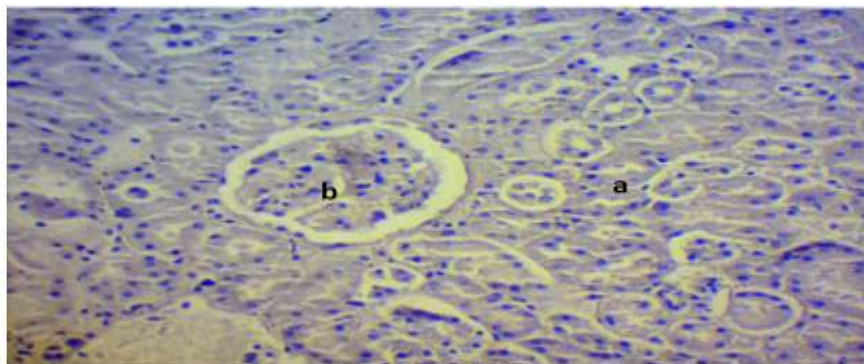


Figure (10) H&E Stain X200, Section in kidney of animal treated with half dose of Iraqi probiotic showed: Cellular swelling of epithelial cells of proximal tubules (a) with normal glomeruli (b)



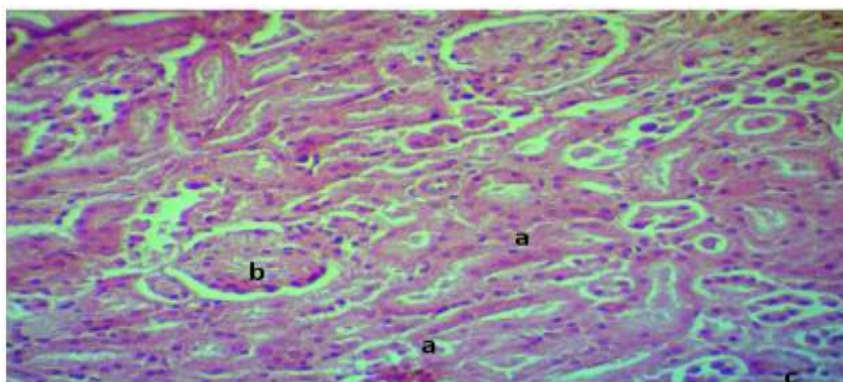


Figure (11) H&E Stain X200, Section in kidney of animal treated with full dose of Biomin showed: All tubules degenerated & necrosis(a)with atrophied glomeruli, edema present in the interstitial layers (b)

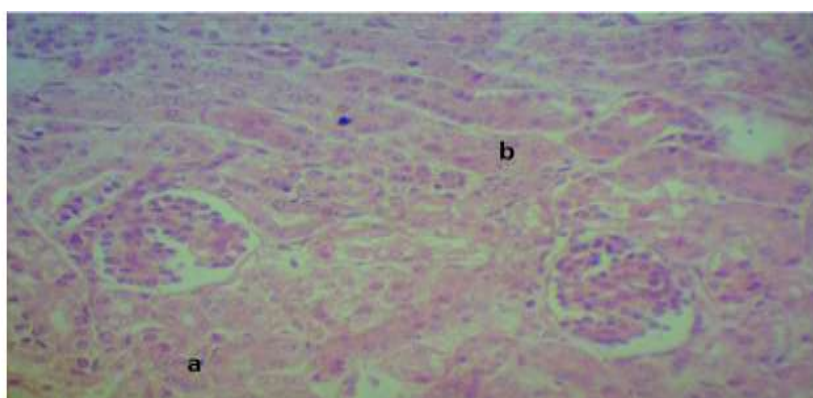


Figure (12) H&E Stain X200, Section in kidney of animal treated with full dose of Iraqi probiotic showed: Normal cortex consists normal hyper cellular glomeruli(a) normal proximal epithelial tubules (b)all tissue without any abnormal pathological changes

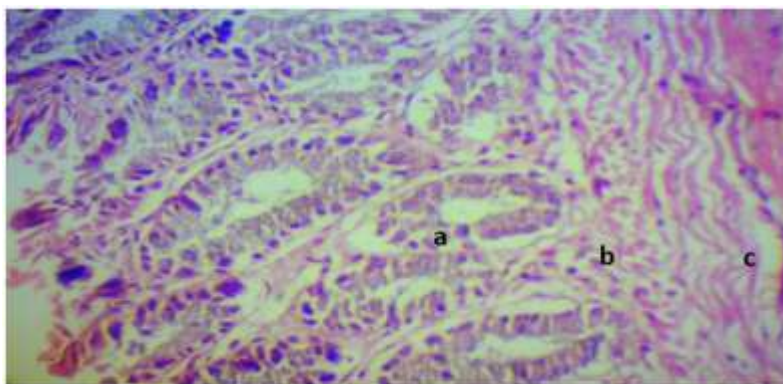


Figure (13) H&E Stain X400, Section in intestine of control negative group showed: Normal villi(mucosa) (a)with normal submucosa layer(b)normal muscle layer (c)

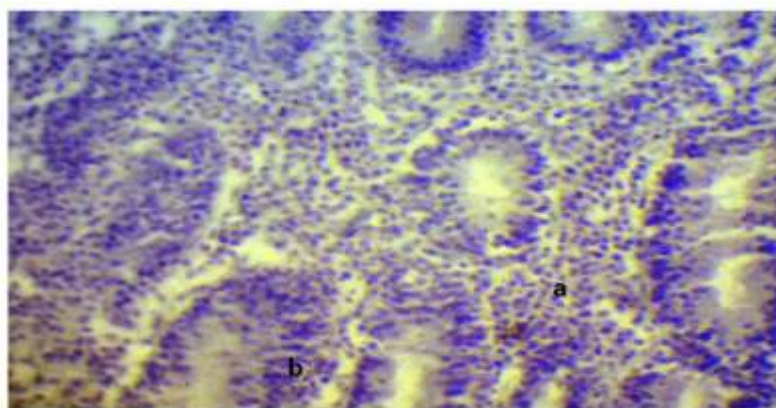


Figure (14) H&E Stain X400, Section in intestine of control positive group showed: Very sever inflammatory cells infiltrated mucosa & submucosa layers mostly lymphocytes (a)a villus in mucosa necrotic(b)

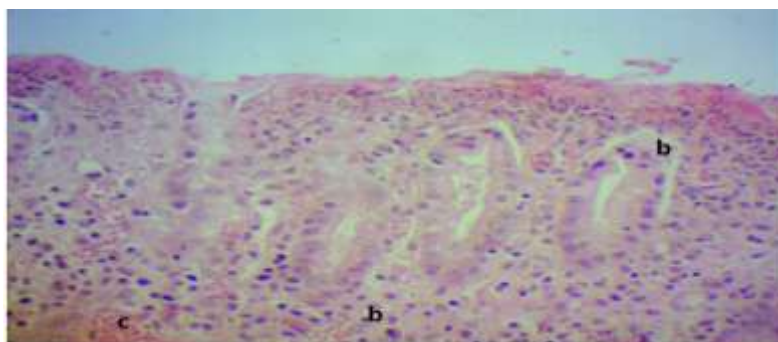


Figure (15) H&E Stain X200, Section in intestine of animal treated with half dose of Biomin showed: Fibrin & inflammatory cells present in mucosa layer (a)infiltrated with neutrophils causing thickening of mucosal layer(b)congestion & hemorrhagic (c)in mucosa &submucosa layers. Diffuse lymphocytic infiltration in submucosal layer (d)

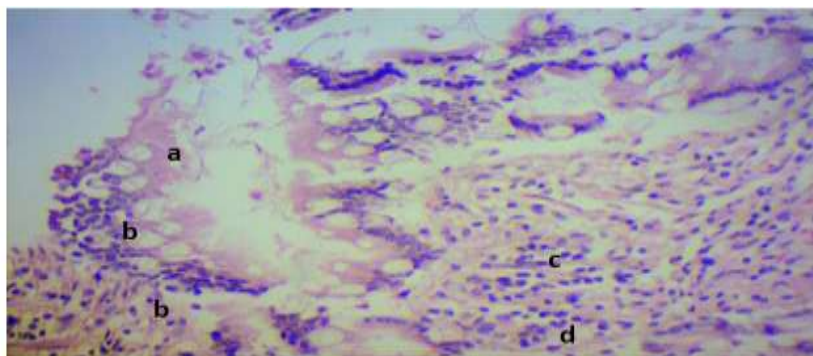


Figure (16) H&E Stain X200, Section in intestine of animal treated with half dose of Iraqi probiotic showed: Sloughing of mucosal layer(a)increase in goblet cells size & number with present of mucin (b)granulation tissue present in sub mucosa layer fibroblast(c)some lymphocyte cell infiltration(d)

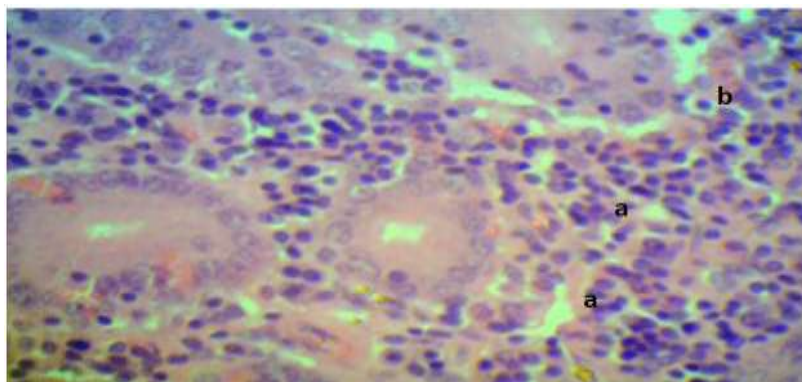


Figure (17) H&E Stain X200, Section in Intestine of animal treated with full dose of Biomin showed: Sever lymphocytic infiltration in mucosal layer (a)some are eosinophilic cells(b)with damage in villi

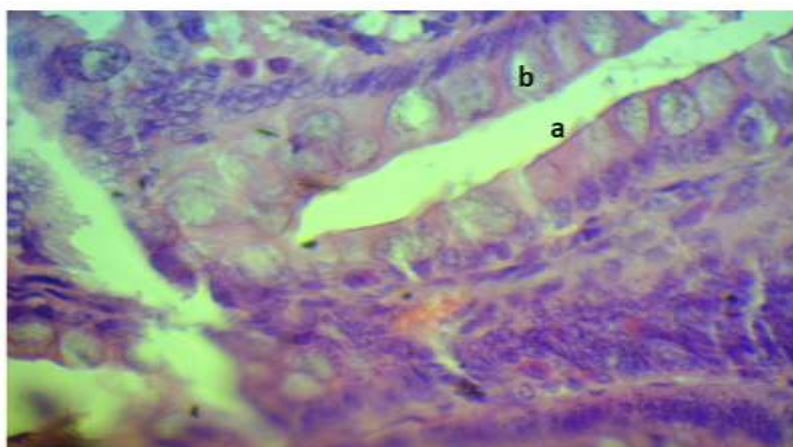


Figure (18) H&E Stain X200, Section in Intestine of animal treated with full dose of Iraqi probiotic showed: All villi elongated(a) with increased goblet cells in size number(b)with heavy basophilic mucin secretion, all layers appear as normal layers

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