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IN VIVO EVALUATION OF LOCAL LACTOBACILLUS ACIDOPHILUS AS A PROBIOTICS IN FEED EXPERIMENT ON SPRAGUE-DAWLEY RATS

Ramy Osama[1]; Raafat El-Sanhouty[1]; Ali Salama[2] and Mahmoud A. Al-Saman[1]

[1]Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city, **Egypt.**
[2]Agric. Microbiology, Faculty of Agriculture, Zagazig University, **Egypt.**

[*]**Corresponding author:** Alisalama1980@gmail.com **Received:** 4 Jan. 2020 **; Accepted:** 2 Feb. 2020

ABSTRACT: This study was carried out mainly in Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city, Egypt, as well as in the laboratories of Agric. Microbiology Dept. at the Faculty of Agriculture, and Animal House at the Faculty of Veterinary Medicine, Zagazig University, Egypt. Adult male healthy Sprague-Dawley rats were used to study the effect of local lactic acid bacteria as a probiotics in animal feed. The important obtained results are the evolution of live body weight over 5 weeks in probiotics-administered groups witnessed relatively higher increases than in control. The obtained results revealed that the oral administration of probiotics gives the best results compared with supplemented of probiotics with diet. Probiotics treatments were associated with significant increases in the level of hemoglobin in rats. These findings may indicate the absence of destruction of mature circulating cells or loss of cells from the circulation by haemorrhage, or reduced RBCs production, Moreover, without different changes were also observed in white blood corpuscles (WBC) in response to the administration of probiotics. The total concentration of serum cholesterol was significantly reduced from 136.2±3.8 mg/dl in the control to a mean value of 88.5±1.8 mg/dl in group treated with *L. acidophilus* oral administration. In contrast, The recorded values of renal parameter (urea and creatinine) indicate significant reduction, for the all treats of probiotic with male rats, The levels of liver enzymes, i.e., aspartate aminotransferase, (AST), alanine aminotransferase (ALT) in rates receiving different probiotic treatments were significantly reduced by the probiotic application, the obtained reduction in results apparently refer to the absence of hepatotoxicity.

Key Words: *Lactobacillus acidophilus*, Probiotics, Sprague-Dawley rats

INTRODUCTION

Lactic acid bacteria (LAB) are Gram- positive, fermentative bacteria and functional classification of nonpathogenic, nontoxigenic that are associated with the production of lactic acid from carbohydrates making them useful for food fermentation. Genus of *Lactobacillus*, *Lactococcus*, and *Streptococcus* are included in this group. *Lactobacillus* has been used in fermented foods for several centuries without adverse effects and is classified as Generally Recognized as Safe (GRAS) because of their long history of safe use, particularly in dairy foods **(Lee *et al.*, 2009).** *Lactobacillus* is important gastrointestinal tract (GIT) residents and it's used as probiotic strains to improve health **(Stefania and Marco, 2017)**.

Probiotic microorganisms have been reported to enhance GIT all over transit, produce vitamins and contribute vitamin availability to the host. Probiotics have wider applications in food, feed, dairy and fermentation industry, as non-pharmacological approaches for health management **(Anandharaj and Sivasankari, 2014).** According to FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) working group experts, probiotics are "live strains of microorganisms that were selected precisely which when supplied to the host in adequate amounts show a health benefit'' **(Markowiak and Slizewska, 2017).**

The GIT of neonatal animals and birds reared naturally are colonized with micro-organisms,

generally originating from the adult (mother). These micro-organisms provide protection from enteric pathogens (**Coconnier *et al.*, 1992)**. Condensation of animal production has decreased the opportunity for natural colonization of the GIT, making animals more susceptible to intestinal pathogens challenge. Probiotics could mimic natural colonization in neonates, or colonize adult animals, preventing pathogenic organisms from colonizing the intestinal mucosa (**Bernet *et al.*, 1994; Hudault *et al.*, 1997**). Certain strains of *Lactobacillus* possess hydrophobic surface layer proteins which help the bacteria to non-specifically adhere to the animal cell surface (**Tuomola and Salminen, 1998; Bibiloni *et al.*, 2001; Johnson-Henry *et al.*, 2007**). Such adhesion of probiotic bacteria to the intestinal epithelium covers the receptor binding sites, preventing pathogenic micro-organisms like *E. coli* O157:H7, *Salmonella*, ……*etc*., from attaching to the epithelium (**Hudault et al., 1997; Johnson-Henry et al., 2007**).

Probiotics can improve broiler chicken growth rates (**Mookiah *et al.*, 2014 and Lei *et al.*, 2015**) and inhibit enteric diseases, including; salmonellosis (**Biloni *et al.*, 2013**), necrotic enteritis and coccidiosis (**Jayaraman *et al.*, 2013**). As feed is the largest cost in poultry production, small improvements in feed use efficiency have a significant economic impact. The improvement in performance and productivity of poultry due to the use of probiotics in feed has been attributed to increased feed intake and improved feed efficiency (**Shim *et al.*, 2012**) but this is not always the outcome. Probiotics can increase feed intake without significant improvement in feed conversion ratio (FCR) **(Afsharmanesh and Sadaghi, 2014)**, and improve FCR without significant difference in feed intake (**Mountzouris *et al.*, 2010; Shim *et al.*, 2012; Zhang *et al.*, 2012; Zhang and Kim, 2014**) and increase feed intake along with significant improvement in FCR (**Landy and Kavyani, 2013**).

Apart from the use of probiotics in formulated animal feed, beneficial bacteria used as silage inoculants may also have a probiotic effect in the rumen (**Weinberg *et al.*, 2004**). However, this response depends on the survival of the silage inoculant in the silage as the pH drops. in dairy animals Probiotics can improve the yield of milk. Dietary supplementation with a combination of *L. acidophilus* NP51 and P. freudenreichii NP24 resulted in a 7.6% increase in average daily milk yield in Holstein cows (**Boyd, West and Bernard, 2011**). **Weiss and McKelvey (2008)** found that dairy cattle fed the probiotic *Propionibacterium* strain P169 had the same milk production as control animals, but with decreased feed consumption, resulting in 4.4% increase in energy efficiency. The aim of this study was to evaluate novel probiotic potential and safety *in vivo* in order to be used as animal health-promoting, functional feeds.

MATERIALS AND METHODS

This study was carried out mainly in Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city, Egypt, and in the laboratories of Agric. Microbiology Dept. at the Faculty of Agriculture, and Animal House at the Faculty of Virtanary Medicine, Zagazig University, Egypt. This work aimed to isolate, identificate and select efficient indigenous strains of Lactic acid bacteria, from different sources as well as, design the feed experiment on adult healthy male Sprague-Dawley rats to study the effect of these lactic acid bacteria as a probiotic in animal feed.

Micro-organism and Culture Preparation

The indigenous two strains of *Lactobacillus acidophilus* used in the present study were previously isolated from yoghurt and suspended mixed pickles samples were collected from different supermarkets in Cairo and Zagazig cities, Egypt and identified based on morphological, phesyological and biochemical characteristics according to **Logan and De Vos (2009).** These strains were genetically identified by sequencing fragments of 16S ribosomal RNA gene and comparing sequences from the GenBank, and confirmed by using matrix-assisted laser desorption/Ionization time of flight mass spectrometry (MALDI –TOF – MS) in peptide and protein analyses according to **Nacef *et al.*, (2016).** The bacterium was maintained on MRS slopes, subcultured monthly and kept at 4°C.

After examining the lactic acid bacteria isolates, 2 strains (*L. acidophilus* RS11 and *L. acidophilus* RS12) were chosen for *in vivo* evaluation of their capability as a probiotic in feed experiment. These strains were separately inoculated into MRS broth and placed in an anaerobic workstation at 35°C for 24 hours. The strains were harvested by centrifugation at 2000 × g for 20 minutes, washed twice with normal saline (0.9% NaCl), and resuspended at 2 × 109 CFU/mL in sterile normal saline. Subsequently, 2 mL of the solution was administered intragastrically to the rats daily.

Animal feeding and experimental design

Forty-two male Sprague-Dawley rats (conventional clean animal grade), aged 4 weeks and weighing 94.1 ± 2.31 grams, were acquired from Laboratory Animal Farm at Faculty of Veterinary Medicine, Zagazig University (Zagazig, Egypt). All animals were kept separately in stainless steel cages under a controlled room temperatures (21 ± 2°C) and humidity 50% - 60%, and sustained on a persistent 12-hour/12-hour light/dark cycle. All rats had ad libitum access to standard rodent chow and filtered water throughout the study. Animals were acclimated for one week prior to use in any.

Experimental design

The 42 of rats were randomly assigned to seven experimental groups; each of 6 rats as follows:

Group 1: rats received normal diet without treatment (Control)

Group 2: rats received normal diet and treated orally administered with L. acidophilus RS11(RS11O)

Group 3: rats received normal diet supplemented with L. acidophilus RS11into feed (RS11F)

Group 4: rats received normal diet and treated orally administered with L. acidophilus RS12 (RS12O)

Group 5: rats received normal diet supplemented with L. acidophilus RS12into feed (RS12F)

Group 6: rats received normal diet and treated orally administered with RS11and RS12 (RS11+RS12O)

Group 7: rats received normal diet supplemented with RS11 and RS12 into feed (RS11+RS12F)

Rats received oral administration of bacteria (100 ml/kg body weight) once daily for four weeks via gastric tube.

Sampling

At the end of the dosing, five animals per group were randomly chosen, weighed and slaughtered to determine all the certain traits. Two separate blood samples were collected from the retro-orbital plexus from each rat: the first blood sample was collected into an EDTA tube for hematological assessments. The second part of the blood sample was collected into a tube (without EDTA) and left at room temperature for 20 min to coagulate; after centrifugation at 3000 rpm for 10 min, the resulted serum was isolated and placed at − 20 °C until used (within 2 weeks) for the estimation of serum biochemical and immune responses indicators. The live body weight (LBW) was measured in replicates at biweekly intervals through collected data by period. After the feeding period, the rats were fasted for 12 hours and euthanized.

Hematological assessments

Using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy), total red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, basophils, eosinophil, neutrophil, lymphocytes, and monocytes were determined according to the methods **Grindem (2011).**

Serum metabolites

To determine serum metabolites, blood samples were left to clot and centrifuged at 3500 rpm for 15 min, after which the serum was sparated and stored at −20 ◦C until analysis. Serum metabolites such; total protein (TP), albumin (Alb), globulins (Glu), A/G ratio, creatinine, uric acid, total cholesterol, total glycerides, HDL: high density lipoprotein; LDL: low density lipoprotein, very low density lipoprotein (VLDL), as well liver enzymes: alanine aminotransferase (ALT) aspartate aminotransferase (AST) were estimated using biodiagnostic kits and a spectrophotometer (Shimadzu, Kyoto, Japan) according **to Abdelnour *et al.*, (2018).**

Immunity induces assay of serum

The serum samples were analyzed to determine the Superoxide dismutase (SOD) activity and reduced glutathione (GSH) determinations were made by the use of commercial kits bought from a spectrophotometer (Shimadzu, Kyoto, Japan), Biodiagnostic Company (29 El-Tahrir St. Dokki, Giza, Egypt), following the methods of **Nishikimi *et al.*, (1972) and Beutler *et al.*, (1963)**, respectively.

Histological studies

Fixation and tissue processing: The formalin preserved liver, kidney and intestine specimens were processed in an automated tissue processor. The processing consisted of an initial 2 steps fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70% ,90 , %and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin, (**Suvarna *et al.*, 2013**) Stained sections were photographed with Microscope digital camera and examined for circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and any other pathological changes in the examined tissues.

RESULTS AND DISCUSSION

Forty two of male Sprague-Dawley rats aged 4 weeks and weighing 94.1 ± 2.31 grams divided into seven groups, six groups received two strains of *L. acidophilus* (RS11 and RS12) as probiotics (single or mixed) oral administration A supplemented to diet beside control and kept under experimental conditions and continuous observation for the following five weeks.

1- Effect of *L. acidophilus* on body weight of rats

The live body weight increased with time in all the experimental animal groups (Table 1). No reductions in body weights were recorded in any group receiving probiotics or not. However, the evolution of live body weight over 5 weeks in probiotics-administered groups witnessed relatively higher increases than in control. The increase in live body weight in the control male rats after 35 days recorded 187.1±2.8 g/rat. Conversely, live body weight in the treated male rats with two strains of *L. acidophilus* after 35 days recorded 212.8±3.5, 207.8±4.7 and 206.6±4.6 g/rat obtained by treatments RS11 (O), RS12 (O) and RS11+RS12(O), respectively. We deduce from this result the oral administration of probiotic, gives the best results compared with supplemented of probiotic with diet. Same results were obtained from many cases, the improvement in growth rate in the probiotic treated animals was associated with increased feed intake (**Landy and Kavyani, 2013 and Lei *et al.*, 2015**) and improved feed use efficiency **(Zhang and Kim, 2014)** compared with untreated animals. Therefore, increased digestibility of feed resulting in improved feed use efficiency could be one of mode of actions for improved growth rate (**Zhao *et al.*, 2013**).

Also, Chang *et al.*, (2001) who observed an increase in daily weight gain and an improvement in feed conversion in pigs using a *Lactobacillus* supplement. The increase in the body weight in rats fed with diet containing the cultured dairy product may be due to the increase in the efficiency in nutrient utilization associated with the availability of more digestible protein and feeding the rats with probiotics resulted in rapid decline of pathogenic coliforms, and significant increase in body weight gain (**Christopher *et al.*, 2006**).

Table 1. Body weight parameters in male Sprague-Dawley rats receiving two *L. acidophilus* strains during 5 weeks (Means±SE)

Items	Control	RS11(O)	RS11(F)	RS12(O)	RS12(F)	RS11+ RS12 (O)	RS11+ RS12 (F)
Week 1	94.0±4.49	95.5±5.33	95.3±3.67	93.0±3.02	93.0±4.07	92.3±3.81	95.17±3.86
Week 2	118.6±3.6	125.3±3.84	119.17±2.2	120.5±1.5	118.5±3.5	118.3±4.6	121.6±4.5
Week 3	140.5±4.9	153.5±3.7	146.6±2.5	144.6±3.8	142.1±2.9	145.8±4.4	144.2±5.9
Week 4	166.8±3.4	179.1±4.2	174.8±2.6	172.8±3.5	168.1±3.7	172.8±4.5	169±4.8
Week 5	187.1±2.8	212.8±3.5	204.1±2.9	207.8 ±4.7	195 ±2.9	206.6 ±4.6	199.1±4.3

(O): Oral administration of Bacteria (F): Bacteria A supplemented to diet
RS11: L. acidophilus RS11 RS12: L. acidophilus RS12

2- Effect of *L. acidophilus* on hematological assessments

The hematological parameters for male Sprague-Dawley rats receiving the tested probiotics are shown in Table (2). Insignificant slight changes in control can be seen in the levels of red blood corpuscles (RBC) in male rats receiving different probiotics doses of *L. acidophilus* (oral administration and supplemented to diet). This may indicate that the tested probiotics not adversely affect the synthesis of RBCs and does not consequently showed insignificant changes when received the tested treatment. In addition, all platelets values are within normal range. Conversely, probiotics treatments were associated with significant increases in the level of hemoglobin in rats. These findings may indicate the absence of destruction of mature circulating cells or loss of cells from the circulation by haemorrhage, or reduced RBCs production (**Nunia *et al.*, 2007**).

In the same results (Table 2), the level of m*ean corpuscular volume* (*MCV*) which is the average volume of red cells had not any significant changes in male rats receiving probiotics compared to the control.

Table 2. Hematological parameters in male Sprague-Dawley rats receiving two *L. acidophilus* strains during 5 weeks

Items	Control	RS11(O)	RS11(F)	RS12(O)	RS12(F)	RS11+ RS12 (O)	RS11+ RS12 (F)
Erythrocytes							
RBCs ($\times10^6$ /mL)	4.1±0.12a	4.13±0.19a	4.2±0.01a	4.1±0.14a	4.4±0.19a	4.03±0.11a	4.1±0.10a
Platelet($\times10^3$/µL)	1004±32a	1007±33a	993±33a	954±22a	999±35a	1057±42a	1045±36a
Hemoglobin (g/dl)	12.2±0.38a	12.8±0.10a	10.7±0.28b	12.2±0.35a	12.0 ±0.36a	12.8±0.46a	12.5±0.57a
Hemoglobin%	87.1±2.4b	83.4±4c	93.9±2a	84.1±1.6c	89.7±2.5b	89.3±3.1b	87.8±4.1b
PCV (%)	36.8±0.4a	36.7±0.31a	37.1±0.25a	36.6±0.24a	36.8±0.1a	37.1±0.13a	37.2±0.16a
MCV(FL)	92.3±2.3a	90.3±1.2a	88.8±1.6a	89.9±0.6a	89.8±1.1a	92.5±2.3a	91.4±1.8a
MCH (pg)	29.6±0.7a	30.9±0.8a	30.7±1.2a	30.5±0.3a	31.8±0.4a	31.7±0.3a	30.6±0.9a
MCHC (g/dL)	31.1±0.8a	34.4±1.6a	34.9±1.7a	33.8±0.8a	34.4±0.9a	34.3±1.1a	33.7±1.4a
Leucocytes							
WBCs ($\times10^3$/ mL)	13.04±0.6a	9.6±0.24c	12.6±0.23a	12.8±0.22a	12.3±0.36a	10.6±0.28b	12.6±0.42a
Basophils	0.25±0.01a	0.27±0.01a	0.24±0.01a	0.23±0.09a	0.25±0.01a	0.27±0.01a	0.25±0.02a
Eosinphils	0.66±0.02a	0.65±0.02a	0.67±0.02a	0.64±0.02a	0.68±0.03a	0.71±0.02a	0.64±0.03a
Lymphocytes	87.7±0.4a	87.2±0.6a	87.9±0.5a	83.1±1.2a	88.1±0.6a	86.1±0.7a	87.6±1.1a
Monocytes	1.43±0.11b	2.41±0.12a	2.26±0.09a	2.17±0.05	2.27±0.09b	2.1±0.04a	1.44±0.15b
Neutrophils %	10.6bc±0.4	9.3c±0.6	9.5c±0.4	12.9a±0.4	9.6c±0.3	11.6ab±0.6	11.2b±0.5

(O): Oral administration of Bacteria (F): Bacteria A supplemented to diet
RS11: *L. acidophilus RS11* RS12: *L. acidophilus RS12*
RBCs: red blood cells; WBCs: white blood cells; HCT: hematocrit
MCV: mean corpuscular volume MCH: mean corpuscular hemoglobin
MCHC: mean corpuscular hemoglobin concentration
Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different (P<0.05).

However, all recorded levels are within the normal range, confirming the previous conclusions that the tested probiotics are not associated with any hazard affecting the status of red blood corpuscles. The changes in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were insignificant and within normal range for probiotics-receiving rats, confirming the previous results and conclusion that the treatment does not adversely affect hemoglobin synthesis.

Moreover, without different changes were also observed in white blood corpuscles (WBC) in response to the administration of probiotics. The absence of any significant elevation in the level of WBC may indicate that probiotics treatments are not associated with any potential inflammatory phenomena. The toxic substances are normally associated with significant increases in the levels of WBC (**Celik and Suzek, 2008; Roy *et al.*, 2010**). As a result, the absence of similar increases in the level of WBC may means the absence of toxic potential. This may also indicate that *L. acidophilus* which used as probiotics in this study did not stimulate the formation of WBC as a xenobiotic substance.

The Basophils, Eosinphils and Lymphocytes (Table 2), which is the average volume of white cells had not any significant changes in male rats receiving probiotics compared to the control. However, all recorded levels are within the normal range, confirming the previous conclusions that the tested probiotics are not associated with any hazard affecting the status of red blood corpuscles.

3- Effect of *L. acidophilus* on serum protein assessments

Total serum protein in two different probiotics strains receiving rats are generally within normal levels for male rats (Table 3), ranging from 5.7 to 7.9 g/ dL in male rats. These values were significantly higher than control referring to a rather beneficial effect of probiotics on liver function. The general metabolism is normally associated with a decrease in the total serum protein (**Roy *et al.*, 2010**). The significant increases in A/G ratio in rats receiving *L. acidophilus* as probiotic do not indicate any adverse effect on immune system since both albumin and globulin were increased by the treatment. But, the increase in albumin synthesis was relatively and slightly higher than the increase in globulin, *i.e.*, globulin production was not negatively affected by the probiotics treatment. Conversely, the enhanced production of both albumin and globulin refer to good liver function and protein metabolism. Moreover,

these changes in A/G ratio do not indicate any emergent diseases in the probiotics treated rats.

4- Effect of *L. acidophilus* on lipid profile assessments

The total concentration of serum cholesterol was significantly reduced from 136.2±3.8 mg/dl in the control to a mean value of 88.5±1.8 mg/dl in group treated with *L. acidophilus* oral administration. In contrast, there was significant difference in the total level of serum cholesterol detected between all groups treated with different probiotics and control group (Table, 3). While, the oral administration with *L. acidophilus* was more efficient in decreasing total serum cholesterol was the most effective in reducing serum cholesterol than supplemented their bacteria to diet. The results of this study are also similar to those carried out by **Abdolamir *et al.* (2010)** who found that probiotics influences lipid profile parameters in which the hyperlipidemic group which did not receive probiotic bacteria registered higher amounts of cholesterol than other groups. In a human study, **Schaarmann *et al.* (2001)** reported that the consumption of the probiotic yoghurt made by *Lactobacillus acidophilus* decreased total cholesterol in hypercholesterolaemic women from 293 mg/dl at the beginning of the experiment to 255 mg/dl after 153 days.

The levels of serum triglycerides (TG) in rats obtained orally the probiotic were significantly lower than in those fed the probiotic sublimated to diet or control. The reduction in triglyceride levels are significant differences with the above-mentioned groups. It is noteworthy from the data in Table (3) that the orally administration of *L. acidophilus* RS11 were more effective in lowering serum triglycerides than all treatments and control. Also, **Salaj *et al.* (2013)** found that the oral administration of *Lactobacillus plantarum* LS/07 resulted in higher decreases of serum cholesterol and LDL cholesterol by 20% and 24%, whereas TG and VLDL levels were decreased by 39%. These results could be explained by confounding variable such as different sources and properties of lactobacilli strains.

As shown in Table (3), there was a significant difference in the plasma HDL cholesterol level between both control groups and other experimental groups at the end of the experimental period. It is clear that the HDL-C contents were greater in all treatments of probiotic groups especially RS11(orally) group since it reached 56.1±0.6 mg/dl.in this respect, **Kikuchi- Hayakawa *et al.* (1998)** reported that consumption of *Bifidobacterium*-fermented soymilk increased the HDL-cholesterol level in hamsters fed on a cholesterol-enriched diet.

Rats obtained the probiotic orally or in feed had significantly lower serum VLDL+LDL-cholesterol than the control group of rats (Table 3). Also, it is noteworthy from the same Table that the serum VLDL+LDL-cholesterol levels in the rats. The same matter was reported by **Abd El-Gawad *et al.*(2005)** who found that the groups of rats fed on a cholesterol-enriched diet supplemented with yoghurt and soy-yoghurt containing *Bifidobacterium lactis* Bb-12 or *B. longum* Bb-46 had significantly lowered levels of serum total cholesterol and very low-density lipoprotein (VLDL)+low-density lipoprotein (LDL) cholesterol than the positive control group (without supplementation). Also, **Salaj *et al.* (2013)** found that the oral administration of *Lactobacillus plantarum* LS/07 resulted in higher decreases of serum cholesterol and LDL cholesterol by 20% and 24%, whereas TG and VLDL levels were decreased by 39%. These results could be explained by confounding variable such as different sources and properties of lactobacilli strains.

5- Kidney Function

The recorded values of renal parameters (urea and creatinine) indicate significant reduction, for the all treats of probiotics with male rats. These reductions in RS11(O) treatment were recorded 1.9±0.08 (g/dL) compared with control (3.3±0.03 g/dL) for serum urea and 0.67±0.02 (mg/dL) compared with control (1.0±0.05 mg/dL) for serum creatinine. These reductions were in the range of 9-30 and 19-40% for serum urea and creatinine in male rats receiving probiotics, respectively. Toxic renal effects are normally manifested with increases in the level of serum urea (**Khalil *et al.*, 1992; Ashour *et al.*, 2007**).

6- Liver enzyme functions

The levels of liver enzymes, i.e.; aspartate aminotransferase, (AST), alanine aminotransferase (ALT) in rates receiving different probiotic treatments (Table 3) were significantly reduced by the probiotic application. Since the elevation of serum aminotransferases (AST and ALT) is taken as an indicator of hepatotoxicity (**Roy *et al.*, 2010 and Ibrahim *et al.*, 2012**), the obtained reduction in these results apparently refer to the absence of hepatotoxicity, i.e., absence of damage or necrosis in the hepatocytes or impairment to liver function in the probiotics receiving groups. The observed reductions in AST and ALT may rather indicate some amelioration in the liver function and performance in the probiotics receiving groups probably as a result of the antioxidant potential of probiotics.

7- Immunity induces assay of serum

Data in Table (4) indicate that superoxide dismutase enzyme and glutathione levels were slightly and significantly increased in the male rats receiving orally doses of *L. acidophilus* RS11. Probiotic consumption has been recommended for immune modulation and general health promotion. The precise mechanisms of immune modulation by

probiotics have not been well elucidated but they are known to influence both specific and non-specific immune responses in animal models and in humans (**Guarner and Malagelada, 2003**). Several studies have demonstrated immunostimulatory effects of probiotics. **Bai *et al.*, (2013)** demonstrated that a probiotic containing *Lactobacillus* stimulated the intestinal T-cell immune system. Similar effects of probiotics on the intestinal immune system of broiler chickens treated with a commercial probiotic product containing *L. acidophilus*.

Table 3. Blood serum proteins, lipid profile, kidney function and liver enzyme functions parameters in male Sprague-Dawley rats receiving two *L. acidophilus* strains during 5 weeks

Items	Control	RS11(O)	RS11(F)	RS12(O)	RS12(F)	RS11+ RS12 (O)	RS11+ RS12 (F)
Blood serum proteins							
TP (mg/dl)	5.7±0.2d	7.9±0.3a	6.2±0.2c	7.2±0.1b	7.8±0.2a	6.4±0.2c	6.8±0.01c
Alb (mg/dl)	3.7±0.15b	3.7±0.12b	2.9±0.03d	3.3±0.16c	4.5±0.23a	4.5±0.11a	4.1±0.1b
Glu (mg/dl)	3.5±0.17b	4.2±0.35a	3.2±0.24b	2.3±0.32c	3.4±0.06b	1.92±0.11d	2.8±0.09c
A/G	1.1±0.09c	0.91±0.10d	0.93±0.07d	1.6±0.32b	1.3±0.10b	2.35±0.09a	1.43±0.07b
Lipid Profile							
Total Cholesterols (mg/dl)	136.2±3.8a	88.5±1.8c	94.4±1.4c	89.5±3.4c	107.5±4b	112.1±2.3b	130.4±0.92a
Total glyceride (mg/dl)	94.1±3a	74.3±2.8d	85.2±2.4b	83.6±1.4c	92.5±1.5b	88.0±1.9b	90.8±2.6b
HDL (mg/dl)	38.6±2.6c	56.1±0.6a	50.2±1.1b	50.6±0.9b	40.9±1.6c	55.3±0.75a	48.9±1.1b
LDL (mg/dl)	64.2±2.9a	30.4±1.8d	37.6±1.6c	32.5±1.4c	44.5±2.2b	28.2±1.5e	36.2±1.1c
VLDL (mg/dl)	31.3±1.1a	24.7±0.9d	28.4±0.8b	27.8±0.5c	30.8±0.5b	29.3±0.6b	30.3±0.8b
Kidney Function							
Creatinine (mg/dL)	1.0±0.05a	0.67±0.02e	0.80±0.05c	0.68±0.02f	0.92±0.02b	0.72±0.02d	0.86±0.03c
Uric acid (g/dL)	3.3±0.03a	1.9 ±0.08d	2.1 ±0.10c	2.2±0.04c	2.6±0.02b	2.7±0.08b	2.8±0.05b
Liver enzyme Function							
ALT	49.1±1.5a	36.8±1.94d	40.5±1.6b	38.7±2.1c	44.5±1.1b	41.6 ±1.3c	42.9±2.2b
AST	130.8±2.1a	104.7±1.9c	118.6±2b	117.3±3.3b	129.5±3.2b	117.7±4b	128.3±1.6a

(O): oral administration of Bacteria (F): Bacteria A supplemented to diet
RS11: *L. acidophilus RS11* RS12: *L. acidophilus RS12*
TP: total protein Alb: albumin Glob: globulin A/G: albumin/ globulin ratio AST: aspartate aminotransferase ALT: alanine aminotransferase TBil: total bilirubin DBil: Direct bilirubin TC: total cholesterol TG: triglycerides HDL: high density lipoprotein LDL: low density lipoprotein VLDL: very low density lipoprotein.
SEM: Standard error of mean Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different (P<0.05).

Table 4. Superoxide dismutase (SOD) and Glutathione (GSH) parameters in male Sprague-Dawley rats receiving two *L. acidophilus* strains

Items	SOD	GSH
Control	0.22±0.04 c	0.22±0.005 c
RS11(O)	0.29±0.06 b	0.34±0.011 a
RS11(F)	0.17±0.05 d	0.28±0.006 b
RS12(O)	0.23±0.02 c	0.25±0.007 b
RS12(F)	0.24 ±0.02 c	0.22±0.005 c
RS11+ RS12 (O)	0.33±0.02 a	0.26±0.008 b
RS11+ RS12 (F)	0.21±0.03 c	0.24 ±0.01 c

(O): Oral administration of Bacteria (F): Bacteria A supplemented to diet
RS11: *L. acidophilus RS11* RS12: *L. acidophilus RS12*

8- Histopathological finding

Histological slices of male rat organs were examined at the end of the experimental period and those of liver and kidney are shown as fellow:

In control treatment: Liver sections (Fig. 1) showed normal hepatic parenchyma with preserved lobular pattern, portal triads structures, vascular tree, kupfur cells and stromal component. Kidney sections revealed normal nephron units with preserved glomerular and tubular structures. The blood vessels and the stroma were within normal limits with normal histomorphology. Most of intestinal sections showed apparently normal mucosa, submucosa, musculosa and serosa.

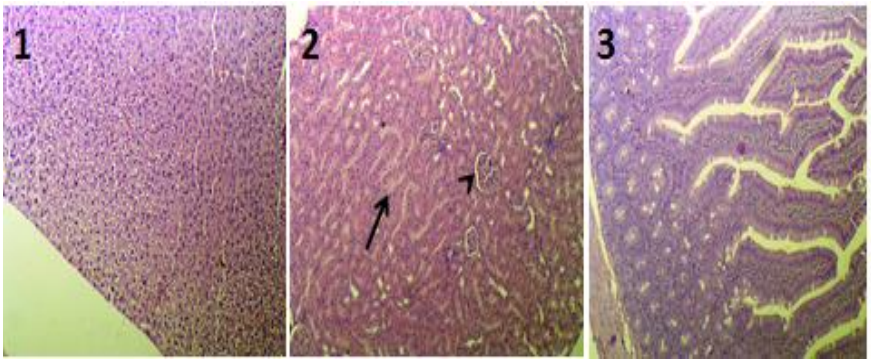

Fig. 1. Photomicrograph of Sections from liver (1) showing normal hepatic parenchyma. Kidney (2) showing normal glomerular **(arrow head)** and tubular structures **(opened arrow).** Intestine (3) showing normal intestinal layers. **H&E X 100 (1,2,3).**

In orally administration of *L. acidophilus* RS11 treatment: Examined sections from liver Fig. (2) showed mild to moderate congestion of the hepatic blood vessels surrounded by mild round cells aggregations. Kidney sections revealed apparently normal renal parenchyma .however, A few tubules showed degenerative changes of their epithelial lining beside presence of hyaline casts in few renal tubular lumen. Most of intestinal sections showed apparently normal mucosa, submucosa, musculosa and serosa. some examined sections from intestine showed hyperplastic changes of lymphocytes in lamina propria and submucosa.

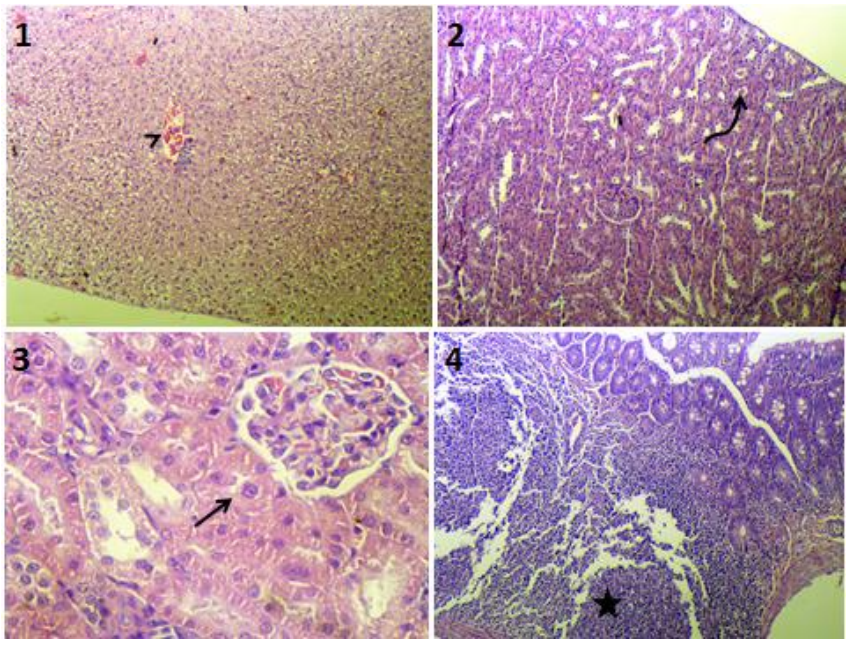

Fig. 2. Photomicrograph of liver (1) showing mild to moderate congestion of the hepatic blood vessels (arrow head) surrounded by mild round cells aggregations. Kidney (2,3) showing degenerative changes in epithelial lining renal tubules (arrow) beside presence of hyaline casts in few renal tubular lumen **(curved arrow).** intestine (4) showing hyperplastic changes of lymphocytes in lamina propria and submucosa **(star)**. .H&E X 100 (1,2), 400 (3,4).

In a supplemented of *L. acidophilus* RS11 to diet treatment: Liver showed moderate congestion of hepatic blood vessels surrounded by moderate round cells infiltration. Fig. (3) Degenerative and necrotic changes in some hepatocytes were also seen. Kidney sections showed apparently normal renal parenchyma. However. Some renal tubular epithelium showed necrotic change. Examined sections of intestine revealed apparently normal mucosal, submucosa and muscular coat layers with branching and widening villus tips beside numerous inflammatory cells infiltration within lamina propria and submucosa were also detected.

In orally administration of *L. acidophilus* RS12 treatment: Liver sections revealed apparently normal hepatic parenchyma with healthy hepatocyte with mild round cells aggregations around hepatic blood vessels (Fig. 4). Sections from kidney showing apparently normal renal parenchyma. However, A few sections revealed congested renal blood vessels. Examined sections from intestine revealed apparently normal intestinal coated layers with branching and widening villus tips. numerous inflammatory cells mainly lymphocytes and eosinophils within lamina propria and submucosa were also detected.

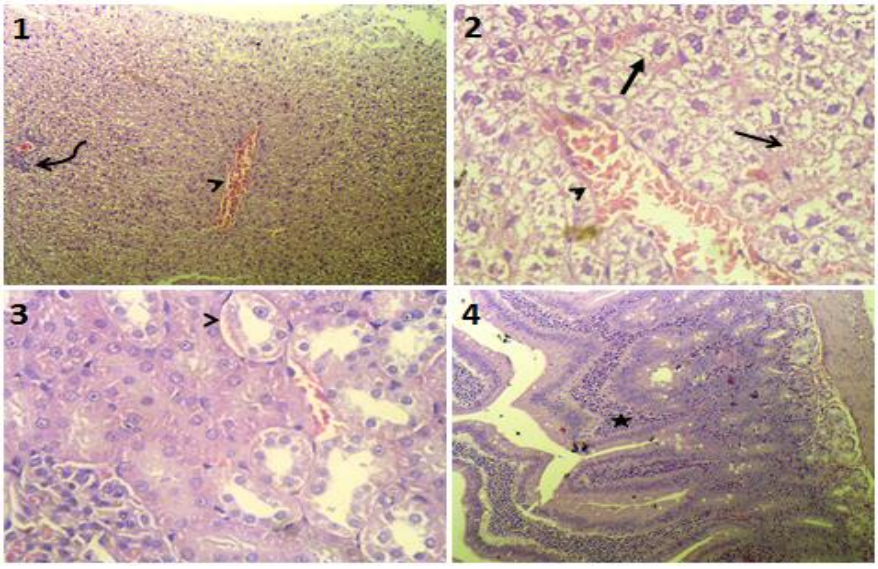

Fig. (3) Photomicrograph of Sections from liver (1, 2) showing moderate congestion of hepatic blood vessels **(arrow heads)** surrounded by moderate round cells infiltration **(curved arrow),** Degenerative **(closed arrow)** and necrotic changes **(opened arrow)** in some hepatocytes. Kidney (3) showing necrosis of some renal tubular epithelium **(arrow head)**. Intestine showing apparently normal layers with numerous inflammatory cells infiltration within lamina propria and submucosa **(star).** H&E X 100 (1,4), 400 (2,3).

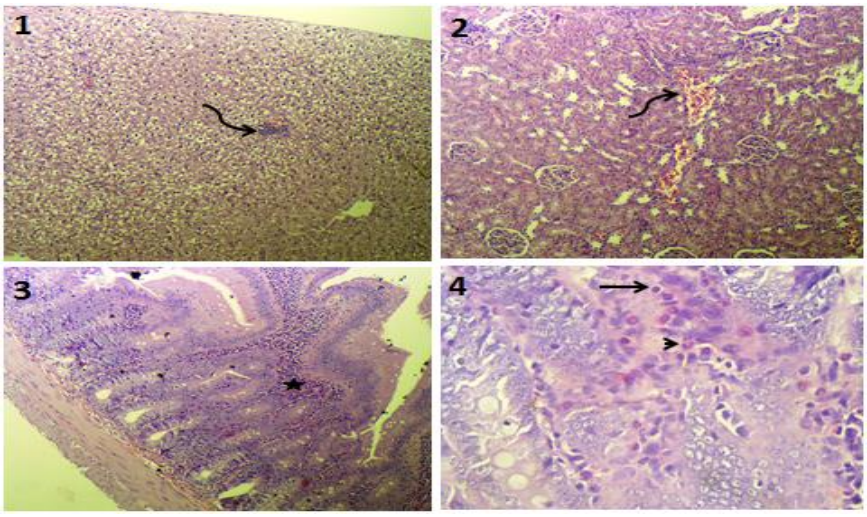

Fig. (4) Photomicrograph of Sections from liver (1) showing perivascular round cells aggregations **(curved arrow)**. Kidney (2) showing congested renal blood vessels **(curved arrow).** Intestine (3,4) showing apparently normal layers with numerous inflammatory cells **(star)** mainly lymphocytes **(opened arrow)** and eosinophils (**arrow head)** within lamina propria and submucosa. H&E X 100 (1,2,3), 400 (4).

In a supplemented of *L. acidophilus* RS12 to diet treatment: Liver sections showed apparently normal hepatic parenchyma with preserved lobular pattern, portal triads structures, vascular tree, kupfur cells and stromal component (Fig. 5). Some hepatocytes showed degenerative changes mainly cloudy swelling and a few cells showed necrosis. Kidney sections revealed hyaline casts within renal tubules. Examined sections from intestine revealed apparently normal intestinal layers with branching and broad villus tips. Numerous lymphocytes and eosinophils within were also detected in lamina propria and submucosa.

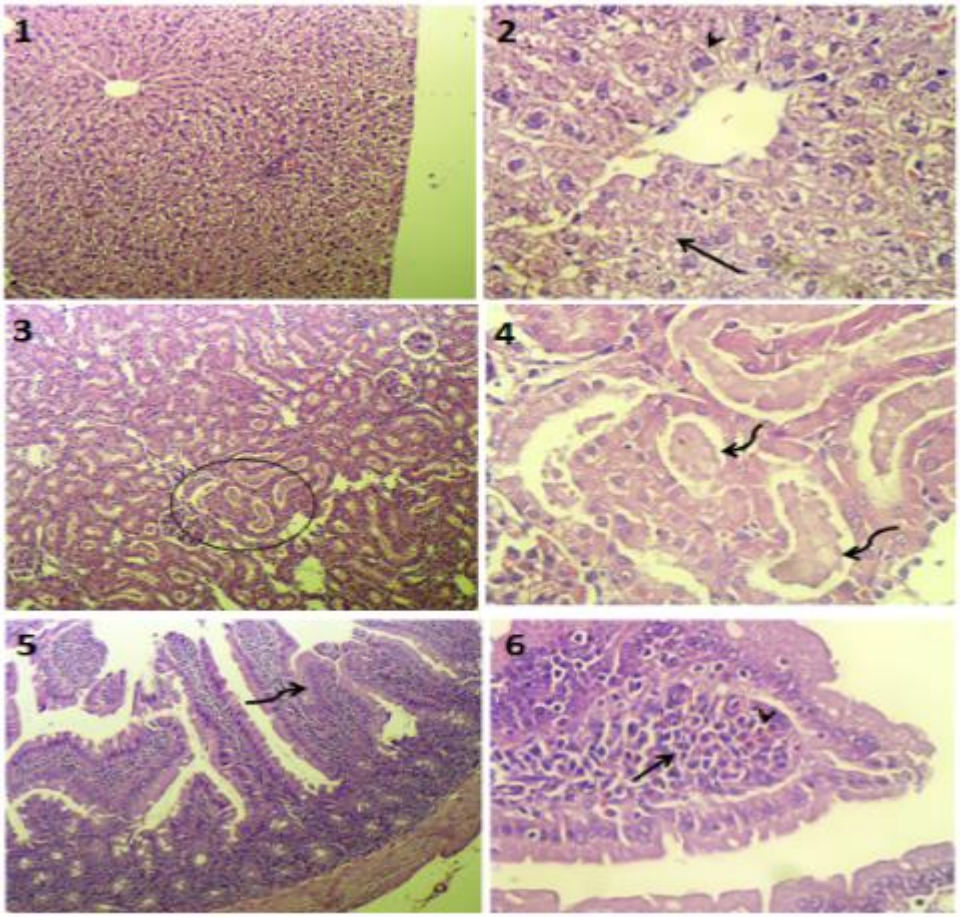

Fig. (5) Photomicrograph of Sections from liver (1,2) showing cloudy swelling **(arrow head)** and few necrotic cells **(opened arrow).** Kidney (3,4) showing intratubular hyaline casts **(curved arrow)**. Intestine (5,6) showing branched villus tips **(curved arrow)** with lymphocytes **(opened arrow)** and eosinophils (**arrow head)** within lamina propria and submucosa. H&E X 100 (1,3,5), 400 (2,4,6).

In orally administration of mixed *L. acidophilus* RS11 and RS12 treatment: Liver sections showed apparently normal hepatic parenchyma with preserved lobular pattern (Fig 6). Some hepatocytes showed degenerative changes mainly cloudy swelling and a few cells showed necrosis. Sections from kidney showing apparently normal renal parenchyma, however. A few sections revealed congested renal blood vessels. Intestine revealed apparently normal layers with branching and broad villus tips.

In a supplemented of mixed *L. acidophilus* RS11 and RS12 to diet treatment: Hepatic parenchyma showed active or binucleated hepatocytes. congested hepatic blood vessels and small necrotic hepatic area were also detected (Fig. 7). Kidney revealed hyaline casts within some renal tubules beside cloudy swelling of some renal tubular epithelium. Examined sections from intestine revealed apparently normal intestinal layers with branching and broad villus tips. Numerous lymphocytes and eosinophils were also detected in lamina propria and submucosa.

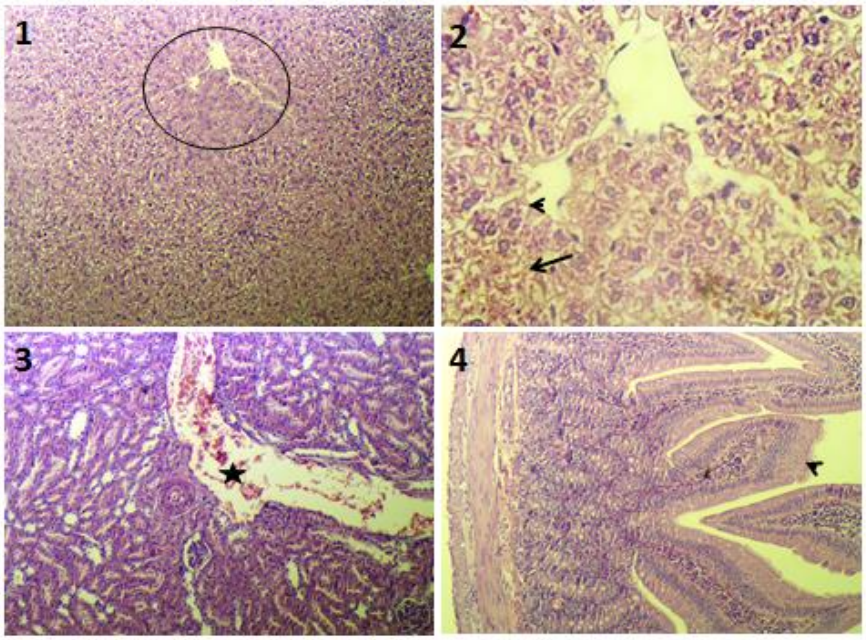

Fig. (6) Photomicrograph of Sections from liver (1, 2) showing cloudy swelling **(arrow head)** and few necrotic cells **(opened arrow).** Kidney (3) showing congested renal blood vessels **(star).** Intestine (4) showing branched and broad villus tips **(arrow head)**. H&E X 100 (1,3,4), 400 (2).

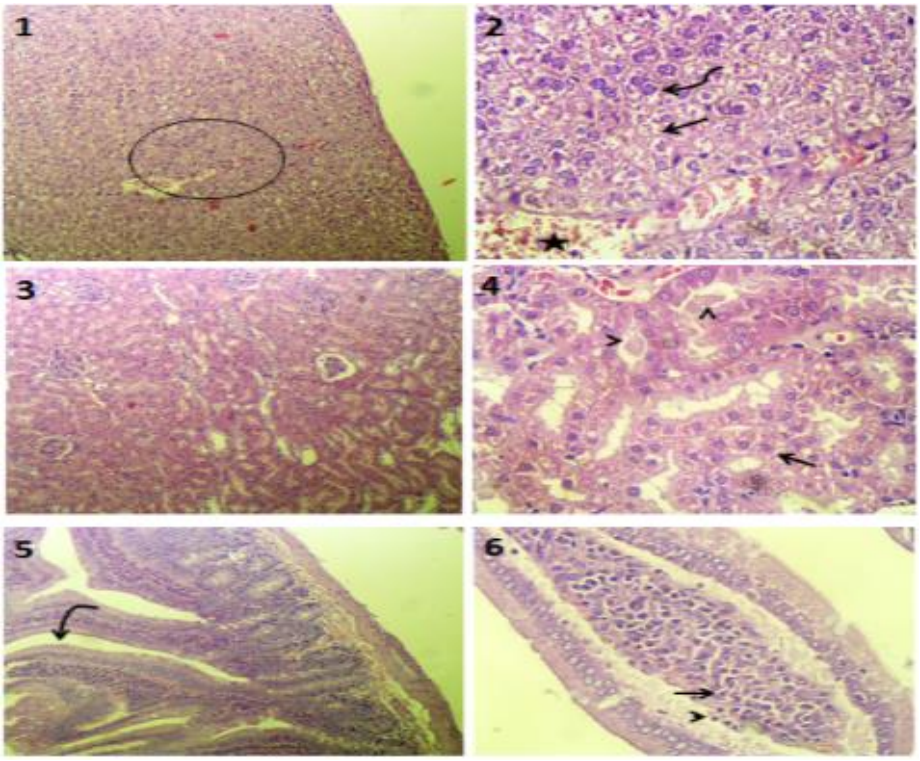

Fig. (7) Photomicrograph of Sections from liver (1,2) showing congested hepatic blood vessel (star), active binucleated hepatocytes (**curved arrow)** and few necrotic cells **(opened arrow).** Kidney (3,4) showing intratubular hyaline casts **(arrow heads)** with cloudy swelling of renal tubular epithelium **(opened arrow)**. Intestine (5,6) showing branched villus tips **(curved arrow)** with lymphocytes **(arrow head)** and eosinophils (**open arrow)** within lamina propria and submucosa. H&E X 100 (1,3,5), 400 (2,4,6).

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