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Using of Iraqi probiotic to detoxify Patulin in albino mice

Shatha Ali Shafiq

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

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ABSTRACT: The present study was to evaluate the histological and biochemical toxic effects of Patulin mycotoxin on liver and kidney tissues of males white mice that extracted from the fungal isolate *Penicillium expansum* at concentration (3.5 mg / kg body weight (given single and repeated doses of Patulin orally administration and the second aspect of this study using of Iraqi probiotic (local product) as detoxifying agent of Patulin. Mice were divided into four groups five mice for each group, taking into consideration liver and kidney weights. Groups of mice were treated as follows. T1: given the toxin once and sacrificed after two days. T2: given the toxin twice for one week. T3: given the toxin twice a week for two weeks. T4: The last group was treated by Patulin for five days orally with addition Iraqi probiotic (2 %) w/ v in drinking water for fifteen days. Each treated group has its corresponding control which received 1% Dimethyl Sulphoxide (DMSO). The results of the treatment of mice males Patulin of 3.5 mg / kg, in three groups (T1,T2,T3) had caused histological changes in the liver tissue represented by degeneration and necrosis in hepatocytes and had increased with increasing of repeated doses of toxin especially at T3 group that it revealed histological changes represented by congestion of central vein with inflammatory cells in their lumen, lymphocytes infiltration and hemorrhage especially near portal space . The results of liver enzymes showed significant decrease in (Aspartate Aminotransferase) AST, (Alanine Aminotransferase) ALT in treated mice with Patulin (repeated doses) which were involved T2 and T3 group compared with the other groups , while the results of enzymatic activity of (Alkaline phosphatase) ALP showed non-significant decrease in all groups . No obvious significant difference in the concentration of Creatinine in all groups, while urea concentration recorded significant decrease in T2 and T3 groups as compared with T1 and T4 groups. Patulin exhibits Histopathological changes coincided with biochemical changes observed in experiments on male mice and the effect of Patulin depended on time of exposure. The other part of the present study was to investigate the biological degradation of Patulin represented by T4 group using by Iraqi probiotic 2% (w/v) which had an active influence to protect the liver and kidney tissues from the toxic effect of Patulin.

KEYWORDS: Patulin, Iraqi probiotic, Histological changes, Biochemical tests.

1 INTRODUCTION

Patulin 4-hydroxy-4H-furo[3,2c] pyran-2(6H)- one is a mycotoxin, has an empirical formula C7H60 4 and molecular weight 154 (Magan &Olsen, 2004). It is a lactone metabolite of several genera belonging to *Penicillium*, *Aspergillus, Paecilomyces*and and *Byssachlamys* , growing on food products especially apples and apple derived products and sometimes in peaches, pears, apricots, grapes, cheese, and grain, or their products , and can be present in intact (fresh) fruits, juices, wines, canned fruits and dried fruit products (Askar, 1999 ; Barkai-Golan & Paster, 2008), . *Penicillium expansum* has been identified as the major producer of Patulin from the species of *Penicillium* which is responsible for the decay in pomaceous fruits (apples and pears). The WHO (1996) recommended a maximum concentration of 50 µg/L in apple juice. In European Union (2002) limit was set to 50 µg/L in both apple juice and 10 µg/kg in products for infants and children.

Patulin is extremely toxic, exhibits mutagenic properties, and is possibly carcinogenic. Strong antibiotic activity has been described, including activity against Gram positive and negative bacteria (Patro 2011) . Exposure to Patulin is associated with immunological, neurological and gastrointestinal outcomes(Alves *et al.* 2000; Baraldi *et al.,* 2003), and has been shown to be toxic to liver and other tissues in animals, such as causing hepatotoxicity, ulceration, congestion, and hemorrhagic lesions (El-Sawi *et al.* 2012) .The toxicity of Patulin is related to its ability to alkylate several enzymes, and inhibit protein

Corresponding Author: Shatha Ali Shafiq

synthesis, forms adducts with DNA and is mutagenic (Casas Lopez *et al*. 2004). Many experimental studies have been carried out using Various bacterial genera most commonly used in probiotic preparations are lactic acid bacteria (LAB) which comprise mainly four genera (*Lactococcus, Lactobacillus, Leuconostoc, Pediococcus*). Other probiotic genera *Bifidobacterium, Esherichia, Enterococcus, Bacillus* and *Streptococcus, Propionbacterium freudenreichii* has also been to have probiotic properties, some fungal strains belonging to *Saccharomyces* such as *S. boulardii* (a strain of *S. cerevisiae*) has also been used these probiotic had been used as detoxifying agents of mycotoxins *in vivo* and *in vitro* (Cabo *et al.,* 2002 ; Topcu *et al*. 2010 ; Dalie *et al.,* 2010 ; Hawer , 2012) . In recent years, only a few studies have been published on the *in vivo* toxicity of patulin . Most of the toxicological studies have used *in vitro* models . The aim of our study was undertaken to establish an *in vitro* model to assess the biochemical and histopathological effects of Patulin in liver and kidney organs of male mice and test the ability of Iraqi probiotic as a biological detoxifying agent to inhibit or reduce the toxic influences of Patulin *in vivo* .

2 MATERIAL AND METHODS

PREPARATION OF PATULIN CONCENTRATIONS

Patulin was obtained from College of Science – department of biology - Mustansiriyah university after extracted from a fungal isolate *Penicillium expansum* that produced Patulin mycotoxin isolated from dried fruits , then was estimated of patulin concentrations after extraction using by HPLC (High Performance Liquid Chromatography) according to (Dombrink-Kurtzman & Blackburn,2005).

PROBIOTIC

Probiotic was obtained from Department of plant protection College of Agriculture – Baghdad university , probiotic is Iraqi product each one gram involves numerous microorganisms :- *Lactobacillus acidophilus* 10^8 Colony Forming Unit (CFU), *Bacillus subtilis* 10^9 (CFU) ,*Lactobacillus* spp. 10^8 (CFU , *Saccharomyces cerevisiae* 10^9 (CFU).

EXPERIMENTAL DESIGN AND ANIMALS

At the beginning, the dose of Patulin was done after dissolving it in 1 % Dimethy sulphate saline (DMSO), the final dose which is considered an acute dose that affect the mouse extensively without killing it was 3.5 mg /kg body weight and the result was recorded after experimenting on five mice for this purpose . Groups of mice were treated as follows: -

T1: given the toxin once and sacrificed after two days.

T2: given the toxin twice for one week.

T3: given the toxin twice a week for two weeks.

T4: The last group was treated by Patulin for five days administrated orally with addition Iraqi probiotic (2 %) w/ v in drinking water for fivteen days . Each treated group has its corresponding control which received 1% Dimethyl Sulphoxide (DMSO). During the experiment, the mice were housed in Plexiglas cages. They were given standard mice diet and had free access to tap water. At the end of the study, the mice were killed by cervical dislocation. The liver and kidney were removed and weighed.

HISTOLOGICAL AND BIOCHEMICAL TESTS

Dissected liver and kidney specimens were removed for both histological and enzymes activity studies. Tissues were fixed in 10% neutral buffered formalin embedded in paraffin and cut into sections which stained with h hematoxylin and Eosin (H&E) for light microscopy.

On the other hand , the liver and kidney cell homogenized were used to estimate the specific activity of liver enzymes(ALS , ALT , ALP) and Creatinine and Urea concentration in homogenized of kidney cells using by colorimetric method (Shafiq & Abdul-Jalil , 2009) .

3 RESULTS AND DISCUSSION

Results showed in Table -1- significant increased ($P<0.01$), in the means of liver weights after treatment with single dose of Patulin (3.5 mg /kg body weight) especially, in T2 and T3 groups compared with control group, T1 group , In addition to

(T4) group which represented by the interaction between single dose of Patulin and Iraqi probiotic (2%) w/v .While means of Kidney weights not appeared any significant difference in all groups. The fact that the liver is the first and most affected organ that is responsible for most metabolic activities while kidneys are less sensitive than the liver in increasing of weight (Gashlan 2008 ; Puel *et al.* 2010) .

Table (1) weights of Organs (mg) in male mice treated with Patulin

Study groups	Organs weight (mg) Mean ± S.D.	
	Liver	Kidney
Control group	1.10 ± 0.05	0.22±1.00
T1	1.08± 0.10	0.21±0.09
T2	1.17 ± 0.08 *	0.21±0.13
T3	1.32 ± 0.08*	0.20±0.10
T4	1.11 ± 0.12	0.21±0.07

HISTOPATHOLOGICAL STUDY OF LIVER AND KIDNEY

Histological figures of organs (liver and kidney) from orally administration mice with single dose and repeated doses of Patulin 3.5 mg / kg body weight were varied in three groups . Liver sections from control mice were presented in Figures 1 shows the liver consists of a central vein and the normal architecture of hepatocytes, arranged in parallel tapes naturally with the presence of glycoprotein granules. The results of the treatment of mice males Patulin of 3.5 mg / kg ,in three groups (T1,T2,T3) had caused histological changes in the liver tissue represented by degeneration and necrosis in hepatocytes and had increased with increasing of repeated doses of toxin especially at T3 group that it revealed hisological changes in liver sections of treated mice with toxin , where represented by congestion of central vein with inflammatory cells in their lumen , lymphocytes infiltration and hemorrhage especially near portal space figures (2) , (3) and (4) .

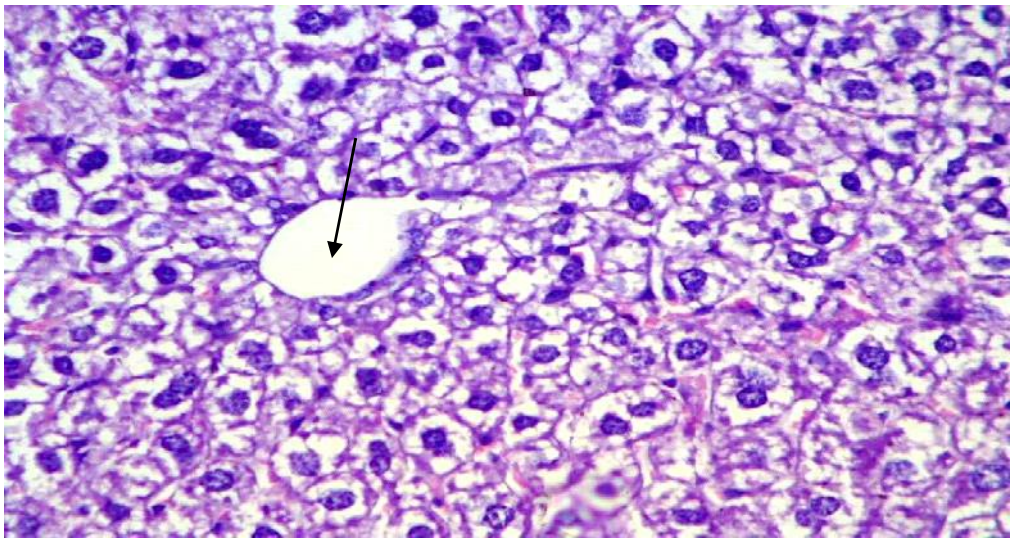

Figure-1-Liver histomicrograph of control male mice shows normal architecture and central vein (H&E, 400X).

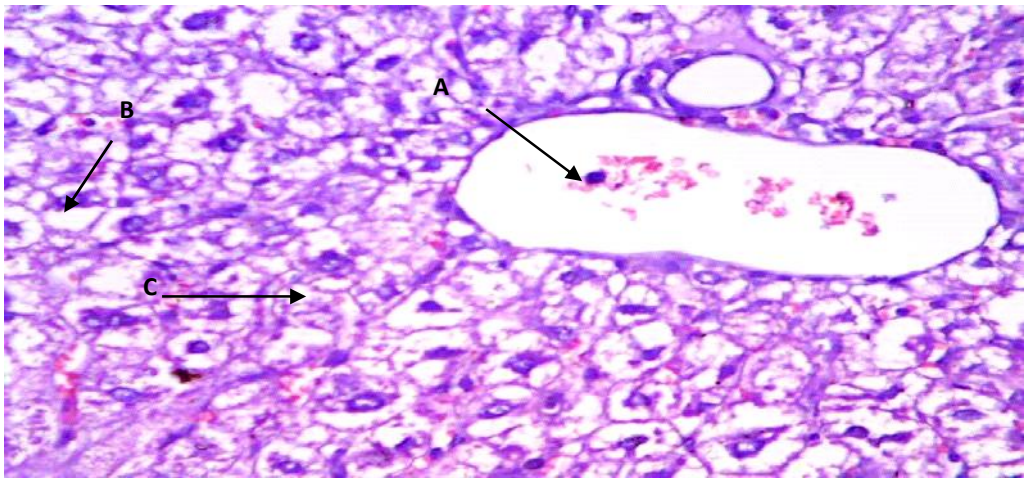

Figure -2- Liver histomicrograph of T1 group male mice shows A: central vein congestion with inflammatory cells in their lumen , B: necrosis, C: degeneration (H&E,400X).

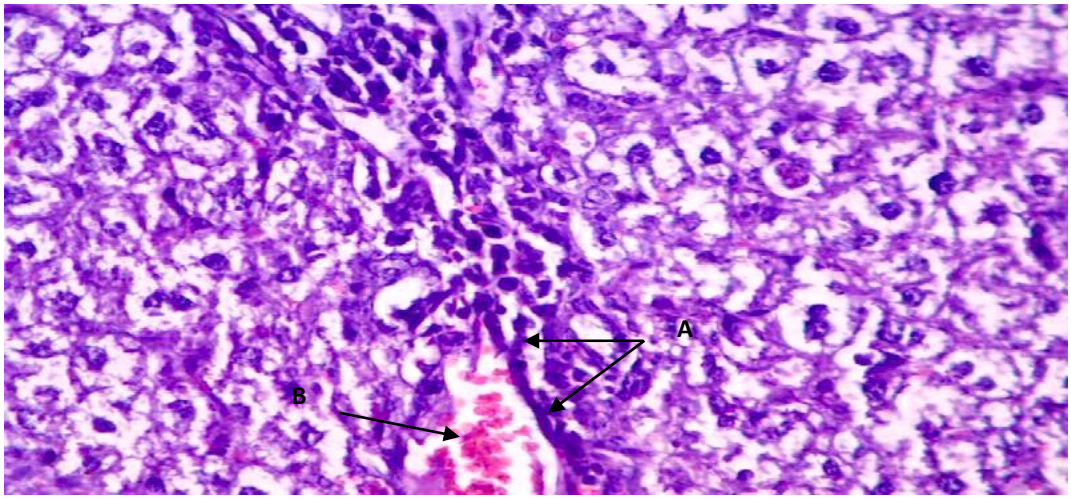

Figure -3- Liver histomicrograph of T2 group male shows A: necrosis and degeneration near portal space, B: inflammatory cells (H&E,400X).

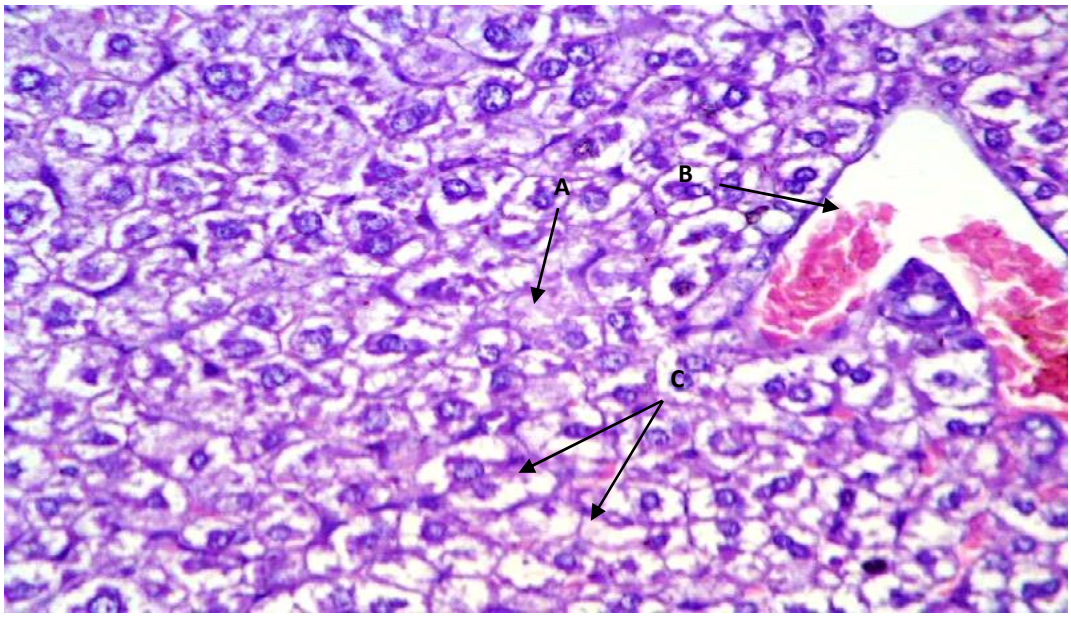

Figure -4- Liver histomicrograph of T3 group mice shows A: degeneration , B: congestion of central vein with inflammatory cells, C: few in glycoprotein granules (H&E,400X).

While histological changes that found in kidney of mice treated with Patulin alone in comparison to control group including degeneration Kidney sections from control mice males shows normal renal tubules and normal glomuerul capillaries as shown in Figure (5), and normal epithelial cells lining of the renal tubules. While kidney sections from mice treated with Patulin alone in both groups T2 and T3 revealed acute tubular degeneration characterized by swelling of epithelial cells lining of the renal tubules or cytoplasmic vacuolation of their cells as shown in figures (6) and (7) . No variation in histological nature of kidney sections in T1 group .

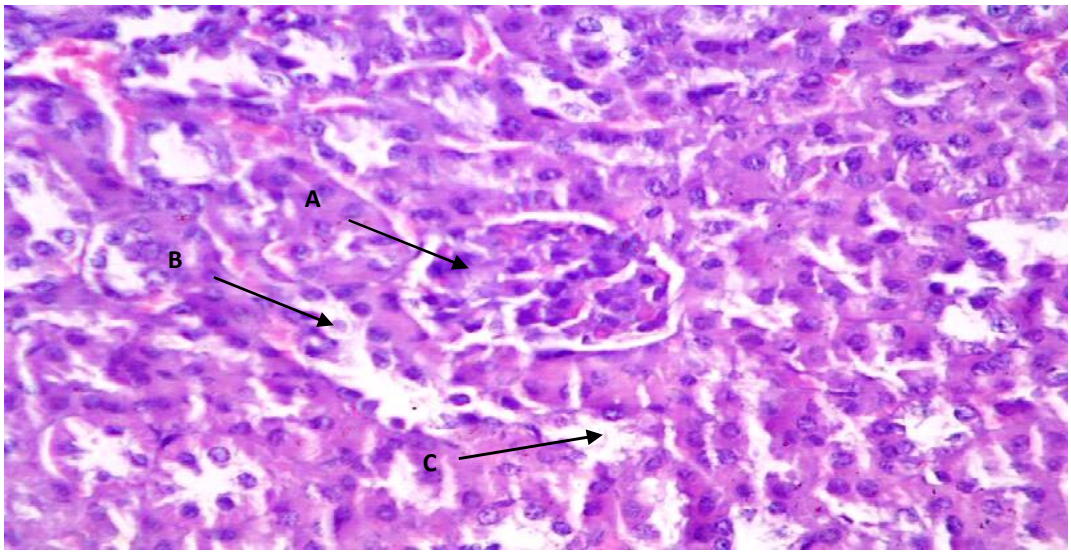

Figure -5- Kidney histomicrograph of control mice shows A: glomuerul capillaries , B: Distal tubules , C: proximal tubules ,(H&E, 400X).

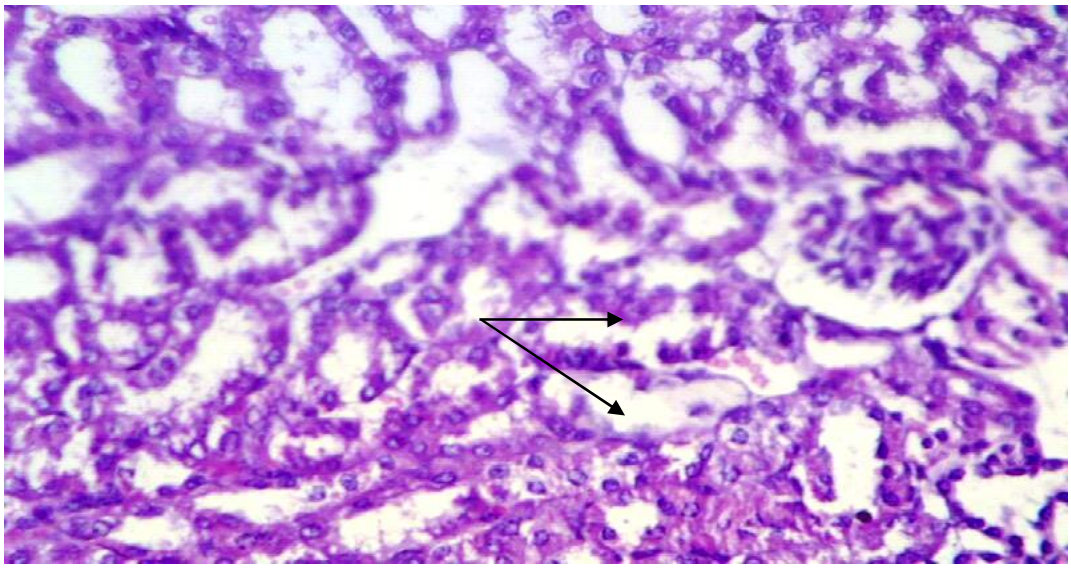

Figure -6- Kidney histomicrograph of T2 group male shows degeneration characterized by swelling of epithelial cells lining of the renal tubules (H&E,400X).

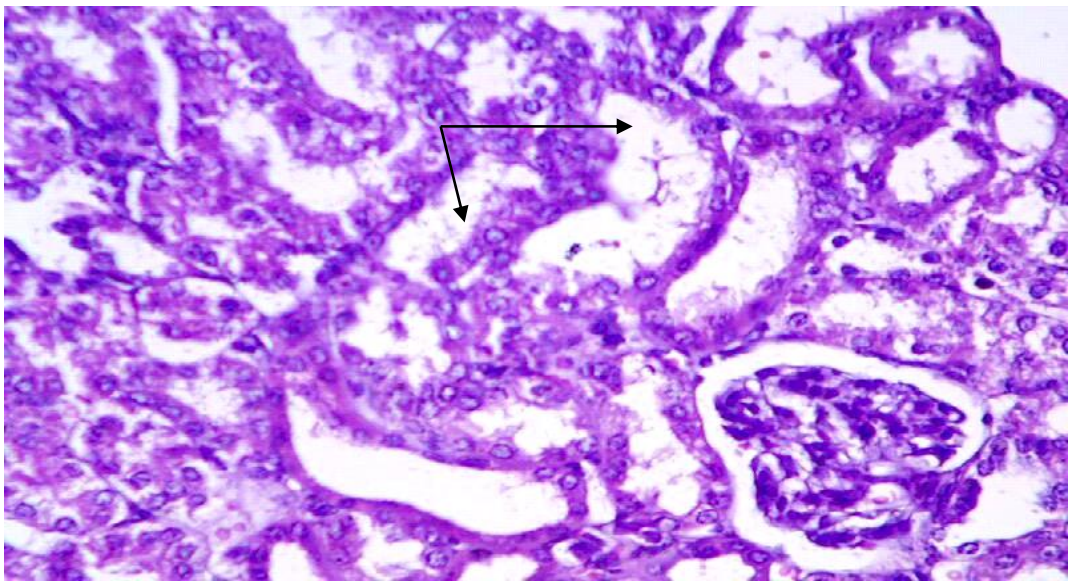

Figure -7- Kidney histomicrograph of T3 group male shows degeneration characterized by swelling of epithelial cells lining of the renal tubules (H&E,400X).

Metabolism of Patulin mainly takes place in liver, leading to the events of histological changes represented by acute inflammation in hepatocytes , and swelling of the cells, causing relax and expand the blood vessels which lead to pooling of blood in central vein , vascular congestion, degeneration and necrosis in hepatocytes Otherwise , the decrease of Patulin toxicity in (T1) group could be explained either by the possibility of metabolic conversion of Patulin into less cytotoxic compound in liver or based on its excretion via urine or faces (Moss, 2002; Speijers, 2004).Theses changes closely resemble to the results obtained by ((Gashlan, 2011 ; El-Sawi et al., 2012 ; Al-Hazmi , 2012) .

As shown in Figures (8) and (9) liver and Kidney sections from mice treated with Patulin in combination with Iraqi probiotic showed normal liver and kidney architecture,which was proved in this study by improvement of histological changes in liver and kidney cells . The reason may be due to the secretions of microorganisms embedded in structure Iraqi probiotic of chemical substances such as lactic acid or antibiotic production Bacterioins (Reuterin that produced by *L.reuteri* (El-Ziney *et al.,* 999) that is responsible for reduction or inhibition the mode of action of mycotoxins in general and Patulin in particular.

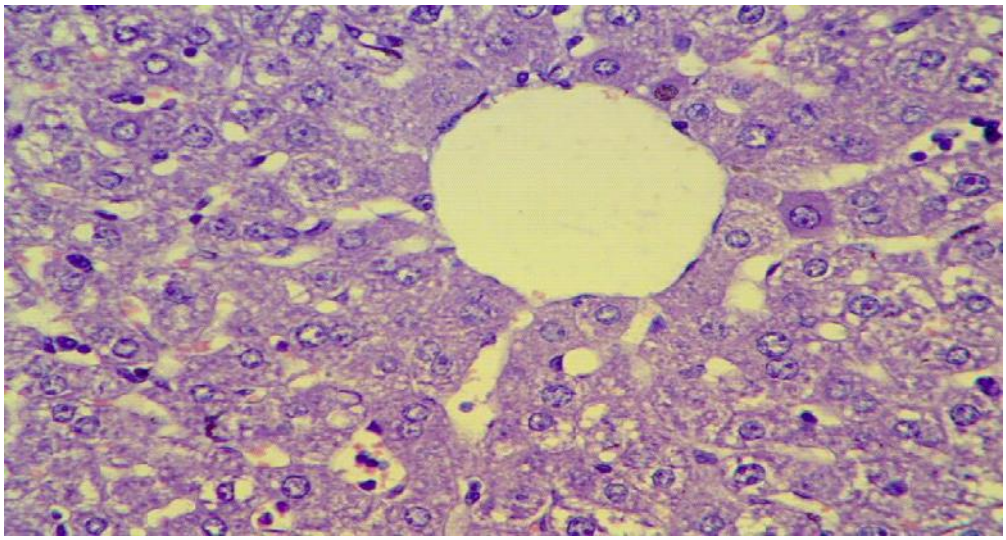

Figure (8) Liver histomicrograph of mice treated with Patulin in combination with Iraqi probiotic shows normal hepatocytes and central vein (H&E, 400X).

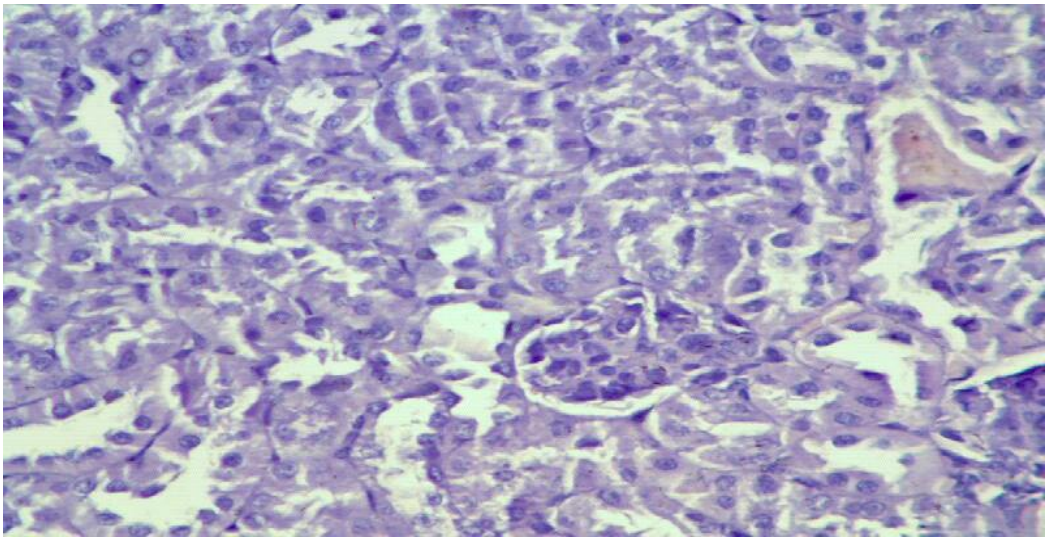

Figure (9) Kidney histomicrograph of of mice treated with Patulin in combination with Iraqi probiotic shows normal glomuerul capillaries, normal renal tubules , (H&E, 400X).

BIOCHEMICAL TESTS

The results of enzymes activity are denoted in Table 2 , significant decrease ($P < 0.01$) in enzymes activities AST, ALT in treated mice with Patulin alone and (repeated doses) which were represented of T2 and T3 group compared with control and T1 group , while the enzymatic activity of ALP , results show non significant decrease in all groups .

Table -2- Effect of Patulin alone and in combination with Iraqi probiotic on some biochemical tests in male mice

Type of test	Treatment groups				
	Con.	T1	T2	T3	T4
ALS(IU)	20.33± 0.09	22.00± 0.11	17.66 ± 0.1*	17.66 ± 0.09*	21.00 ± 0.08
ALT(IU)	22.00 ± 0.0	21.33± 0.07	17.00± 0.12*	16.66± 0.1*	20.44± 0.12
ALP(IU)	13.33± 0.0	11.33± 0.09	11.00± 0.08	12.00± 0.0	12.33± 0.08
Urea(dL)	27.166± 0.1	26.33± 0.12	24.83± 0.10	23.66± 0.09*	25.99± 0.10
Creatinine(dL)	0.600± 0.09	0.56± 0.10	0.60± 0.11	0.63± 0.12	0.61± 0.09

The significant decrease of liver enzymes observed in the present study in treated groups could be explained in view of histological finding which showed cellular membrane damage that increase the permeability of the membranes then leakage these enzymes into the blood serum, the reason due to the ability of mycotoxin in inhibition of protein synthesis and oxidation of phospholipids in cellular membranes that make it more permeability. (Glodman &Shields, 2003 ; Shafiq &Abdul-Jalil , 2009). On the other hand, no significant differences in the concentration of Creatinine in all groups , may be due to glomuerul capillaries were not affected by Patulin through histological examination of the kidneys and Creatinine test reflects the work of glomuerul capillaries(El-Sawi *et al*., 2012) . The regard to the concentration of urea in the same Table, results of statistical analysis showed no significant differences ($p < 0.01$) in T1 and T2 groups except in T3 group recorded significant decrease in urea concentration The reason may be due to damage of kidney tissue represented by necrosis, degeneration and protein catabolism, causing a decrease in urea concentration in homogenizer of kidney cells and leakage it into the blood serum. While the results of the effect of Patulin with Iraqi probiotic on biochemical parameters including AST, ALT,ALP in liver tissue , urea and Creatinine in kidney tissue of male mice which represented by T4 group ,the results were revealed the ability of Iraqi probiotic to protect of liver and kidney tissues from the damage resulting from Patulin mycotoxin . Numerous studies have pointed to the importance of the using of probiotic as bio-agents to remove or destroy the mycotoxins in general (Biernasiak et al., 2006 ; Shafiq, 2015),whenever there was a diversity of microorganisms and even within the same species in the structure of probiotic, that will lead to exhibit the important role of probiotic in detoxification of mycotoxins .

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