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# Histopathological and Biochemical Changes in Kidney and Liver Organs of Rat's Received Apple Juice Contaminated by Patulin Mycotoxin

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**ABSTRACT**

Fruit juices are popular drinks as they contain antioxidants, vitamins, and minerals that are essential for human being and play important role in the prevention of heart diseases, cancer, and diabetes. Consumption of fresh juices increased dramatically due to their freshness, high vitamin content, and low caloric consumption. Fruit and vegetable markets are known to contain several species of fungi. The present study was to evaluate the histological and biochemical toxic effects of Patulin mycotoxin (that formed by *Penicillium expansum* fungus) on liver and kidney organs of female white rats when received fresh Apple juice at 3 ml/rat each 48 hr (as drink). The obtained data resulted that, no animal mortality was observed in any treated group. Fresh Apple juice associated Patulin mycotoxin with or with out *Penicillium expansum* fungus showed non-significant decreased all body weight gain of rats during first week period where decreased significant all body weight gain of rats during second week period and increased significant all tested biochemical parameters i.e. urea, GOT and GPT but, showed non-significant increased with creatinin in serum of all treated groups compared with control group which was in normal limit. Also, the results shown several changes in liver and kidney tissues and malformed symptoms represented by degeneration and necrosis in hepatocytes and had increased with increasing of repeated doses of toxin especially at second group after two weeks period compared with untreated rats (control group).

***Key words:*** Fresh Apple juice, *Penicillium expansum*, Patulin, Rats, Liver, Kidney, Biochemical, Histopathological

## Introduction

The mycotoxin patulin is a secondary metabolite. Among the most efficient producers of patulin are *Aspergillus clavatus, A. giganteus, A. terreus, Byssochlamys nivea, P. carneum, P. dipodomyicola, Penicillium expansum, P. griseofulvum, P. marinum, P. paneum* and several dung associated Penicillia. *Alternaria alternata, Fusarium culmorum, Mucor hiemalis, Trichothecium roseum* and many others produce patulin. *Penicillium expansum* is the most important patulin producer (Frisvad *et al*., 2004b and Hudson *et al*., 2012).

*Penicillium expansum*, the blue mold that causes soft rot of apples, pears and other fruits, is recognized as one of the most common producers of the mycotoxin patulin. Patulin occurs mainly in damaged fruits, although the presence of mould does not necessarily indicate the presence of patulin. Patulin is also regularly found in unfermented apple juice, although it does not survive the fermentation into cider products. Patulin is quite toxic at high concentration in laboratory settings, but evidence for natural poisoning is indirect and inconclusive. Nevertheless, a provisional maximum tolerable daily intake has been established in many countries worldwide (Bennett, and Klich, 2003 and Dimitrios *et al*., 2012).

Patulin is one of the smallest of the group of toxic metabolites known as polyketides. The biosynthesis of patulin occurs over a much narrower range of $w^a$ than growth of the mould and it is most stable at low pH so the environment of fresh fruit is ideal for its production Magan *et al*., 2004) and Moss, (2008). Patulin [4-hydroxy-4H-furo (3, 2-c) pyran-2 (6H)-one], a mycotoxin (structure shown in Figure 1), is heat resistant, stable in dilute acid, and labile in alkali. It is produced by approximately 60 species of molds belonging to over 30 genera. Many fungi found in spoiled food such as *Aspergillus* spp. and *Penicillium* spp., including *A. clavatus, A. giganteus, A. terreus, P. urticae, P. expansum* and *Byssochlamys nivea*, produce patulin (Magan *et al*., 2004).

It is a lactone metabolite of several genera belonging to *Penicillium, Aspergillus, Paecilomyces* 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,

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canned fruits and dried fruit products (Askar, 1999 and Barkai-Golan and Paster, 2008). 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.

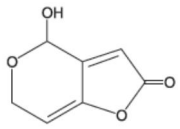


**Fig. 1:** Chemical structure of patulin **(**Cited from Chao-Ling, *et al*., 2000; Magan, *et al*., 2004 and Moss, 2008).

Although patulin was studied first as a potential new antibiotic, research demonstrated the toxicological properties of this compound. It is reported to be acutely toxic to mice, teratogenic to chicken embryos and immunosuppressive in mice and rabbits. Furthermore, it is able to inactivate enzymes, mainly as a consequence of its strong affinity for sulfhydryl groups (Escoula *et al*., 1988). However, it is unlikely that the toxicity is systematic since patulin is degraded quickly after absorption in the gastro-intestinal tract, and will probably only lead to local toxic effects. The International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence for carcinogenicity of patulin in experimental animals.

Patulin caused gastrointestinal effects as, distension, ulceration and haemorrhage. In chronic studies in rats, patulin caused neurotoxicity, immunotoxicity and genotoxicity. Reproductive and teratogenicity *in vivo* studies showed that patulin was embriotoxic. Regarding its potential as a human carcinogen, it has been classified as group 3 (Group 3- Not classifiable as to its carcinogenicity to humans) by International Agency for Research on Cancer (IARC). Recent studies have also demonstrated that patulin alters the intestinal barrier function. Patulin has electrophilic properties, thus high reactivity to cellular nucleophiles. It is believed to exert its cytotoxic and chromosome damaging effects mainly by forming covalent adducts with essential cellular thiols especially with sulfhydryl groups. These properties, led to cause cross linking with proteins, enzyme inhibition, depletion of glutathione and genetic damage including chromosomal aberrations (Ester *et al*., 2013).

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 and 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 synthesis, forms adducts with DNA and is mutagenic (Casas Lopez *et al*., 2004). In recent years, only a few studies have been published on the *in vivo* toxicity of patulin.

The aim of this study was to establish the histopathological and biochemical effects of Patulin in kidney and liver organs of white female rats.

## Material and Methods

*Juice preparation for analysis:*

*Penicillium expansum* was isolated from fresh apple juice (Embaby *et al*., 2015). Surface sterilized apple fruits were inoculated by pure cultural of *Penicillium expansum* fungus then, incubated at 22±1ºC for 15 days to producing patulin as shown in Fig. (2). Under clean conditions berries were squeezed using handle machines, crushed by a processor to obtain the fruit juices and juice were extracted using the sterile stainless steel blender. The resultant juice was filtered through pasteurized double layers of cheesecloth in opened presterilized juice bottles at room temperature according to Torkamani and Niakousari, (2011) and Kamal *et al*., (2014).

**Fig. 2:** Patulin production formed by *Penicillium expansum* fungus in Golden and Red apple fruits

*Experimental animals:*

Three months old Sprague–Dawley female rats (100–120 g were purchased from the Animal House Colony, Giza, Egypt) and were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolize able energy 12.08 MJ) in artificial illuminated and temperature controlled room free from any other source of chemical contamination at the Animal House Laboratory, National Research center, Dokki, Egypt, which were housed under constant environmental conditions (22± 1°C room temperature; 65 ± 5% relative humidity; 12-h light/dark). After an acclimatization period of 1 week, the animals were divided into three groups (4 rats/ group) and housed in filter-top polycarbonate cages. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Egypt. Food consumption was approximately 15 g/female/day according to Liebert *et al*., (2006). They were allowed to freely consume tap water and were fed according to the indicated experimental diets. The animals were adapted to the environmental conditions for 7 days prior to the start of the experiment. Measurements of BW were monitored every day throughout the 3 weeks exposure (Madrigal-Santilla *et al*., 2006).

*Experimental design:*

On experimental day, annulated rats were assigned to three groups of 4 animals each and received a single oral dose of 3 ml/Kg, using apple juice as drink. Animals within three treatment groups were maintained on their respective diets for 2 weeks as follows: group 1, received apple juice (drinking) extracted from the infected apple parts which contain *Penicillium expansum* spores and mycellim (as drink); group 2, treated orally with apple juice extracted from the infected apple fruits without *Penicillium expansum* spores or mycellim (as drink) and group 3, untreated control which received normal water. The animals were observed daily for signs of toxicity. Body weights were recorded during the experimental period. The experimental determinations were made in rats fed on diets for two weeks with the same basic according to Madrigal-Santilla *et al*. (2006). The weight of each rat was determined at the end of the experiment for two weeks.

*Blood samples:*

All animals were not fed on the day of the sacrifice. After the experimental period (14 days), the blood samples were collected from retro-orbital venous plexus for biochemical analysis in 15 ml-polypropylene tubes containing heparin as anticoagulant and centrifuged at 3000 rpm for 10 min to separate the plasma and used for biochemical serum analysis of kidney and liver function. Also, Blood draws (150-200 µl) were made using 1 ml syringes connected to the jugular cannula. Blood samples were collected into anticoagulant tubes that contained ethylene-diamine-tetra-acetic acid (EDTA) and were stored frozen at -20°C Fig. (3). Blood samples obtained from treated and untreated rats were analysis (Mangano *et al*., 2001).

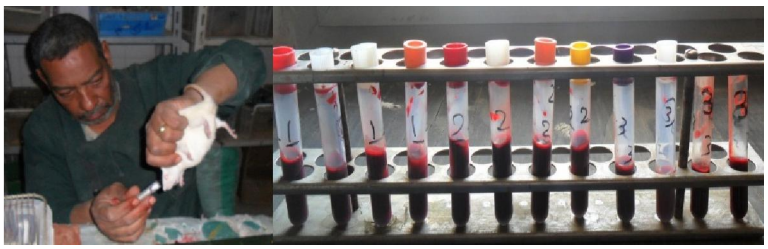

**Fig. 3:** Blood collection

*Biochemical evaluation of experimental animals:*

All the biochemical determinations were carried out using commercial kits according to the kits manufactures unless explained as follow:-

*Determination of Urea:*

Determination of urea level in serum by using the enzymatic colorimetric method of Barham and Trinder, (1972) by using kit obtained from BioMérieax SA (France).

*Determination of Creatinine:*

Creatinine was determined in serum using commercial kits purchased from Stanbio Laboratory, Inc. (San Antonio, Texas, USA) according to the methods of Bartles *et al*. (1972).

*Histopathological studied:*

At the end of treatment period, and after blood samples were collected, all animals were killed and the kidney and liver samples were collected for histopathological examination, all rats were deadened using ether and

sacrificed (with painless) by decapitation (guillotine) Fig. (4). The liver and kidney tissue of each animal was dissected and immediately frozen in liquid nitrogen (freeze clamping). All samples were stored at - 80°C until analysis (Aoudia *et al.,* 2008). Samples of each organ were homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate (Lin *et al*., 1998). This homogenate was centrifuged at 1700 rpm and 4 $^{O}$C for 10 min and the supernatant was stored at -80 $^{O}$C to the next day until analysis. This supernatant (20%) was used for the determination of lipid peroxidation in the liver and kidney tissue according to the method described by Esterbauer *et al*. (1991). The homogenate was further diluted to give 5% homogenate (w/v), centrifuged at 3000 rpm for 5 min at 0 $^{O}$C and used for the determination of total antioxidant capacity according to the method described by Koracevic *et al*. (2001). Other liver and kidney samples were excised and fixed in natural formalin 10% and were hydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin. Serial sections (5 mm thick) were cut and stained with hematoxylin and eosin (H&E) Drury and wallington, (1980) for histopathological investigation. Kidneys and livers were fixed by following standard methods of dehydration and clearing. A small piece of kidneys and livers were sectioned for 6 µm thick of the control and treated samples in Histopathological laboratory, Histopathology Department, National Research Centre, Dokki, Egypt. Liver and Kidney specimens from all animals (Treated with two types of apple juices which containing patulin (PT) as well as un-treated) were dissected immediately after death, and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 µm thick were cut and stained with Haematoxylin and eosin (Drury and Walligton, 1980) not found for histopathological investigation. Images were captured and processed using Adobe Photoshop version 8.0.


**Fig. 4:** Kidneys and livers collection

*Fixatives used for study:*

Tissues were fixed by neutral buffered 10% formalin and then embedded into paraffin for histological study by light microscope.

*Staining used for the study:*

Haematoxylin and eosin stain (H and E) stain gives clear cytoplasm differentiation and nuclear and gives good idea about histological structure of the samples of the study and reveals some pathogenic changes (Saddiq and Kalifa, 2011).

*Histopathological examination*:

All sections of kidneys and livers (5 to 6 µm thick) of the control and treated samples which were stained (with hematoxylin-eosin) and mounted on the glass microscope slides using standard histopathological techniques then, examined by light microscopy (Drury and wallington, 1980; Omar, 2012 and Saddiq, 2012).

## Results

**I-Effect of *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) on body weight of rats**

The obtained data show that, no animal mortality was observed in any treated group. Data in Table (1) indicated that, all animals received Apple juice contaminated with the mycotoxin Patulin (Pat) with or free *Pencillium expansum* fungus showed a non-significant decreased body weight gain of rats during first week period where decreased significant all body weight gain of rats during second week period compared to the control group (Un-treated). The average of body weight of animals for control group was 147.5g then decreased to 135g with the first group of animal's rat which received Apple juice contaminated with *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) group (1) causing 12.5 g of loss equal 8.47 percentage of loss for the first weak. Also the obtained results noted that, (second group) rats received Apple juice contaminated with Patulin (Pat) only (free *Pencillium expansum* fungus) gave average 130g of body weight compared with control group (3) which record 147.5g with 17.5g of loss equal 11.86 loss percent in the same period time (the first weak).

On the other hand data presented that, decreased significantly all body weight gain of rats during second week period. The average of body weight animals for control group (3) in the second weak was 177.5 g and reduced significantly to 120g with the first group of animal's rat which received Apple juice contaminated with

*Pencillium expansum* fungus and their mycotoxin Patulin (Pat) group (1) causing 57.5 g of loss equal 32.39 percentage of loss. Also, the obtained results noted that, (second group) rats received Apple juice contaminated with Patulin (Pat) only (free *Pencillium expansum* fungus) gave average 125g of body weight compared with control group (3) which record 177.5g causing 52.5g of loss equal 29.58 loss percent in the same period time.

**Table 1:** Effect of *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) on body weight gain of rats for two weeks

| Received period | Animal groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control group | First group | | | Second group | | |
| | Bw. (g) | Bw. (g) | L(g) | %L | Bw. (g) | L(g) | %L |
| First week | 147.5 | 135 | 12.5 | 8.47 | 130 | 17.5 | 11.86 |
| Second week | 177.5 | 120 | 57.5 | 32.39 | 125 | 52.5 | 29.58 |
| LSD: ⟶ 0.05 | For first week:- Control 3rd&1stgroup-P=0.2148 n Control 3rd &2nd group – P=0.1089 n 1st & 2nd group – P=0.526 n | | | | For second week:- Control 3rd&1stgroup-P=0.0014s Control 3rd&2nd group – P=0.0038 s 1st & 2nd group – P=0.7012n | | |
| | First week & Second week Control group 3rd– P= 0.0233 n First 1stgroup-P= 0.1629 n Second 2nd group – P= 0.6648 n | | | | | | |

*Bw. = Body weight (g) L = Loss (g) % L = % Loss s =significant n = non-significant*
***Group 1** = Animals received Apple juice contaminated with Pencillium expansum fungus and their mycotoxin Patulin (Pat)*
***Group 2** = Animals received Apple juice contaminated with the mycotoxin Patulin (Pat) free Pencillium expansum fungus*
***Group 3** = Un-treated (Control group)*

**II-Biochemical studied (serum analysis):**

Changes in biochemical parameters caused by the mycotoxin Patulin (Pat) were recorded in Table (2). The effects of received Apple juice contaminated with the mycotoxin Patulin (Pat) with or with out *Pencillium expansum* fungus on serum biochemical parameters of rats compared with control group indicated that, control group was in normal limit, but when rats received Apple juice contaminated with the mycotoxin Patulin (Pat) with or without *Pencillium expansum* fungus were found to induced significantly ($P \leq 0.05$) increase all biochemical parameters i.e. urea, GOT and GPT but, showed non-significant increased with creatinin compared with control group which was in normal limit. Data cleared that liver and kidney function of the control group was in normal rang and represented 7.63 m mol/L for urea, 0.87 mg/ dl for creatinin, 45.00 U/L for GOT and 31.00 U/L for GPT respectively.

The obtained data presented that, mycotoxin Patulin (Pat) with or with out *Pencillium expansum* fungus were found to be increased significantly kidney function as Urea m mol/L from 7.63 to 12.25 m mol/L with the first group (1) animals which received Apple juice contaminated with *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) and to 10.53 m mol/L with the second group (2) animals which received Apple juice contaminated with the mycotoxin Patulin (Pat) only (with out *Pencillium expansum* fungus). Also, increased non-significantly creatinin from 0.87 mg/ dl to 1.19 mg/ dl with the first group (1) animals which received Apple juice contaminated with *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) and to 1.03 mg/ dl with the second group (2) animals which received Apple juice contaminated with the mycotoxin Patulin (Pat) only (with out *Pencillium expansum* fungus). Increase significantly GOT from 45.0 U/L to 50.00 U/L for the first group (1) animals and 54.33 U/L for the second group (2) animals. Finally Patulin (Pat) was found to be increased significantly GPT from 31.0 U/L to 43.0 and 34.67 U/L for the first and second group animals respectively.

**Table (2):** Effect of *Pencillium expansum* fungus and their mycotoxin Patulin (Pat) on serum biochemical parameters in rats

| Parameter | | Group (1) | Group (2) | Group (3) |
|---|---|---|---|---|
| Urea | m mol/L | 12.25 | 10.53 | 7.63 |
| Creatinine | mg/ dl | 1.19 | 1.03 | 0.87 |
| GOT | U/L | 50.00 | 54.33 | 45.00 |
| GPT | U/L | 43.00 | 34.67 | 31.00 |
| LSD: ⟶ 0.05 | For Urea:- Control 3rd &1st group-P=0.0010s Control 3rd &2nd group – P=0.0098s 1st & 2nd group – P=0.069n | | For Creatinine:- Control 3rd&1st group-P=0.6951 n Control 3rd &2nd group – P=0.8438 n 1st & 2nd group – P=0.853 n | |
| | For GOT:- Control 3st&1stgroup-P=0.0007s Control 3st &2st group – P≤0.000s 1st & 2st group – P=0.0021 s | | For GPT:- Control 3st&1stgroup-P≤0.0001 s Control 3st &2st group – P=0.0033 s 1st & 2st group – P≤0.0001 s | |

***GOT** = Glutamate oxaloacetate transaminase*
***GPT** = Glutamate pyrovate transaminase*
*s =significant n = non-significant*

**Histopathological results of liver group:**

The biochemical results reported in the current study were confirmed by the histological results. the results that, shown several changes in liver and kidney tissues and malformed symptoms of female white rats when received fresh Apple juice contaminated Patulin mycotoxin with or with out *Penicillium expansum* fungus after two weeks period compared with untreated rats (control group). Histological figures of liver sections from orally administration female rats with 3 mlg/rat doses of Apple juice contaminated Patulin with or without *Pencillium expansum* and body weight were varied in two groups other than control group. Liver sections from control rats were presented in Fig. (5) shows the liver consists of a central vein and the normal architecture of hepatocytes. The results of the treatment female rats received Apple juice contaminated with Patulin at 3 mlg/rat in two groups 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 second group that it revealed hisological changes in liver sections of treated rats with toxin , where represented by congestion of central vein with inflammatory cells in their lumen , lymphocytes infiltration and hemorrhage especially near portal space figures (6) and (7).

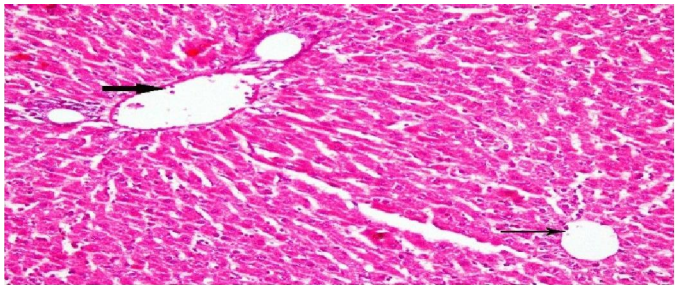

**Fig. 5:** A photomicrography for (control group) showing normal liver tissue. The liver is divided histologically into lobules. The center of the lobule is the central vein (thin arrow) and at the periphery of the lobule there are the portal triads (thick arrow) (H&E 100).

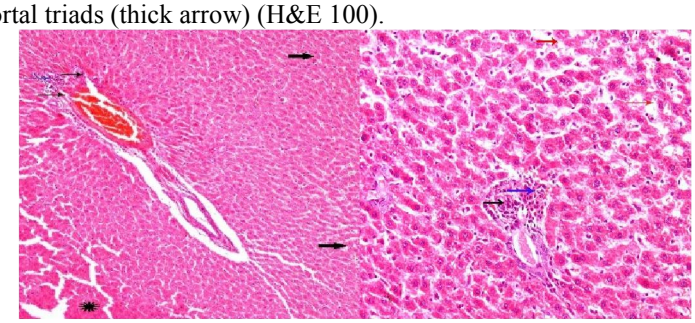

**Fig. 6:** A photomicrography for (first group) of hepatic tissue showing in the left one bile duct proliferation (the black thin arrow ) , and a focal of inflammatory cells (the blue arrow), also there are areas of necrotic hepatic cells (*) other hepatic cells are vacuolated cells (the thick black arrow ) , On a higher magnification in the right photo showing bile duct proliferation (the black thin arrow ) , and a focal of inflammatory cells (the blue arrow), also there are areas of inter-hepatic edema (the red arrow) (H&E 100& 200).

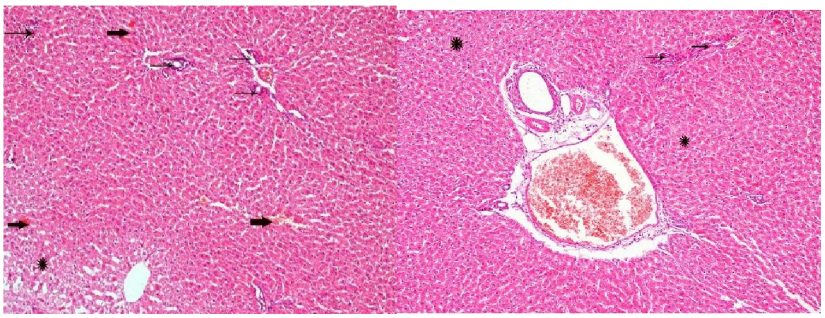

**Fig. 7:** A photomicrography for (second group) of hepatic tissue showing in the left picture inflammatory cells around the bile ducts (thin arrow) but there is no bile duct proliferation with focal areas of hemorrhage (thick arrow), also there are areas of inter-hepatic edema and vacuolated hepatic cells (*), The same finding also seen on a higher magnification in the right picture (H&E 40 & 100).

**Histopathological results of kidney group:**

While histological changes that found in kidney of mice treated with Patulin 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 (8), and normal epithelial cells lining of the renal tubules. While kidney sections from mice treated with Patulin in both first and second group revealed tubular degeneration characterized by swelling of epithelial cells lining of the renal tubules or cytoplasmic vacuolation of their cells as shown in figures (9) and (10).

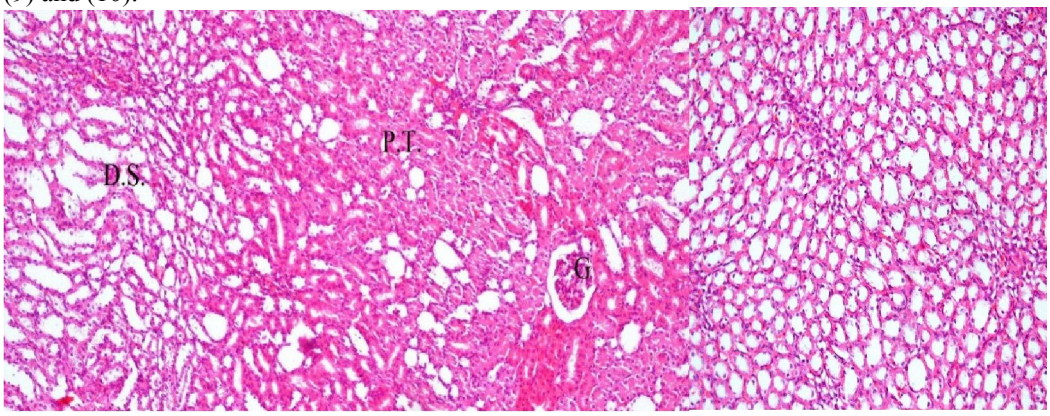


**Fig. 8:** A photomicrography for (control group) showing normal Kidney tissue. The left picture showing the glomeruli (G) then the proximal convoluted tubules (P.T) and the inner layer is the distal convoluted tubules (D.S), the right picture showing the distal tubules with well formed epithelial lining by higher magnification (H&E 100&200).

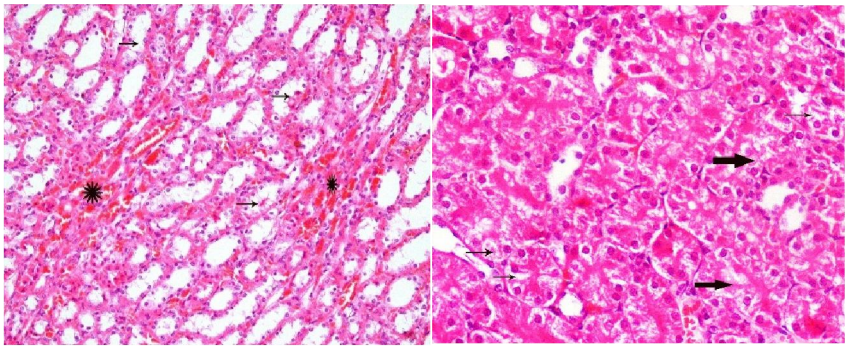

**Fig. 9:** A photomicrography for (first group) of kidney tissue showing in the left one areas of congested tubules with areas of hemorrhage and necrosis (*) other tubules lined by vacuolated epithelial cells (thin arrow) , On a higher magnification in the right photo showing the area of proximal conveluated tubules with inter-tubular depris and some of their epithelial lining are shedded inside the tubules (thick arrow) with other tubules lined by vacuolated epithelial cells (thin arrow) (H&E 200& 400).

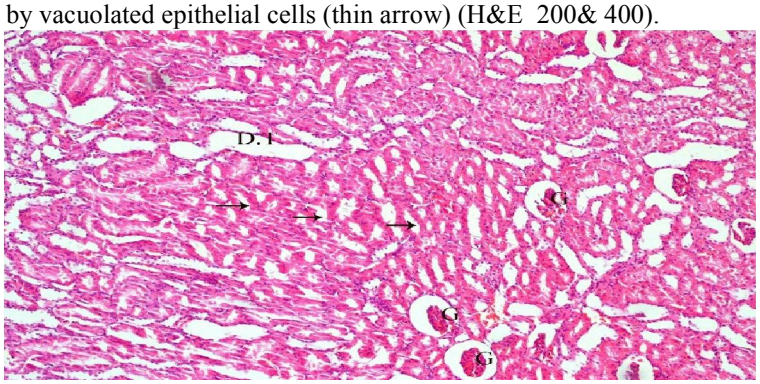

**Fig. 10:** A photomicrography for (second group) of kidney tissue showing the proximal conveluated tubules with mild oedema (thin arrow) also the distal tubules appears normal (D.T) with an intact epithelial lining of both tubes of tubules , on the other hands the glomeruli (G) looks to be degenerated (H&E 100).

## Discussion

The obtained data presented that, all animals received Apple juice contaminated with the mycotoxin Patulin (Pat) with or without *Pencillium expansum* fungus showed a significant decrease in body weight gain compared to the control group (Un-treated) and induced significantly ($P \leq 0.05$) increase all biochemical parameters i.e urea, creatinin, GOT and GPT in serum of all treated groups. Also, Data indicated that control group was in normal limit.

Similar results were obtained by many investigators; Mézes, (2008) reported that, mycotoxins may cause blood abnormalities. There are some clinical signs, which may appear in rabbit, such as severe pain in the abdomen, several blood abnormalities, e.g. high urea and creatinine levels, calcium-phosphorus imbalance, abnormal levels of liver enzymes (AST, ALT and GGT), feed refusal, weigh loss. Exposure to PAT caused a decrease in the cells antioxidant capacity as noted by depleted intracellular GSH and a corresponding increase in intracellular ROS. The innate cell survival mechanisms to adapt to oxidative stress was activated following PAT exposure via the up regulation of AO transcription factors, NRF2 and PGC1α; and AO gene expression, SOD, CAT, GPx and SIRT3 in HEK293 cells. El-Sawi *et al*., (2012) found that, levels of AST and GGT were increased significantly in serum of all treated groups compared with control group but ALT was increased significantly in treated group after one week only.

The results of the treatment female rats received Apple juice contaminated with Patulin at 3 mlg/rat in two groups had caused histological changes in the kidney and liver tissues represented by degeneration and necrosis in hepatocytes and had increased with increasing of repeated doses of toxin especially at second group that it revealed hisological changes in liver sections of treated rats with toxin , where represented by congestion of central vein with inflammatory cells in their lumen , lymphocytes infiltration and hemorrhage especially near portal space

These changes closely resemble to the results obtained by Moss, (2002); Speijers, (2004); El-Sawi *et al*., (2012); Gashlan, (2008) and Al-Hazmi, (2012) they found 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.

**Conclusion:**

Juices squeezed from fresh fruits contain microorganisms which are potentially hazardous to public health. The selling and consumption of juices are never stopped on nutritional grounds as well as livelihood of street vendors. Apple juice associated patulin may have a destructive effect on the biochemical and histopathological microscopic picture of hepatic and renal tissues in affected kidney and liver rats. Exposures of patulin indicate that hepatic alteration was produced in manner related to dose duration and crude venom may used as new therapeutic approach to detoxify hepatocytes from patulin. It is alarming situation for suitable agency to take some necessary action, make guidelines to prevent potential food poisoning from juices that contain pathogenic microorganisms in juices. Mycotoxin analysis is an important subject with social relevance. Pasteurization and sterilization treatments were the most suitable for elimination all Juice microbial of its presence in human diet.

## References

Al-Hazmi, M. A., 2012. Patulin in apple juice and its risk assessment on albino mice, Toxicol., and Health, 30(4)

Alves, I., N. G. Oliveira and A. Laires, 2000. Induction of micro nuclei and chromosomal aberrations by the mycotoxin patulin in mammalian cells. Role of ascorbic acid as a modulator of Patulin Castagno city. Mutagenesis, 15: 229-234.

Aoudia, N., E. Tangni and Y. Larondelle, 2008. Distribution of ochratoxin A in plasma and tissues of rats fed a naturally contaminated diet amended with micronized wheat fibers: Effectiveness of mycotoxin sequestering activity. Food and Chemical Toxicology 46, 871–878.

Askar, A., 1999. Patulin in apple juice and children's apple food. Fruit Proces; 3:74-7.

Baraldi, E., M. Mari, E. Chierici, M. Pondrelli, P. Bertolini, and C.Pratella, 2003. Studies on thiabendazole resistance of *Penicillium expansum* of genetic pear: pathogenic fitness and genetic characterization. Plant Pathology, 52:362-370.

Barham, D. and P. Trinder, 1972. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst. 97(151):142-145.

Barkai-Golan, R. and N.Paster, 2008. Mycotoxins in fruits and vegetables. Academic Press is an imprint of Elsevier, London, UK, and pp.395.

Bartels, H., M. Bohmer and C. Heirli, 1972. Serum creatinine determination without protein preciptation. Clin. Chem. Acta. 37, 193–197.

Bennett, J.W. and M.Klich, 2003. Mycotoxins. Clinical Microbiology Reviews, Vol.16, No.3, pp.497-516, 0893-8512.

Casas, L. J. L., P. E. M. Rodriguez, F. M. A. Vilches, P. J. A. Sanchez, S. J. M. Fernandez, and Y. Chisti, 2004. Lovastatin inhibits its own synthesis in *Aspergillus terreus*. Journal of Industrial Microbiology and Biotechnology 31: 48–50.

Chao-Ling, L. A. I., F. You-Min and Y. S. Daniel, 2000. Detection of Mycotoxin Patulin in Apple Juice. Journal of Food and Drug Analysis, 8(2): 85-96.

Dimitrios, I. T., D. Myrto, P. A. Polymnia and C. T. Eleftherios, 2012. Review; Biological control strategies of mycotoxigenic fungi and associated mycotoxins in Mediterranean basin crops. Phytopathologia Mediterranea, 51, 1, 158−174.

Drury, A. A. and E.A. Wallington, 1980. Carleton`s histological technique.5th Ed., Oxford University press, New York, Toronto.

El-Sawi, N.M., H. M. Gashlan, S. H. H. Younes, R.F. Al-Massabi, and S. Shaker, 2012. Biochemical and histological studies on the effect of the Patulin mycotoxin on male rats' liver and treatment by crude venom extracted from jelly fish. Life Science Journal, 9(4): 1143-1153

Escoula, L., M. Thomsen, D. Bourdiol, B. Pipy, S. Peuriere and F. Roubinet, 1988. Patulin immunotoxicology: effect of phagocyte activation and the cellular and humoral immune system of mice and rabbits. International Journal of Immunopharmacology, 10, 983–989.

Esterbauer, H., R.J. Schaure, and H.Zollner, 1991. Chemistry and biochemistry of 40 hydroxy-nonenal malonaldehyde and related aldehydes. Free Rad. Biol. Med. 11, 81–128.

Ester, P., V. M. Liliana, G. C. Jesús, and L. Joan-Maria, 2013. Occurrence of patulin in organic and conventional apple juice. Risk assessment. Recent Advances in Pharmaceutical Sciences III, 131-144.

Europeanunion, 2002. Patulin information leaf from Fermented. J. Eur. Commun., 141:12-15.

Frisvad, J.C. and R. A. Samson, 2004. Polyphasic taxonomy of Penicillium subgenus Penicillium. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology 49, 1–174.

Gashlan, H. M., 2008. Biochemical Studies of Patulin on Liver Functions in Male Albino Mice, Journal of Applied Animal Research, 34(1): 93-96.

Hudson, C. do A., C. Elissa, M. B. César, C. T. Eduardo, and M. L. Jane, 2012. Determination of Patulin in Apple Juice from Fuji Apples Stored in Different Conditions in Southern Brazil. Journal of Food Research; Vol. 1, No. 3.

Kamal R. A., R. Dhiman, N. K. Aggarwal, V. Kumar, and M. Kaur, 2014. Microbes Associated with Freshly Prepared Juices of Citrus and Carrots. International Journal of Food Science Volume, Article ID 408085, 7 pages.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 54(5):356-361.

Liebert, J., I. Matławskab, W. Bylka and M. Murias, 2006. Protective effect of Aquilegia vulgaris L. on aflatoxin B1-induced hepatic damage in rats. Environmental Toxicology and Pharmacology 22, 58–63.

Lin, C.C., Y.F. Hsu, T.C. Lin, F.L. Hsu and H.Y. Hsu, 1998. Antioxidant and hepatoprotective activity of punicalagin and punicalin on carbon tetrachloride-induced liver damage in rats. J. Pharm. Pharmacol. 50, 789–794.

Madrigal, S. E., B. E.Madrigal, M. R. Ma´rquez, and A. Reyes, 2006. Antigenotoxic effect of *Saccharomyces cerevisiae* on the damage produced in mice fed with aflatoxin $B_1$ contaminated corn. Food and Chemical Toxicology 44, 2058–2063.

Magan, N. and M. Olsen, 2004. Mycotoxins in food detection and control, Wood head Publishing Ltd, Cambridge, UK. 488pp.

Mangano, N.G., V.M. Cutuli, A. Caruso, E. De Bernardis, M. Amico-Roxas, 2001.Grapefruit juice effects on the bioavailability of cyclosporin-A in rats. European Review for Medical and Pharmacological Sciences; 5: 1-6.

Mézes, M., 2008. Mycotoxins and other contaminants in rabbit feds. Proceedings of the 9th World Rabbit Congress Verona (Italy) - June 10-13, 157-177.

Moss, M. O., 2002.Mycotoxin review-1.*Aspergillus* and *Penicillium*. Mycologist. 16(3):116-119.

Moss, M.O., 2008. Fungi, quality and safety issues in fresh fruits and Vegetables. Review Article, Journal of Applied Microbiology 104, 1239–1243.

Omar, N., 2012.Effect of some aflatoxins on a lymphatic organ (spleen) of male albino rats (Histopathological Study) .The Egyptian Journal of Hospital Medicine .July. Vol., 48: 357– 367.

Patro, M.S.K., 2011. Comparative study of various mycotoxins against few bacterial test organisms. International Journal of Pharmacy and Pharmaceutical Sciences, 3(5): 288 -291.

Saddiq, A. A., 2012. Antifungal and prophylactic activity of pumpkin (*Cucurbita moschata*) extract against *Aspergillus flavus* and aflatoxin $B_1$ African Journal of Microbiology Research Vol. 6(41), pp. 6941-6947, 27.

Saddiq, A. A. and S. A. Kalifa, 2011. Impact of fungal content of some Arabic nuts to induce kidney toxicity and agonistic action of natural resources. African Journal of Microbiology Research Vol. 5(9), pp. 1046-1056, 4 May.

Speijers, G. J. A., 2004. Patulin. In: Mycotoxins in foods: detection and control. Edited by: Magan, N. and Olsen, M. CRC Press. Wood head publishing limited, Cambridge, England. 340, 344.

Torkamani, A. E. and M.Niakousari, 2011. Impact of UV-C light on orange juice quality and shelf life, International Food Research Journal 18(4): 1265-1268.