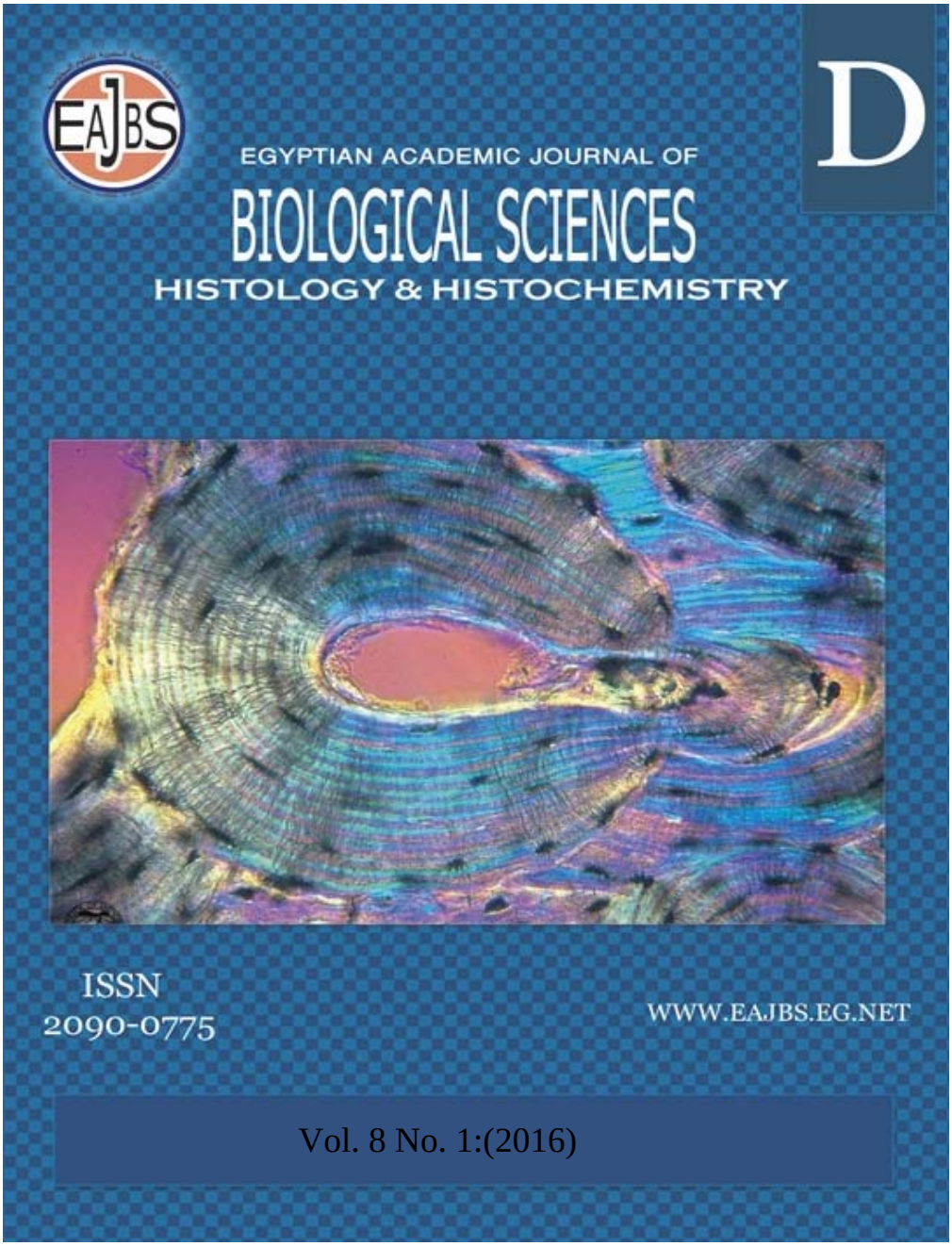

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Protective And Creative Effects of *Panax ginseng* Aqueous Crude Extract in Histopathological Changes of BALB/c Mice Exposed to Aflatoxins

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ABSTRACT

Histopathological changes in liver, kidney and spleen of BALB/c mice were studied. Results indicated that animals treated with crude extract of aflatoxins (9mg /kg b.w) then with *Panax ginseng* aqueous crude extract (150mg / kg b.w.) (Post treatment), and animals treated with ginseng extract (100mg / kg b.w.) then treated with crude extract of aflatoxins (9 mg / kg b.w.) (Pretreatment) by oral gavage appeared the best results as shown in histpathological sections compared with control. Mouse liver treated with(9mg/kg b.w) of crude aflatoxins showed degenerative effect and necrotic cells with inflammatory cells infiltration near the central vein. Pathological changes at the groups treated with *P. ginseng* extracts(150 and 50 mg) then (9 mg/kgb.w) crude aflatoxins showed degenerative effect inside hepatocyte with depletion of glycoprotein granules and apoptotic cells. While treating with (100mg/kg) crude extract was showed look-like normal structure appearance of hepatic tissue. Pathological changes at the groups treated with AFs extract (9mg/kgb.w) then crude extracts of *P. ginseng*(150 mg/kgb.w) showed mild degenerative changes of hepatocyte cells compared with (100mg/kgb.w) dose which showed normal cells. Whereas, (50 mg/kgb.w) showed certain necrosis area of hepatocyte and inflammatory cells infiltration. Pathological changes in mouse kidney treated with (150mg/kgb.w) crude extract of *P. ginseng* then treated with (9mg/kgb.w) AFs extract showing apoptotic and degenerative changes of renal epithelial cells, while pretreatment with (100mg/kgb.w) crude extract showing certain epithelial cells of renal tubule with some apoptotic cells, whereas(50mg/kg) crude extract of plant extract shows normal appearance with mild apoptotic cells and congestion. The post treatment animal's kidney with (150mg/kgb.w) plant extract shows few cells of renal tubules and congestion while post treatment with (100mg/kgb.w)crude extract of *P. ginseng* showing normal structure appearance which consist of glomeruli and renal tubules. While few cells of renal tubules and congestion which appeared in kidney waspost treated with (50mg/kgb.w).

Histopathological examination of mouse spleen treated with(9mg/kgb.w) AFs crude extract showing degenerative and necrosis of splenic parenchymal tissue and shrinkage of white pulp with hemorrhage. Pretreatment animal with (150mg/kgb.w)crude extract of *P. ginseng* showing splenic tissue with hyperplasia of white pulp surrounded by hemorrhage, while pretreatment with(100mg/kg b.w) revealed hyperplasia of white pulp surrounded by reactive fibrosis. Whereas(50mg/kg b.w) crude extract of *P. ginseng* pretreatment showing look-like normal structure appearance of white and red pulp. On the other hand, the post treatment with (150mg/kg b.w) or (100mg/kg b.w) crude extract showing widening of white pulp and reduction in red pulp. Otherwise, the post treatment with(50mg/kg b.w) crude extract of *P. ginseng* showing look-like normal structure with slight widening of white pulp.

INTRODUCTION

Exposure to aflatoxin can lead to several health-related conditions including acute and chronic aflatoxicosis, aflatoxin-related immune suppression, liver cancer, liver cirrhosis, as well as nutrition-related problems in children such as stunted growth in many areas, due to widespread high-level consumption, aflatoxin contamination through food and feed is unavoidable due to the absence of alternative food and feed resources.

When ingested, aflatoxin binds to liver proteins. The metabolic products may persist for 2 to 3 months or longer and can be detected through blood tests (Wagacha and Muthomi, 2008). Aflatoxins can cause disease in vertebrates animal when introduced *via* a natural route, ingested, absorbed through the skin or inhaled (Luttfullah and Hussain, 2011).Ginseng (Panax ginseng C.A. Meyer) is one of the most widely used medical plants, particularly in traditional oriental medicine, and has a wide range of pharmacological and physiological actions (Ko,1998; Slifman, 1998). Previous studies have shown that ginseng enhances immune response (Kim, 1999), antioxidant activity (Abdel-Wahhab and Ahmed, 2004), anti-tumor activity in humans and in laboratory animals (Keum, 2000, 2003, Moreover)it was found that ginseng protects from toxic substances (El-Kady, 2006) and human diseases by several different mechanisms (Yokozawa, 1988). Moreover, recent epidemiological studies have demonstrated that ginseng intake is associated with a reduced risk of environmentally related cancers (Xiaoguang, 1998). However, few studies have examined its preventive or therapeutic potential against the toxic effects of environmental contaminants such as mycotoxins.

MATERIALS AND METHODS

Aspergillus flavus Isolate

Pure *Aspergillus flavus*isolates were obtained from the Department of Biotechnology, College of Science/ University of Baghdad, they were cultured on rice medium.

Aflatoxins production in rice medium, extraction and analysis using High Performance Liquid Chromatography (HPLC)

Selective isolates of *A. flavus* (were cultured initially on PDA media) were grown in 250ml Erlenmeyer flask (80 flasks) containing 25g of sterile rice medium Then a discs of 5 mm diameter of fungi culture was punctured in the rice media to prepare the inoculums for *A. flavus*. Then the flasks were incubated for 21 days at 28°C (Odette *et al*., 1966).

After incubation time, the moldy rice was soaked overnight with 75 ml of chloroform in dark place. Then the soaked medium was homogenized with electric homogenizer for 15 min. The extracted solution was filtered through gauze then was filtered through a What man No.1 filter paper. The residue was washed with 50 ml of chloroform, and then was filtered. Chloroform fractions were pooled and evaporated to dryness at 50°C. Dried extracts stored at 4°C until use (Kosalec *et al*., 2005).

Detection and Quantification of aflatoxin

It was prepared by diluting in absolute methanol, 1mg /ml for B2 and G2, 10mg/ml for B1and G1. Then 100 µl aliquot of each aflatoxin solution was combined in a 2-ml vial and mixed well. This mixture was further diluted in series to 100,000 folds in water: methanol (7:3v/v) and stored at -70 ºC in deep freeze until using (Gupta *et al*., 2012).

Laboratory Animals and Experimental Design

Seventy eight of Swiss albino mice (male) were purchased from the National Centre for Drug Control and Research /Baghdad were used, their ages were ranged (8-12) weeks and weighting (25-30)g. The mice were acclimatized for two weeks before treatment. They housed in plastic cages containing hard wood chips for bedding, in controlled animal house at 25± 2Cº, 4/10 hour's light / dark cycle. The animals were given water and fed with suitable quantity of complete diet

The animals were divided into twelve groups and were orally administered, as shown below:

Control groups (consisted of six groups) each group contained (5) mice.

Group 1: Animals without any treatments.

The flowing groups were treated by oral gavage with:

Group 2: Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks).

Group 3: Crude extract of ginseng (150mg / kg b.w.) for ten days.

Group 4: Crude extract of ginseng (100mg / kg b.w.) for ten days.

Group 5: Crude extract of ginseng (50mg / kg b.w.) for ten days.

Group 6: Methanol 10% two times in a week (for two weeks).

The pre and post treatment groups either with crude extract of ginseng or crude extract of aflatoxins were divided into six groups, each group contained (8) mice («**1**»refers to pretreated groups (which treated with ginseng aqueous crude extract for ten days then were treated with crude extract of aflatoxins (9mg/kg b.w) twice a week for two weeks). Whereas «**2**»refers to post treated groups (which treated with crude extract of aflatoxins twice a week for two weeks then they were treated with crude ginseng extract for twenty one days).The letters a ,b and c refer to concentration of *P. ginseng* extract (150 ,100 and 50mg/kg b.w) respectively.

Group 1-a: Animals were pretreated with (150mg / kg b.w) plant aqueous crude extract.

Group 1-b: Animals were pretreated with (100mg / kg b.w) plant aqueous crude extract.

Group 1-c: Animals were pretreated with (50mg / kg b.w) plant aqueous crude extract.

Group 2-a: Animals were post-treatedwith (150mg / kg b.w) plant aqueous crude extract.

Group 2-b: Animals were post-treated with (100mg / kg b.w) plant aqueous crude extract.

Group 2-c: Animals were post-treated with (50mg / kg b.w) plant aqueous crude extract.

Tissue processing and sectioning

Tissue processing, sectioning and staining were carried out according to Luna (1968). At the end of each experiment, and after taking blood samples the mice were abdominal sacrificed. Samples from liver, kidney and spleen were dissected out, and washed in saline solution for removal blood. Then the organs were fixed in plastic containers containing 10ml of formalin 10 % for 24 hours. After that the organs samples were dehydrated for one to two hours in each ascending concentration of alcohol (70%, 90% and 100% v/v). The dehydrated tissues were cleared in xylene for one to two hours. Then the samples were submerged in melting paraffin wax for one to two hours, left to cool and then embedded in paraffin wax and cut by a microtome into section of 4-5 µm thickness. The sections were floated out in a warm water bath to lay on slides and dried on oven 50-55 º C and stained with haematoxylin and eosin (H & E).

RESULTS AND DISCUSSION

Histopathological effects inmouse liver

Liver sections from control group expressed normal structure appearance of hepatocytes arranged as a thread around central vein as in figure 1-a, while the section in figure 1-b showed mild degenerative change of hepatocyte cells of 10% methanol treated group whereas other hepatocytes were normal. Whilst figure 1-c appeared histological examination of AFs (9 mg/kg b.w) treated group which showed degenerative effect and necrosis of hepatocytes with inflammatory cells infiltration near the central vein.

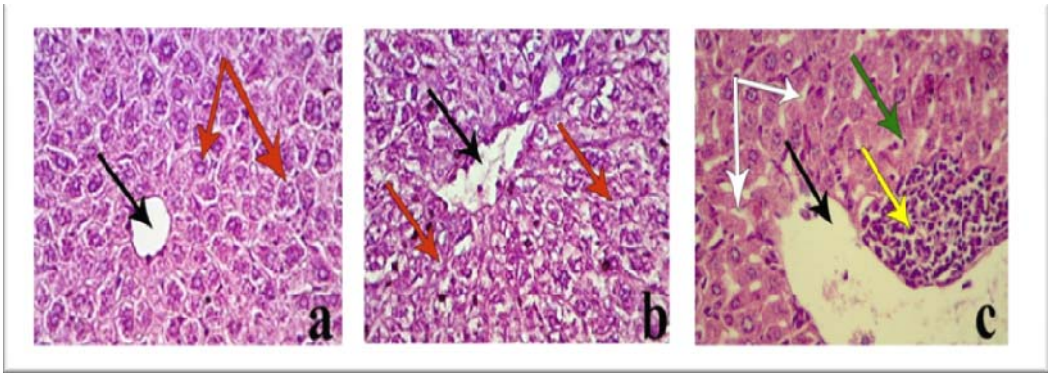

Fig. 1: Histological sections of albino mouse liver -H&E stain-(● Hepatocytes; ● Central vein).a. Control mouse shows normal tissue (X20).b. Mouse treated with methanol 10% shows no clear lesions (X20). c. Mouse treated with crude aflatoxins (9 mg/kg b.w) shows: (○ Inflammatary cells, ● Necrotic cells, ○ Degenerative cells) (X40).

The pretreated animal with (150mg/kg b.w) for ten days, showed degenerative effect inside hepatocyte cells with depletion of glycoprotein and apoptotic cells. Whereas histopathological section of mouse liver pretreated with (100mg/kg b.w) shows look-like normal appearance of hepatic tissue. While mouse liver pretreated with (50mg/kg b.w) appears degenerative effect with depletion of glycoprotein granules and apoptotic cells (Figure 2-a,b,c).Furthermore, the liver of post-treated animals with (150 mg/kg) for twenty one days showed mild degenerative changes of hepatocyte cells, while other cells were normal as in Figure 2-d. Whilst the section of (100mg/kg b.w) treated animal showed dispersed area of necrosis with inflammatory cells infiltration as exhibited in Figure 2-e. However Figure 2-f shows histopathological section of liver wich treated with (50mg/kg b.w) was manifested certain area necrosis of hepatocyte cells, degenerative cells and inflammatory cells infiltration.

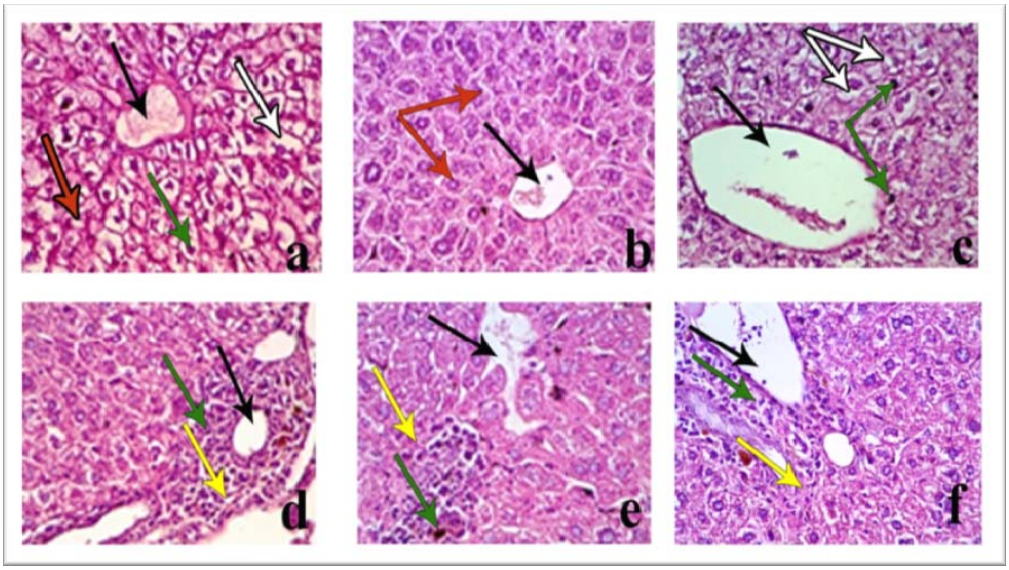

Fig. 2: Histological sections of pretreated (a,b,c) and post-treated(d,e,f) albino mice liver show: (● Hepatocytes, ● Central vein) - H&E stain-a. (150mg/kg b.w) shows: ○ Degenerative cells, ● Necrotic cells (X40).b.(100mg/kg b.w) shows: Normal threads of hepatocyte (X40).c. (50mg/kg b.w) shows: ○ Degenerative cells, ● Apoptotic cells (X40).d. (150mg/kg b.w) shows: ○ Inflammatary cells, ● Apoptotic cells (X40).e. (100mg/kg b.w) shows: ○ Inflammatary cells, ● Necrotic cells (X40).f. (50mg/kg b.w) shows: ○ Inflammatary cells, ● Necrotic cell.

Histopathological examination revealed that administration with aflatoxins induces degenerative changes in the liver characterized by periportal damage, hepatic cords degeneration, and increased incidence of vacuolar degeneration and few dilated sinusoids.

Devendran and Balasubramanian, (2011) showed that many histological changes of liver include degenerative reversible lesion, mild parenchymatous degeneration characterized by granular appearance of hepatocytes cytoplasm, severe hydrophilic and vacuolar degeneration.

The vast majority of hepatocytes had significant cytoplasmic visualization with disseminated necrotic cells on rats treated with 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm aflatoxin B1 for 8 days.

In the present study, mice treated with ginseng extract before orafter the mycotoxins treatment showed various degrees of protectionagainst the histological changes and fibrosis in the liver. Similarto the current histological results, Wu *et al.*, (2001) proved thatthe red ginseng had an anticarcinogenic effect on the developmentof liver cancer induced by diethyl nitrosamine (DEN) in rats.

Histopathological effects in kidney

Histological examination of control mouse kidney expressed normal structure of renal tissue which consists of glomeruli and renal tubules (proximal and distal convoluted tubules)as in Figure 3-a.Whereas in Figure 3-b, the section revealed no lesions or other pathological abnormalities in animal administered with 10% methanol. Whilst Figure 3-c appeared histological examination of AFs (9 mg/kg b.w) treated group which showed appearance of apoptotic cells, degenerative cells and shrinkage of glomeruli.

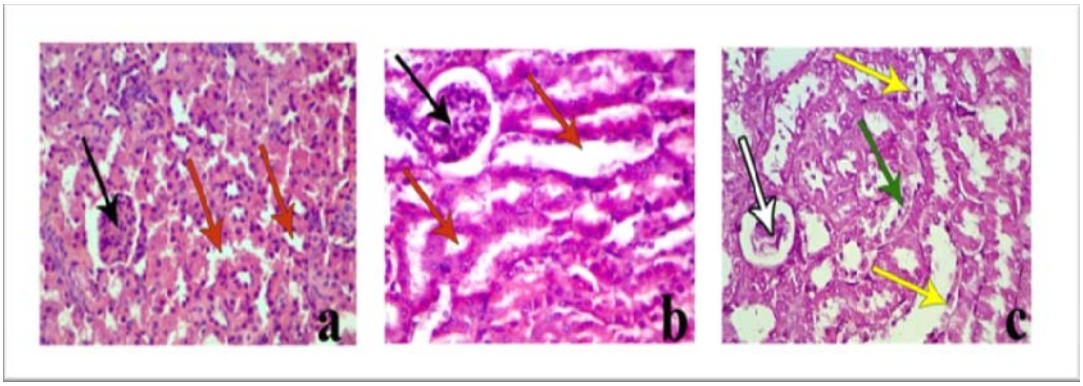

Fig. 3: Histological sections of albino mouse kidney -H&E stain- show (Renal Tubules, Glomeruli.)a. Control mouse shows normal tissue (X20).b. Mouse treated with methanol 10% shows no clear lesions (X20).c. Mouse treated with crude aflatoxins (9 mg/kg b.w) shows (Apoptotic cells, Degenerative cells, Shrinkage Glomerulus) (X40).

The pretreated animal with ginseng extract (150mg/kg b.w) showed apoptotic and degenerative changes of renal epithelial cells as in Figure 4-a. Whereas figure 4-b appears histopathological section of mouse kidney pretreated with (100mg/kg b.w) showed certain epithelial cells of renal tubule with mild apoptotic cells. While Figure 4-c shows histopathological section of animal kidney pretreated with (50mg/kg b.w) showing look-like normal appearance with some apoptotic cells and congestion.

The post-treated mice with (150 mg/kg b.w) ginseng extract showed normal kidney architecture with mild

congestion as in Figure 4-d. Whilst the section of (100mg/kg b.w) treated animal look-like normal kidney parenchymal which consists of glomeruli and renal tubules with congestion as exhibited in Figure 4-e. However Figure 4-f demonstrates the histopathological section of kidney post-treated with (50mg/kg b.w) manifesting normal cells of renal tubules with moderate congestion. Different parts of the kidney were exposed to aflatoxins especially the aflatoxin B1 and its metabolites leading to nephrotoxicity before it is excreted in the urine (Coulombe, 1994; Sharma, 2011). The kidney is susceptible to many toxic agents due to the high amount of blood it receives and about 20-25% of blood that flows in at rest coupled with the large amounts of circulating toxicants that reach the kidneys (Harriet, 2003).

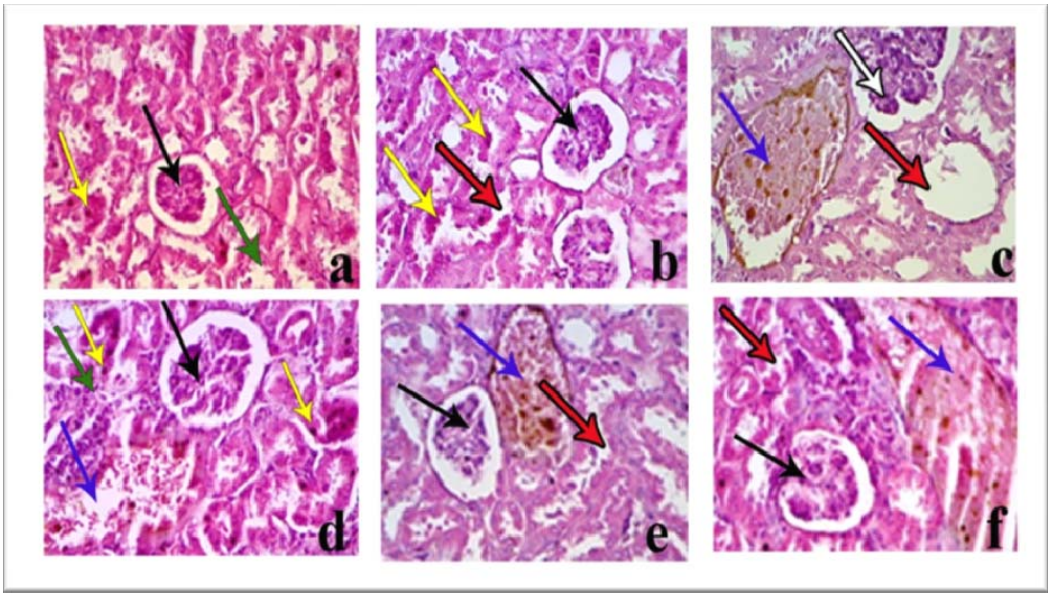

Fig. 4: Histological sections of pretreated (a,b,c) and post-treated (d,e,f) albino mice kidney (● Glomeruli, ● Renal tubules) -H&E stain-a. (150mg/kg b.w) shows normal tissue (X40).b. (100mg/kg b.w) shows: ○ Apoptotic cells (X40).c. (50mg/kg b.w) shows: ○ Apoptotic cells , ● Congestion (X40).d. (150mg/kg b.w) (X40),e. (100mg/kg b.w) (X40).f. (50mg/kg b.w) (X40) show normal tissue with mild congestion ●.

Also they have high oxygen and nutrient requirements because of their workload and therefore filters one-third of the blood reaching them and re-absorb 98-99% of the salt and water. Results obtained by Fareed and Inaam (2006) showed that histological changes of kidney in female albino rats treated with 80µg/kg aflatoxin include shrinkage of the cells condensation and fragmentation of nucleus, hypereosinophilia of cytoplasm, while Saddiq and Kalifa, (2011) showed that treated of female rats with aflatoxin B1 (2 mg/kg b.w) cause a histological changes of kidney including degeneration of the cells forming of proximal and distal convoluted tubules. Helal *et al*., (2012) showed many histological changes of rabbits' kidney treated with aflatoxins including, moderate congestion of glomerular tuft and renal blood vessels and in some cases showed perivascular cuffing represented by aggregation of lymphocytes around the renal blood vessels.

The results of present study appeared many inflammatory cells, degenerative cells, apoptotic cells, shrinkage of glomeruli and congested

blood vessels were appeared in kidney sections treated with aflatoxins (9 mg /kgb.w). These findings were also detected by previous researchers who proved that orally administration of aflatoxins to rats induced damage in glomeruli andtubules (Rastogi *et al*., 2001). Lee *et al*., (2002) stated that ginseng might reduce cell damage induced by toxic substances and stabilize cell membranes by providing protection against toxic agents induced tissue injury. On the other hand, Kim *et al*., (2010) mentioned that ginseng has a potent antiapoptotic effect as it increases the expression of the antiapoptotic gene Bcl-2.Other researchers were reported that the protective effect of ginseng was due to its antioxidant property (Zhu *et al*., 2001). Zhang *et al*., (2010) and Sen *et al*., (2012) reported that ginseng increases the intracellular concentration of glutathione and superoxide anions, which they inactivate nitric oxide, and increases antioxidant enzymes. Whereas Hassan and Abdel-Wahhab (2006) stated that ginsenoside has been known to protect kidney from apoptosis and DNA fragmentation caused by chemical and cancer drugs.

Histopathological effect in spleen

Histological examination of the control spleen of mice revealed normal structure of splenic parenchymal tissue, consisting of lymphoid tissue, white and red pulp as showed in figure 5-a.While histological sections in spleen treated with 10% methanol showed no lesions or other histopathological changes of organic tissue (Figure 5-b). Whereas Figure 5-c appeared histological examination of AFs (9 mg/kg b.w) treated group showing degeneration and necrosis of splenic parenchymal tissue and shrinkage of white pulp with hemorrhage. Other section showed loss of spleen tissue cellularity.

The pretreated animal with (150mg/kg b.w) ginseng extract showing hyperplasia of white pulp surrounded by hemorrhage, that was clear in figure 6-a. Whereas histopathological section of mouse spleen pretreated with (100mg/kg b.w) was revealed hyperplasia of white pulp surrounded by reactive fibrosis (collagen fibers) as showing in figure 6-b. While in figure 6-c showing histopathological section of mouse spleen pretreated with (50mg/kg b.w) showing look-like normal structure appearance which consists of white and red pulp.

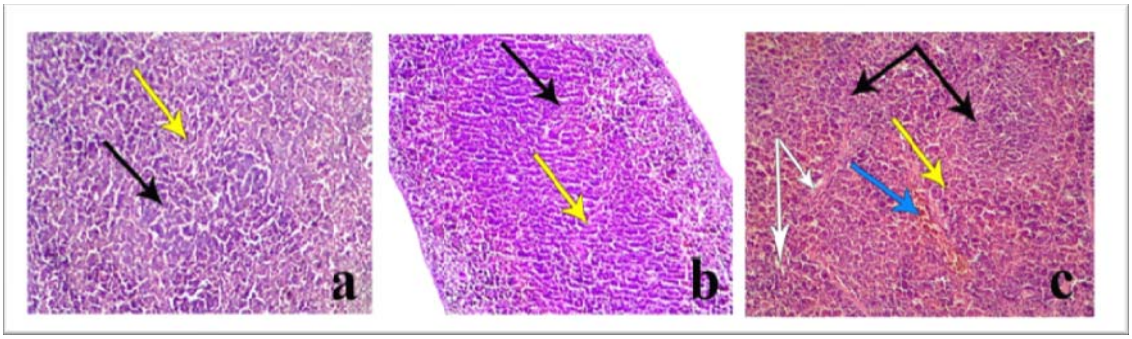

Fig. 5: Histological sections of albino mouse spleen -H&E stain- shows (● white pulp; ○ red pulp)a. Control mouce shows normal tissue (X40).b. Treated with methanol 10% shows no clear lesions (X20). c. Ttreated with crude aflatoxins (9 mg/kg b.w) shows: (● Haemorrage; ○ Necrtic Cell) (X40).

Histopathological section of the mouse spleen post-treated with (150 mg/kg b.w and 100mg/kg b.w) ginseng extract showing widening of white pulp and reduction in red pulp as clear in Figure 6-d and e. However the section of the mouse spleen post-treated with (50mg/kg b.w) revealed look-like normal

structure with slight widening of white pulp (Figure 6-f).

Wei *et al.*,2013 reported that the spleen is a principal peripheral lymphoid organ and plays an important role in protective immune reactions. It is involved in humoral and cellular immune responses through its role in the generation, maturation and storage of lymphocytes (Sandford *et al.*, 2011).

Also, it is a center of activity of the reticuloendothelial system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections (Brender *et al.*, 2005). Wang *et al.*, (2013) reported that an increased apoptotic splenocytes was observed in AFB1 treated animals, which revealed one mechanism of AFB1-induced immunosuppression.

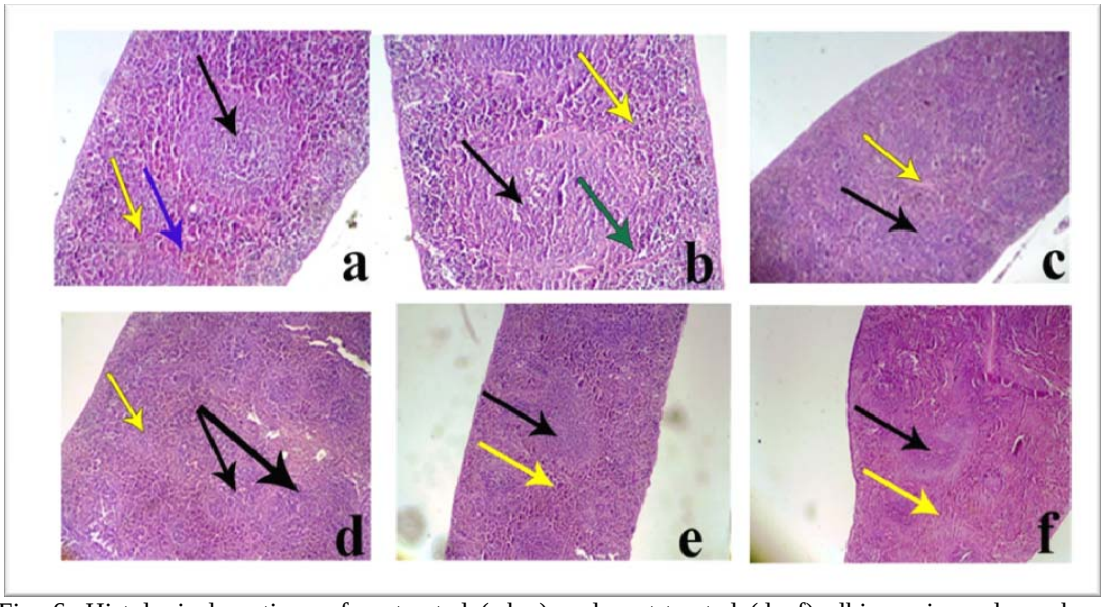

Fig. 6 :Histological sections of pretreated (a,b,c) and post-treated (d,e,f) albino mice spleen show (●White pulp, ○Red pulp) -H&E stain-a. (150mg/kg b.w) shows: ● Haemorrage (X40). b. (100mg/kg b.w) shows: ● Fibrosis (X40) c. (50mg/kg b.w). d. (150mg/kg b.w)e. (100mg/kg b.w) f. (50mg/kg b.w) (X20), ● normal structure.

Furthermore, previous studies have clarified that oxidative stress would induce mitochondrial dysfunction, nuclear translocation, DNA binding, and transcriptional activity of p53, and then activate the course of cell-cycle arrest and cell apoptosis (Kume *et al.*, 2006; Rosa et *al.*, 2011). The results of present study is in accompanied with Omar, (2012) how showed that feeding of albino rats on diet containing aflatoxins at concentration of 0.1ppm for 2,4,6 weeks, lead to histological changes of spleen including lymphocytic degeneration, fatty changes with numerous hemorrhagic areas. Also agreed with Al-Azawee, (2006) showed that mice treated with 2ppb aflatoxin B1daily for 28 weeks have a histological changes of spleen including diffusion of cancer cells in the red pulp with accumulation of mononuclear cell, also free hemosiderin accumulation in the red pulp, also there is a lymphoid hyperplasia which look like nodules elucidation for accumulation of hemosiderin was that sinusoid and pulp filled with blood with marked hyperplasia of endothelium of sinusoids.

The present results were compatible with Sathyanath *et al*., (2013) were reported that pretreatment with *P. ginseng* attenuated, but did not prevent the severity of histopathological changes, and loss of spleen tissue cellularity, loss of integrity between cell to cell

adhesions. The severity of these histopathology changes were not attenuated by *P. ginseng* post-treatment.

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