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MODULATION OF AFLATOXINS TOXICITY IN RATS BY ETHANOLIC POMEGRANATE PEEL (*Punica granatum.L*) EXTRACT

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ABSTRACT

This work was conducted to investigate the effect of ethanolic pomegranate peels (PP) extract on liver and kidney structure and function in rats experimentally exposed to different doses of aflatoxins (AFS). An experiment was conducted for 6 weeks, 36 male rats were divided into 6 groups (6 rats in each). G_1 served as vehicle treated control, G_2 received PP extract via gavage (50 mg/kg body weight (b.w.) daily). G_3 received AFS via gavage (1 mg/kg b.w.) twice/week. G_4 received AFS via gavage (2 mg/kg b.w.) twice/week. G_5 received AFS twice/week (1 mg/kg b.w.) + (PP) extract daily (50 mg/kg b.w.). G_6 received AFS twice/week (2 mg/kg b.w.) + (PP) extract daily (50 mg/kg b.w.). Liver enzymes (Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP)), Total Protein (TP), albumin and renal biochemical parameters (creatinine, urea and uric acid) were estimated in serum at 3rd and 6th week post treatment , while pathological examination of liver and kidney was performed at the end of experiment. Result demonstrated that both aflatoxins administrated levels caused elevation in AST, ALT, ALP, creatinine, urea and uric acid and a decrease in TP and albumin in dose and time dependant manner, while synergistic administration of PP extract and AFS resulted in limitation of the negative effects of AFS on the tested parameters especially with low AFS dose and longer time. Pathological examination revealed that the severity of damage in liver and kidney correlated with aflatoxins' doses. While PP extract administration was able to protect liver and kidney against low AFS dose and minimize the damage of liver and kidney induced by high AFS dose.

Keywords: Aflatoxin, Pomegranate, Pathological examination, Liver enzymes and renal biochemical parameters

INTRODUCTION

Aflatoxins (B_1, B_2, G_1 and G_2) are a group of toxic compounds produced by toxigenic strains of *Aspergillus flavus* and *Aspergillus para*siticus. These fungi can grow on a wide variety of food commodities (Wilson and Payne, 1993). Humans are exposed to AFS directly by the consumption of contaminated food or indirectly by the consumption of animal products (i.e., fish, meat, milk and eggs) derived from animals that consumed AFS contaminated feed *(*Abdelhamid *et al.,* 1994 and Bennett and Klich, 2003). AFS are well known as hepatotoxic, hepatocarcinogenic and mutagenic agents. These effects are mainly due to adduct formation with DNA, RNA and protein. In addition, it also causes lipid peroxidation as well as oxidative damage to DNA. AFB_1 possess genotoxic potential in a variety of test systems. Other aflatoxin has not been so extensively investigated, but in

a variety of studies B_2, G_1, G_2 and M_1 have all shown evidence of genotoxicity (Verma, 2004). The AFS present in food and feed are hazardous to both human and animal health (Do and Choi, 2007).Therefore, development and application of intervention strategies that are highly effective and economically feasible against dietary AFB1 contamination and aflatoxicosis are critical for improving human and animal health, especially in high-risk areas.

AFS toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) during the metabolic processing of AFB_1 by cytochrome P450 in the liver (Preetha *et al*., 2006).

Ueno et al. (1991) indicated that active oxygen scavengers reduce mutation induced by various mutagens. It has been suggested that compounds which possess antioxidants activity can inhibit mutation and cancer because they can scavenge a free radicals or induce antioxidants enzymes (Hochstein and Atallah, 1988). Dietary intake of natural antioxidants could be an important factor in body's defense mechanism against many mutagens and carcinogens, also many antioxidants are being identified as anticarcinogens. Many plant polyphenols, have been shown to act as potent antimutagenic and anticarcinogenic agents (Tanaka *et al.,* 1993 and Yen and Chen, 1994).

The pomegranate, *Punica granatum* L., is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity (Julie, 2008). Negi *et al.* (2003) showed that the pomegranate peel (PP) extracts have both antioxidant and antimutagenic properties and may be exploited as biopreservatives in food applications and neutraceuticals. PP extract contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid. It has been used in preparation of tinctures, cosmetics, therapeutic formulae and food recipes (Nasr *et al*., 1996). Guo *et al.* (2003) found that PP had highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits commonly consumed in China. Moreover, Li *et al. (*2006) concluded that PP extract appears to have more potential as health supplement rich in natural antioxidants than the pomegranate pulp extract.

In this study, we investigated the modulation of ethanolic PP extract on toxic effects of AFS in male Albino rats.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade. Chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) and Solvents were purchased from Merck (Darmstadt, Germany).

Microorganism

Aspergillus flavus NRRL (3145) was obtained from the National Research Center (Dokki, Giza).

Standard Aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 were obtained from Sigma Chemical Company (St. Louis, Mo USA).The purity of these materials were checked by thin layer chromatography [silica gel aluminium sheets 20x20 cm purchased from Merck (Darmstadt, Germany) using a mixture of diethyl ether: methanol: distilled water (96: 3: 1, v/v/v) as a running solution] and each gave one spot.

Aflatoxin production and assays

Preparation of media for *Aspergillus flavus* growth

a) Potato-Dextrose-Agar Media (PDA): This medium was prepared according to ***Shotwell et al.*** (***1966***) as follows:

Flask 1 contained**:** Dextrose (20g), $CaCO_3$ (0.2g), $MgSO_4.7\ H_2O$ (0.2g) and distilled water (100 ml)

Flask 2 contained**:** Agar (15g) and distilled water (400ml)

Flask 3 contained**:** Potato (Spilled and sliced) (200g) and distilled water (500ml)

The contents of flask 3 were brought shortly to 121 ºC in autoclave and filtered through cheesecloth. The solution was brought up to original volume simultaneously. The agar in flask 2 was melted and the solution in flask 1 was heated to boiling. Contents of the three flasks were mixed together and autoclaved for 15 min. at 121 ºC.

b) Yeast Extract Sucrose Media (YES): This medium yields high amount of aflatoxin especially B_1 and G_1 with *A. flavus.* It was prepared according to method of *Davis et al. (1966)* as follows: Yeast extracts (20g), Sucrose (200g), $FeSO_4.7H_2O$ (10mg), ZnSO4.7H2O (5mg), $MnSO_4.4H_2O$ (1mg) and distilled water (1000ml) were dissolved, mixed and autoclaved for 15 min at 121ºC.

Fungal growth

Inoculum was prepared by incubating PDA slant tubes (1.5x15 cm) inoculated with A. flavus spores for 7 to 21 days at 28 ºC. Spores of 10-day old (A. flavus culture) were scrapped loose with a loop after adding 3 ml sterile distilled water to each slant (Shotwell *et al.*, 1966). The Spores scraped by adding sterile distilled water to the surface growth on agar slant and an aliquot amount from the resulting spore suspension (1 ml) was added to conical flasks (2 liters) containing yeast extract media. Mycelial mats after 10-day incubation at 30ºC were broken with a glass rod and collected by filtration through filter paper. Culture filtrates were extracted with chloroform (1:2, v/v). (Abd El-Mageed, 1987). The chloroform extract was evaporated

until obtaining dry film in rotary evaporator. Then the obtained dry film containing AFS was reconstituted with dimethyl sulfoxide (DMSO) and used for quantitative analysis of AFS and then preparation of AFS doses.

Determination of aflatoxins concentration in solution

The concentration of AFS in DMSO solution was determined using HPLC technique (Agilent 1100 Series U.S.A with column C_{18}, Lichrospher 100 RP-18, 5µm×25cm) as follows: The mobile phase consisted of water: methanol: acetonitrile (54:29:17, v/v/v) at a flow rat of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460nm (Flourcenses detector), respectively (Roos *et al.*, 1997).

Preparation of aflatoxins dose

The DMSO containing AFS solution consisted of a mixture of aflatoxin B_1, B_2, G_1 and G_2 at a total concentration of 34mg AFS/ ml as a ratio of 8: 2: 4: 1, respectively used to prepare 2 final concentrations of AFS, 1mg AFS/5ml DMSO and 2mg AFS/5ml DMSO.

Pomegranate peels preparation and extraction.

Peel was obtained from juice stores. It was air dried and milled using laboratory mill to pass a 1.0 mm-size, and then dried again in a cabinet oven with air circulation at 60°C for 16h. Then kept in refrigerator prior to extraction.

PP was extracted according to the method of Panovska *et al.* (2005). Ten g of dry powder were extracted with 100 ml of 70% ethanol in a screw-capped flask and shaken at room temperature for 24 h. The extracts were centrifuged at 5000 *rpm* for 10 min while the residue was re-extracted under the same conditions twice and filtered through a Büchner funnel with filter paper (Whatman No.1). The 70% ethanol extracts were concentrated under reduced pressure, lyophilized to obtain powders, and stored at 4 °C.

Animal and treatment

Thirty-six male Albino rats weighing 110±5g (provided by the Laboratory Animal Center, Faculty of Veterinary Medicine, Cairo University) were housed in stainless steel cages in animal house in Regional Center for Foods and Feeds, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt under controlled light and temperature conditions 12/12 hours light: dark cycle, 22-33 ^{O}C). During the acclimation period (1 week) and experimental period (6 weeks), a basal diet and tap water were supplied ad libitum. The basal diet consisted of casein 20%, corn oil 10%, wooden fibers 5%, salt mixture 4%, vitamin mixture 1% and starch 60% (Lane – Peter and Pearson, 1971). Rats were divided into six groups(G) and treated for 6 week as follow: G_1: served as vehicles treated control (received 5ml DMSO/ kg b.w. daily + 5ml DMSO/ kg b.w. twice/ week on Monday and Thursday, orally by gavage); G_2: received orally 50mg PP extract powder/ kg b.w. suspended in 5ml DMSO daily + 5ml DMSO/ kg b.w. twice/week on Monday and Thursday; G_3: received orally low dose of AFS (1mg AFS suspended in 5ml DMSO/ kg b.w.) twice/ week on Monday and Thursday + 5ml DMSO/ kg b.w. daily; G_4: received orally high dose of AFS (2mg AFS suspended in 5ml DMSO/ kg b.w.) twice/ week on Monday and Thursday + 5ml DMSO/ kg b.w. daily; G_5: received orally low dose of AFS (1mg AFS suspended in 5ml DMSO/ kg b.w.) twice/ week on Monday and Thursday + 50mg PP extract powder

suspended in 5ml DMSO/ kg b.w. daily; and G_6: received orally high dose of AFS (2mg AFS suspended in 5ml DMSO/ kg b.w.) twice/ week on Monday and Thursday + 50mg PP extract powder suspended in 5ml DMSO/ kg b.w. daily.

At the 3rd week and 6th week of the treatment period, blood samples were collected from the retro-orbital venous plexus under light CO_2 anesthesia and the sera were separated and stored at−20 °C until analysis. Rats were scarified at the end of experiment and liver and kidney were removed for histological examination.

Biochemical assays

The Alera Clinical Chemistry (ACE) System Automatic Analyzer (Alfa Wasserman Corporation) was employed to measure the following parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, creatinine, urea and uric acid.

Histopathological techniques

Samples for histopathological examination were fixed in 10% neutralized formalin for 2 days, washed in tap water, dehydrated in ascending grades of ethyl alcohol and finally cleared with xylene and embedded in paraffin wax. The paraffin blocks were five – micron cutted and stained by Haematoxylin and Eosin as described by Pearse (1968).

Statistical analysis

The data obtained in the present work represented in tables as mean ± standard error. Statistical analysis for the collected data was done according to the procedure outlined by Gomez and Gomez (1984). The treatment means were compared using the least significant difference test (LSD) at the 5% level of probability as outlined by Waller and Duncan (1969) using the Duncan test institute program.

RESULTS

Biochemical parameters

Liver function

The *in vivo* study revealed that rats administrated AFS orally (G_3 and G_4) significantly increased the serum activity of ALT, AST and ALP but decreased the TP and albumin levels in dose and time dependant manner when compared with control (P<0.05) (Table 1). While no significant differences were found in their levels in serum of rats administrated with PP extract (G_2) comparing with control one (P<0.05) in both experimental times. Simultaneous administration of rats with PP extract and different doses of AFS (G_5 and G_6) resulted in reduction of elevated levels of ALT, AST and ALP, elevation of depleted level of TP and restore level of albumin to normal (comparing to groups treated by AFS alone and control group). The ameliorating effect of PP extract on liver function was time dependent and more obvious on low AFS dose.

Table (1): Activity of ALT, AST and ALP and concentration of TP and albumin in serum of rats during experimental periods (means ± SE).

Parameters / Groups	ALT (U/L)		AST (U/L)		ALP (U/L)		TP (mg/dl)		Albumin (mg/dl)	
	3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks
G_1	52.3± 0.89^{g}	60.3± 3.38^{fg}	87.0± 4.36^{h}	107.3± 2.19^{fgh}	349.3± 19.67^{h}	524.30± 15.07^{g}	7.84± 0.05^{ab}	8.09± 0.09^{a}	3.85± 0.07^{ab}	3.98± 0.12^{a}
G_2	54.0± 2.08^{g}	59.0± 2.64^{fg}	90.67± 3.18^{gh}	112.3± 5.04^{fg}	368.0± 9.45^{h}	534.3± 17.14^{g}	7.93± 0.17^{ab}	8.15± 0.25^{a}	3.85± 0.06^{ab}	3.90± 0.10^{ab}
G_3	126.3± 10.36^{c}	140.67± 10.27^{c}	189.67± 5.78^{c}	236.3± 16.3^{b}	668.0± 11.01^{d}	847.30± 24.34^{b}	6.98± 0.24^{de}	6.74± 0.29^{e}	3.63± 0.13^{b}	3.62± 0.06^{b}
G_4	164.67± 12.34^{b}	189.67± 5.78^{a}	246.0± 8.62^{b}	306.0± 12.2^{a}	785.3± 21.18^{c}	1020± 30.61^{a}	6.84± 0.06^{e}	6.62± 0.25^{e}	3.30± 0.17^{c}	3.27± 0.10^{c}
G_5	84.0± 3.79$^{d e}$	77.0± 1.52^{ef}	160.0± 2.65^{d}	121.67± 4.63^{ef}	587.0± 5.86^{ef}	544.3± 23.88^{fg}	7.47± 0.17^{bcd}	7.67± 0.17^{abc}	3.75± 0.04^{ab}	3.80± 0.05^{ab}
G_6	98.0± 5.55^{d}	86.3± 1.76^{de}	198.67± 6.44^{c}	142.3± 6.23^{de}	631.3± 3.71^{de}	591.0± 15.17^{ef}	7.20± 0.20^{cde}	7.55± 0.14^{a-d}	3.73± 0.16^{ab}	3.68± 0.06^{ab}
$LSD_{0.05}$	18.353		22.17		51.45		0.55		0.3	

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at *P* <0.05

Kidney function

The current results presented in Table 2 clearly indicated that rats treated by different doses of AFS showed significant increase in serum levels of creatinine, urea and uric acid comparing to that of control group ($P<0.05$). The increase of their levels was parallel to dose of AFS and time of administration. While there were no changes in their levels in serum of group treated by PP extract comparing to control group ($P<0.05$). The co-administration of PP extract and AFS in G_5 and G_6 rats resulted in reduction in increased levels of creatinine, urea and uric acid in serum in both times of treatment (comparing to groups treated by AFS alone and control group).

Table (2): Cretinine, urea and uric acid concentration in serum of rats during experimental periods (means ± SE).

Parameters / Groups	Creatinine(mg/dl)		Urea(mg/dl)		Uric acid(mg/dl)	
	3weeks	6weeks	3weeks	6weeks	3weeks	6weeks
G_1	0.42± 0.01^{g}	0.47± 0.01^{g}	40± 1.52^{g}	51.3± 1.20^{ef}	1.26± 0.04^{f}	1.36± 0.06^{ef}
G_2	0.45± 0.01^{g}	0.49± 0.03^{fg}	42.3± 1.76^{g}	52.3± 0.33^{ef}	1.28± 0.04^{f}	1.32± 0.07^{ef}
G_3	0.81± 0.02^{d}	0.99± 0.04^{c}	66.0± 2.30^{c}	80.0± 2.08^{b}	2.19± 0.12^{c}	2.80± 0.21^{b}
G_4	1.14± 0.10^{b}	1.33± 0.11^{a}	84.0± 2.08^{b}	99.0± 2.51^{a}	3.02± 0.08^{b}	4.16± 0.03^{a}
G_5	0.63± 0.01^{ef}	0.54± 0.01^{efg}	56.0± 2.08^{de}	49.0± 2.08^{f}	1.71± 0.13^{d}	1.63± 0.09^{de}
G_6	0.82± 0.03^{d}	0.65± 0.02^{e}	65.3± 2.85^{c}	59.3± 1.45^{c}	1.85± 0.03^{d}	1.74± 0.13^{d}
$LSD_{0.05}$	0.143		5.73		0.299	

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at *P* <0.05

The major reduction in their levels was noticed at 6 week of administration and with low AFS dose. No significant differences were noticed in ability of PP extract to reduce toxic effect of high dose of AFS on urea and uric acid between 3rd and 6th week of experiment.

Histopathological examination

Liver

Microscopical examination of rat´s liver from G_1 (vehicle treated control) revealed the normal marked histological structure of hepatic lobule (Fig.1). Also no histological changes were noticed in liver of rat from G_2 which treated with PP extract (Fig. 2).

Conversely, the examined sections from G_3 which treated by low AFS dose showed vacuolar degeneration of hepatocytes (Fig. 3), kupffer cells activation, individual cell necrosis of sporadic hepatocytes (Fig. 4) and focal hepatic necrosis (Fig. 5). Moreover, liver of rat of G_4 which treated with high AFS dose showed dramatic changes. Liver showed karyomegaly of some nuclei, apoptosis of hepatocytes (Fig. 6), portal infiltration with leucocytes (Fig. 7), cytokaryomegaly of hepatocytes, oval cells proliferation (Fig. 8) and mitotic figures (Fig. 9).

Non significant marked histopathological changes were noticed in liver of rat of G_5 (Fig. 10) which treated with low AFS dose and PP extract. Meanwhile, liver of G_6 (treated by high AFS dose and PP extract) showed kupffer cell proliferation and necrosis of sporadic hepatocytes (Fig. 11).

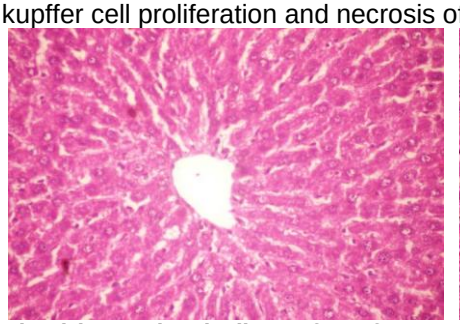

Fig. (1): Section in liver of rat from G_1 showing the normal structure of hepatic lobule (H and E X 200).

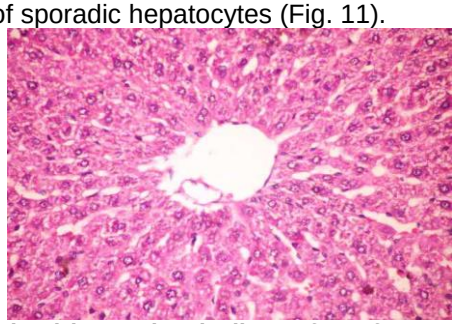

Fig. (2): Section in liver of rat from G_2 showing non significant histopathological changes (H and E X 200).

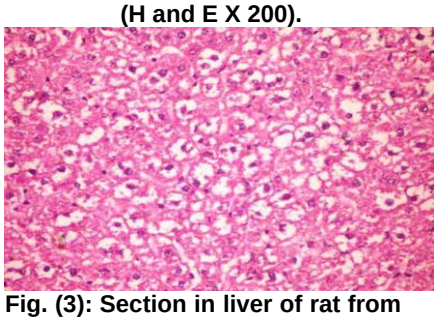

Fig. (3): Section in liver of rat from G_3 showing vacuolations of hepatocytes (H and E X 200).

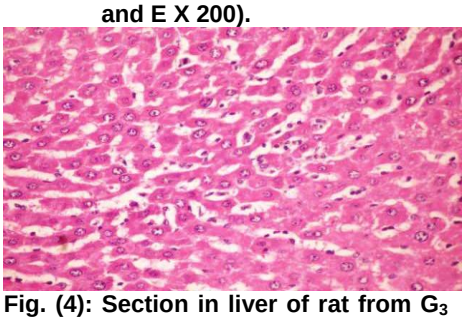

Fig. (4): Section in liver of rat from G_3 showing kupffer cells activation and necrosis of sporadic hepatocytes (H and E X 200).

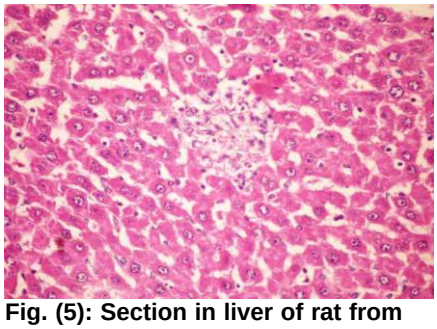

Fig. (5): Section in liver of rat from G_3 showing focal hepatic necrosis (H and E X 200).

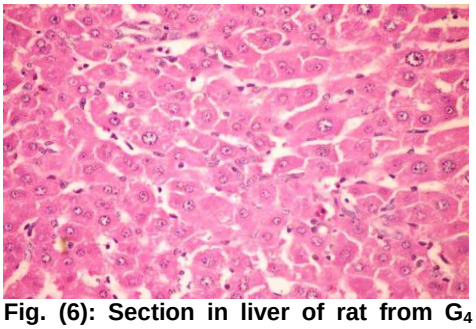

Fig. (6): Section in liver of rat from G_4 showing karyomegaly of some nuclei and apoptosis of hepatocytes (H and E X 200).

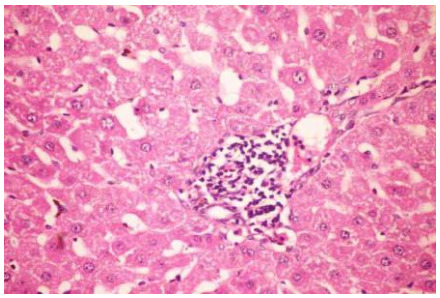

Fig. (7): Section in liver of rat from G_4 showing portal infiltration with leucocytes (H and E X 200).

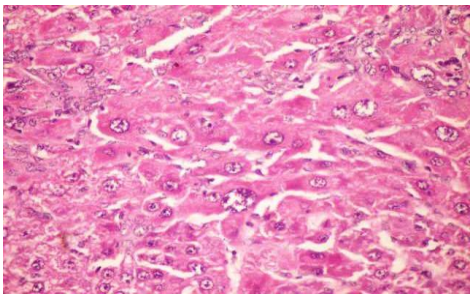

Fig. (8): Section in liver of rat from G_4 showing cytokaryomegaly of hepatocytes associated with oval cell proliferatin (H and E X 200).

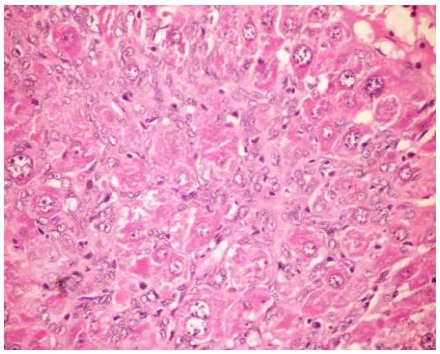

Fig. (9): Section in liver of rat from G_4 showing oval cell proliferatin, apoptosis of hepatocytes and mitotic figures (H and E X 200).

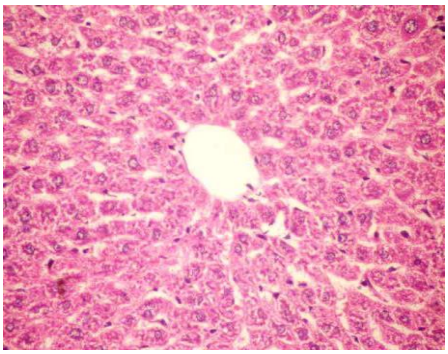

Fig. (10): Section in liver of rat from G_5 showing no marked histopathological changes (H and E X 200).

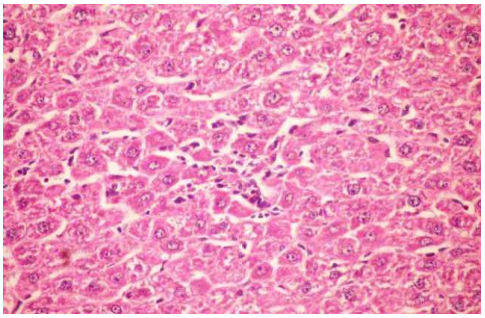

Fig. (11): Section in liver of rat from G_6 showing kupffer cell proliferation and necrosis of sporadic hepatocytes (H and E X 200).

Kidneys:

Microscopically, kidneys of rat from G_1 (vehicle treated control) revealed normal histological structure of renal parenchyma (Fig. 12). Also, kidneys of rat from G_2 which received PP extract revealed apparent normal renal parenchyma (Fig. 13).

Mean while, kidneys of rat from G_3 which treated by low AFS dose showed vacuolations of renal tubular epithelium (Fig. 14) and interstitial nephritis (Fig. 15). Moreover, section from G_4 which treated with high AFS dose revealed tubulointerstitial nephritis (Fig. 16, 17), cystic dilatation of some renal tubules (Fig.16) and dilatation of Bowmen's space (Fig. 17).

However, kidneys of G_5 (treated by low AFS dose and PP extract) revealed no histological changes (Fig. 18). While examined kidneys of rat from G_6 which treated with high AFS dose and PP extract showed vacuolations of renal tubular epithelium (Fig. 19) and focal tubular necrosis associated with leucocytic cells infiltration (Fig. 20).

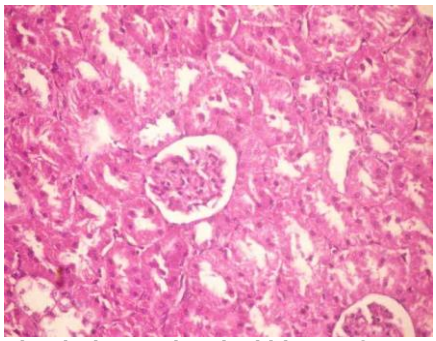

Fig. (12): Section in kidney of rat from G_1 showing the normal histological structure of renal parenchyma (H and E X 200).

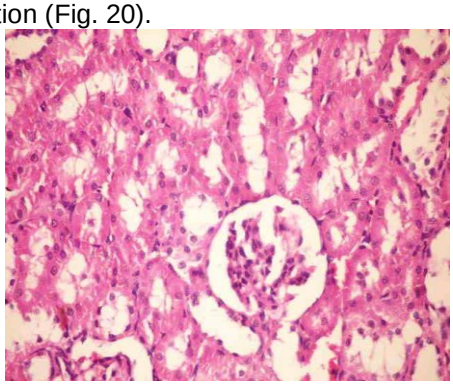

Fig. (13): Section in kidney of rat from G_2 showing apparent normal renal parenchyma (H and E X 200).

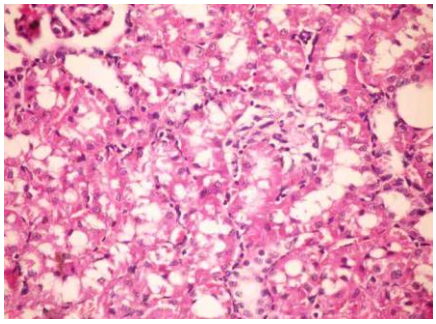

Fig. (14): Section in kidney of rat from G_3 showing vacuolations of renal tubular epithelium (H and E X 200).

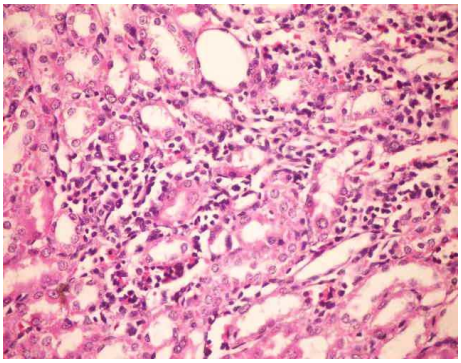

Fig. (15): Section in kidney of rat from G_3 showing interstitial nephritis (H and E X 200).

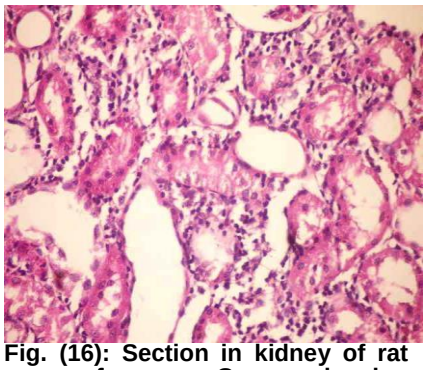

Fig. (16): Section in kidney of rat from G_4 showing interstitial nephritis and cystic dilatation of some renal tubules (H and E X 200)

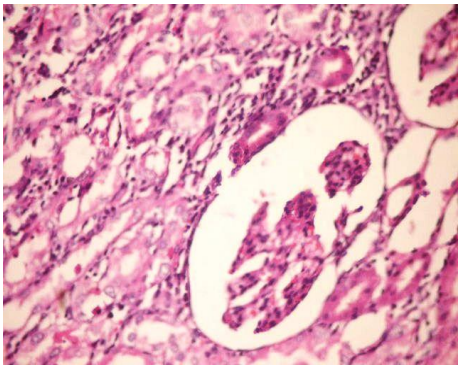

Fig. (17): Section in kidney of rat From G_4 showing interstitial nephritis and dilatation of Bowmen's space (H and E X 200).

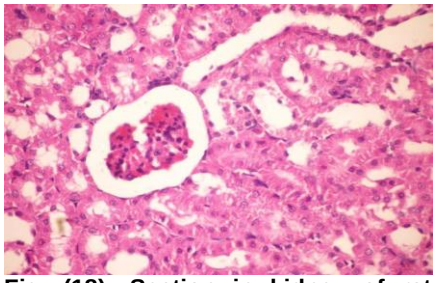

Fig. (18): Section in kidney of rat from G_5 showing no histopathological changes (H and E X 200).

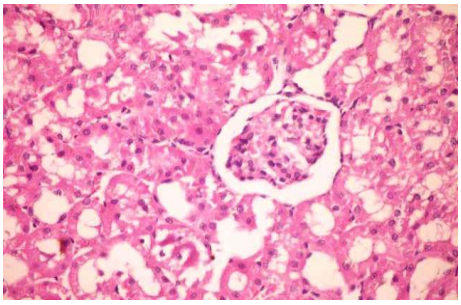

Fig. (19): Section in kidney of rat From G_6 showing vacuolations of renal tubular epithelium (H and E X 200).

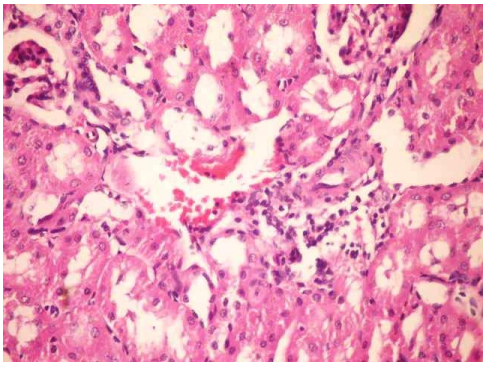

Fig. (20): Section in kidney of rat from G_6 showing focal tubular necrosis associated with leucocytic cells infiltrations (H and E X 200).

DISCUSSION

Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations. In developed countries, sufficient amounts of food combined with regulations that monitor aflatoxin levels in these foods protect human populations from significant aflatoxin ingestion. However, in countries where populations are facing starvation or where regulations are either not enforced or nonexistent, routine ingestion of aflatoxin may occur (Cotty *et al.,* 1994 and Abdelhamid, 2010). Worldwide, liver cancer incidence rates are 2 to 10 times higher in developing countries than in developed countries (Henry *et al.,* 1999).

In clinical diagnosis, determination of transaminases is of great importance. The activity of ALT and AST is a sensitive indicator of acute hepatic necrosis and hepatobiliary disease *(Kaplan, 1987).* In the present study, it was indicated that AFS treatment caused a significant, dose and time-dependent elevation in ALT, AST and ALP activities in serum of rats. The liver is considered to be the principle target organ for AFS and the increase of ALT, AST and ALP activities indicated acute hepatic necrosis *(Abdel-Wahhab and Soher, 2003).* Similar to our results, *Liu et al. (2001)* reported that administration of rats with AFB_1 by gavage resulting in elevation of serum AST and ALT activities. In addition, *Abdel-Wahhab et al. (2002)* reported that feeding rats with diet contaminated with AFS resulting in significant increase in AST, ALT and ALP in serum. Also *Karakilcik et al. (2004)* fed rabbits on diet AFB_1 for 10 week noticed that, the levels of AST, ALT and ALP in serum were significantly elevated. Moreover, *Preetha et al. (2006)* mentioned that oral administration of rats by AFB_1 resulting in a significant increase in ALP, AST and ALT activity in serum and decreased it in liver after 72h of treatment. The results also showed that the AFS oral administration significantly resulted in decrease of serum level of TP and albumin concentrations with increase of AF dose and time of treatment. These results run in agreement with the data of *Dwivedi et al. (1993)* and *Abo-Norag et al.(1995)* who showed that AF- induced reduction in TP and

albumin in rats. The decrease in plasma protein may be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis *(Terao and Ueno, 1978 and Srivastava, 1984).* In addition, *Osuna and Edds (1982)* suggested that such effect may be due to the metabolism of AFS in liver, where it interferes with protein synthesis and RNA production, resulting in decreasing of albumin. Moreover, the low level of TP may indicate protein catabolism and/or kidney dysfunctions *(Abdel- Wahhab and Aly, 2003).* Data presented herein showed that AFS treatment caused significant elevation of serum creatinine, urea and uric acid in dose and time dependent manner. The present results are supported by the results obtained by *Soliman (1994*) in rams*; El-Zahar et al. (1996)* in rabbits and *Hassan (1998)* in hens. The increased level of blood urea and creatinine with decreased level of blood protein may indicate protein catabolism and /or kidney dysfunction *(Abdel-Whhab et al., 1999).* Generally, these results indicate degenerative changes and hypofunction of liver and kidneys *(Kaplan, 1987).*

In the present investigation, treatment of rats by AFS for 6 weeks resulted in hepatotoxicity as revealed by histological study. The degree of severity of pathological lesions depends upon concentration of AFS and time of treatment. Liver of rat treated by low dose of AFS showed vacuolar degeneration of hepatocytes, kupffer cells activation, necrosis of sporadic hepatocytes and focal hepatic necrosis. Meanwhile, liver of rat treated with high AFS dose showed karyomegaly of some nuclei, apoptosis of hepatocytes, portal infiltration with leucocytes, cytokaryomegaly of hepatocytes, oval cells proliferation and mitotic figures. Liver injury by aflatoxicosis was recorded in various studies by *Chou et al. (2000); Hiruma et al. (2001)* and *Preetha et al. (2006)* in rats injected by aflatoxin and *Hassanein and Abdel Gawad (2001)* and *Vinita et al. (2003)* in rabbits injected by aflatoxin. Kidney of rats was also affected by AFS treatment. The severity of pathological lesions was concentration and time dependent. Kidneys of rats treated with 1mg AFS/ kg b.w. showed vaculations of renal tubular epithelium and interstitial nephritis. While, kidneys of rats treated with 2mg AFS/ kg b.w. revealed tubulointerstitial nephritis, cystic dilatation of some renal tubules and dilatation of Bowmen's space. Such results were previously recorded by *Abdelhamid et al. (1995)* in broiler and *Vinita et al. (2003)* and *Abdeen et al. (2004)* in rats. Similar alterations were reported by *Abdelhamid et al. (2002 a, b and 2005)* on rabbits and rats, respectively when fed on aflatoxic diets. Also, *Abdelhamid et al. (1990 and 2002 a)* came to similar histological changes by aflatoxic rabbits.

The toxic effect produced by AFS on different parameters was explained by *Gutteridge and Halliwell (1990)* who mentioned that, AFS treatment resulted in enhancement of lipid peroxidation in rats, which is directly related to free radical mediated toxicity. The targets of oxidative damage are usually critical biomolecules such as nucleic acids, proteins, and lipids. In addition, AFS is oxidized by cytochrome P_{450} enzymes to AF-8,9oxide (AF- epoxide) which can bind to DNA to form adducts *(Choy, 1993)*. Reaction occurs with high region specificity at the N7 position of guanine residues in DNA *(Iyer et al., 1994).*

This study has shown that PP extract alone does not have a negative impact on biochemical parameters and histology of liver and kidney.

The current results revealed that co-treatment with PP extract and AFS resulted in a significant improvement in all the tested parameters as indicated by significant alleviation of toxic effect of AFS on ALT, AST, ALP, T.P, albumin, creatinine, urea and uric acid levels in serum. Especially with low AFS dose and at 6^{th} week of treatment, but it failed to normalize them except albumin, which restored to normal. The protective effect of PP on liver function against many oxidative stresses was previously recorded by *Toklu et al. (2007)* who reported that PP administration was significantly able to decrease the increase of AST and ALT levels as a result of biliary obstruction induced by bile duct legation in serum of rats due to its antioxidant and antifibrotic prosperities. *Parmar and Kar (2007)* mentioned that the treatment of rats with pomegranate peel extract resulted in a decrease of the elevation of serum ALT and creatinine Kinase induced by atherogenic diet. Furthermore, *Masoud and Abo Hagger (2009)* reported that addition of PP powder to diet containing AFS was significantly able to decrease the increase in levels of ALT, AST, ALP, creatinine and urea induced by AFS in serum of guinea pigs.

Present histopathological results revealed that PP extract was able to protect liver and kidney tissue against low AFS dose and minimize the damage of liver and kidney induced by high AFS dose. The beneficial effect of PP extract on tissue of liver and kidneys was previously reported by many studies. Chidambara Murthy *et al. (*2002*)* reported that feeding rats with methanolic extract of PP extract provides protection against carbon tetrachloride inducing liver injury and restoring the normal hepatic architecture with portal triad, portal veins and hepatic artery and vein were visible. In addition, Parmar and Kar (2007) revealed that simultaneous treatment of rats with PP extract ameliorated histological alterations induced by atherogenic diet and liver and kidney tissue were normalized near to those control one. Furthermore, Toklu *et al. (*2007*)* mentioned that pomegranate peel extract with its antioxidant and antifibrotic properties might be of potential therapeutic value in protecting the liver structure from fibrosis and oxidative injury due to biliary obstruction.

The noticed protective effect of PP extract against toxic effect of AFS could be attributed to its high antioxidants content *(*Noda *et al.,* 2002 and Li *et al.,* 2006*)*. The phenolic contents of *P. granatum* peel extract acts as a potent free radical scavenger, reducing the levels of hydrogen peroxide and superoxide anion, and consequently inhibit lipid peroxidation, enzyme inactivation, lipid peroxidation of liver and kidney and provide degenerative disease- protective effects on oxidative DNA damage *(*Kim *et al.,* 2007 and Althunibat *et al.,* 2010*).* More interesting, Li *et al. (*2006*)* showed that PP extract had markedly higher antioxidant capacity than pulp extract in scavenging or preventing capacity against superoxide anion, hydroxyl and peroxyl radicals as well as inhibiting $CuSo_4$-indused LDL oxidation.

In conclusion, the present work suggests that *P. granatum* peel extract might act as a suppressor against tissue damage and inhibit the progression of organs dysfunction induced by AFS.

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تحوير سمية الأفلاتوكسين فى الجرذان عن طريق استخدام المستخلص الإيثانولى لقشر الرمان.

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**** المركزالإقليمي للأغذية و الأعلاف- مركز البحوث الزراعية – الجيزة-مصر**

أجريت الدراسة لمعرفة تأثير المستخلص الإيثانولى لقشر الرمان على خلايا الكبد و الكلى ووظائفهما فى الجرذان المعاملة بمستويات مختلفة من الأفلاتوكسين. تم اجراء تجربة لمدة ٦ أسابيع على ٣٦ جرذ . تم تقسيم الجرذان الى ٦ مجموعات (٦جرذان فى كل مجموعة) .المجموعة الاولى تركت ككنترول (عوملت بالمادة الحاملة) ، المجموعة الثانية عوملت بمستخلص قشر الرمان بالأنبوب المعدى (٥٠مجم / كجم وزن جسم يومياً) ، المجموعة الثالثة عوملت بالأفلاتوكسين عن طريق الأنبوب المعدى (١ مجم / كجم وزن جسم) مرتان فى الأسبوع ، المجموعة الرابعة عوملت بالأفلاتوكسين عن طريق الانبوب المعدى (٢ مجم / كجم وزن جسم) مرتان فى الأسبوع . المجموعة الخامسة عوملت بالأفلاتوكسين مرتان فى الأسبوع (١مجم/ كجم وزن جسم)+ مستخلص قشر الرمان يومياً (٥٠مجم / كجم وزن جسم). المجموعة السادسة عوملت بالأفلاتوكسين مرتان فى الأسبوع (٢مجم/ كجم وزن جسم)+ مستخلص قشر الرمان يومياً (٥٠مجم / كجم وزن جسم). تم تحليل انزيمات الكبد(ALT, AST, ALP) ، البروتين الكلى و الألبيومين وظائف الكلى (creatinine, urea, uric acid) فى المصل عند الأسبوع الثالث و السادس من المعاملة، بينما تم عمل التحليل الباثولوجى للكبد و الكلى فى نهاية التجربة . أظهرت النتائج أن كلاً جرعتى الأفلاتوكسين تسببا فى زيادةALT, AST, ALP, creatinine, urea, uric acid و نقص البروتين الكلى و الألبيومين بطريقة معتمدة على الجرعة و الوقت. بينما أدت المعاملة بمستخلص قشر الرمان مع الأفلاتوكسين فى الحد من التأثير السلبى للأفلاتوكسين خاصة مع الجرعة المنخفضة و الوقت الأطول . أظهرت التحاليل الباثولوجية أن شدة الضرر الحادث فى الكبد و الكلى يتناسب طردياً مع جرعات الأفلاتوكسين. بينما كان مستخلص قشر الرمان قادر على حماية الكبد و الكلى من الجرعة المنخفضة من الأفلاتوكسين و تقليل الضرر الحادث للكبد و الكلى بالجرعة المرتفعة من الأفلاتوكسين .

قام بتحكيم البحث

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