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PROTECTIVE EFFECT OF POMEGRANATES PEEL AGAINST AFLATOXINS INDUCED PATHOLOGICAL ALTERATIONS IN GUINEA PIGS TISSUES.

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ABSTRACT

Aflatoxins (AFs) are acutely toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive to most mammalian species. AFs contamination of food and live stock feeds is an ongoing problem. *Punica granatum L. (*pomegranate) is an important source of bioactive compounds and has been used for folk medicine for many centuries. The aim of our study was to examine the potential protective effect of pomegranate peel (PP) powder against toxic effect of AFs on body organs of guinea pigs (GPs). 105 male GPs were divided into 15 groups. 1st group received basal diet (control G_1). 2nd group received low dose of AFs (25 µg/kg diet), 3rd group received high dose of AFs (50 µg/kg diet) , while 4th, 5th, 6th, 7th groups received 6%, 9%, 12%, and 15% of PP powder in diet respectively, 8th, 9th, 10th, 11th groups received 6%, 9%, 12%, and 15% of PP powder plus low dose of AFs respectively in diet, while 12th, 13th, 14th, 15th groups received 6%, 9%, 12%, and 15% of PP powder plus high dose of AFs respectively in diet. Histopathological examination of liver, kidney and brain was proceeding at 10th and 30th day post treatment. Results revealed that AFs cause sever damage of liver, kidney and brain in time and dose dependant manner, while there are no pathological lesions found in groups treated by PP powder. More over all PP concentration showed protective activity against liver damage in GPs treated by low dose of AFs and reduce effect of high dose of AFs on liver in concentration dependant manner. Also pomegranate peel has protective effect against kidney and brain damage especially at low dose of AFs but there is no difference between PP concentrations in ability of protection of brain. While higher dose of PP showed more protective effect than lower concentration against kidney toxicity by high dose of AF.
Keywords: Aflatoxins, Pomegranate Peel, histopathology, liver, kidney, brain.

INTRODUCTION

Aflatoxins represent a group of similar chemicals produced by the common molds *Aspergillus flavus and Aspergillus parasiticus* (Huff *et al.,* 1986). The presence of AFs in animal feed stuff is recognized as one of the most serious health hazard (Hassanein and Abdel Gawad, 2001). AF producing fungi infests food grain (i.e. maize, groundnuts, wheat, etc.) or other food items usually during storage. AF contaminated food and feed have been reported from all ports of the world (Koirala *et al.,* 2005). AFs have been found to be potent hepatotoxins, highly toxic carcinogenic, teratogenic and mutagenic, which lead to genetic damage (Wogan, 1969). AF affect liver causing cirrhosis, hepatoma, hepatitis as well as affect other organs like Kidney, myocardium, muscles and brain (Shank et al., 1971 and Newbrene, 1983).

Reactive oxygen species are considered to participate in the main mechanism of AF toxicity (Gesing and Karbowink-Lewinska, 2008). AF

cause oxidative damage to lipids and protein in liver, kidney and brain (Madhusudhanan *et al.*, 2004).

Antioxidant is defined as any substance that when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevent oxidation of substrate (Halliwell, 1995). Evidence from animal and human experiments reveals that some natural antioxidant other than ascorbic acid, caratenoids and vitamin E could be absorbed significantly and act as potent antioxidant in vivo (Gao *et al*., 2001; Pataki *et al*., 2002 and Su *et al*., 2003).

Pomegranates *(Punica granatum)* have been used extensively in folk medicine of many cultures (Longtin, 2003). Pomegranates are mentioned in Koran and are valued by Moslems; their book describes paradise as having an abundance of pomegranates. In fact, prophet Mohamed is said to have encouraged his followers to eat them as means of purging their bodies of envy and hearted (Feig, 2005). Pomegranates have a wide spectrum of antibacterial, antiviral, and antiheminthic properties as well as anticancer properties in case of prostate, breast, colon and skin tumors (Louba, 2007). PP contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid. It has been used in the preparation of tinctures, cosmetic, therabeutic formula 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 28kinds of fruits commonly consumed in china. More over Li *et al.*, (2006) concluded that PP extract appear to have more potential as health supplement rich in natural antioxidants than the pomegranate pulp extract.

In the currant study we try to determine the ability of PP powder to suppress the toxicity of aflatoxins on guinea pig organs.

MATERIALS AND METHODS

Materials

a. Pomegranate peel preparation:

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

b. Aflatoxin production:

Aflatoxins were produced through fermentation of yellow corn with *A. flavus* NRRL (3145) as described by Kubena *et al.* (1990). Fermentation was carried out in 2.5-L conical flasks containing yellow corn. Twenty-five milliliters of water were added to each flask; then yellow corn was autoclaved. Each flask was inoculated with 1 ml of an *Aspergillus* spore suspension contains 10^5 cfu and then was incubated at 25^oC with moisture content of 17% for 15 days (Shotwell *et al.,* 1966). After incubation, the yellow corn was autoclaved, dried, and then ground.

Preparation of aflatoxin contaminated diet:

The contaminated yellow corn contained mixture of aflatoxin B_1, B_2, G_1, G_2 at a total 7.65 mg/kg, was added to the basal diet to provide the desired levels as follows:

- Low aflatoxin concentration dose was prepared by adding 3.26 g of contaminated corn to 1kg basal diet to yield a final concentration 25 µg/kg diet.
- High aflatoxin concentration dose was prepared by adding 6.53 g of contaminated corn to 1kg basal diet to yield a final concentration 50 µg/kg diet.

Determination of aflatoxins concentration of contaminated diet:

Aflatoxins' concentration was determined using HPLC technique (Agillent 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 flow rate of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460nm (Florences detector), respectively (Roos *et al,.* 1997*).*

Analysis and Investigation:

The previous and the following determinations were kindly carried out by the different specific laboratories of Regional Center for Food and Feed, Agriculture Research Center:

Methods

a. Animals

21 days old GPs with body weight of approximately 250g were used according to Liu, (1988). The GPs were divided into 15 groups, caged in colonies in stainless steel cages and maintained at 22-24 °C and 45-55% relative humidity as recommended in NRC (1995) in animal house of Regional Center for Food and Feed.

Experimental design and animal diets:

The design of the experimental work was planned by using 3 levels of corn contaminated with AF (0, 3.26, 6.53g/ kg diet) and 5 levels of adsorbent PP (0, 6, 9, 12, 15%) resulted in 15 treatments (table1).

The powdery diets were pellitized after moisturizing with water according to Ostwald *et al*., (1971) as guinea pigs fed pelleted natural-ingredient diets often do not readily accept a powdered purified diet. According to composition of diet formulated by Typpo *et al*., (1985). Fifteen diets were previously prepared for 15-subjected experimental animal groups as follows:

Group 1: Diet 1 (Basal diet)
Group 2: Diet 2 (Diet 1 + 3.26g of low AF contaminated corn).
Group 3: Diet 3 (Diet 1 + 6.53g of high AF contaminated corn).
Group 4: Diet 1 + 6% PP
Group 5: Diet 1 + 9% PP
Group 6: Diet 1 + 12% PP
Group 7: Diet 1 + 15% PP
Group 8: Diet 2 + 6% PP
Group 9: Diet 2 + 9% PP
Group 10: Diet 2 + 12% PP
Group 11: Diet 2 + 15% PP
Group 12: Diet 3 + 6% PP
Group 13: Diet 3 + 9% PP
Group 14: Diet 3 + 12% PP
Group 15: Diet 3 + 15% PP

t1

At the 10th and 30th day of experiment 3 guinea pigs from each group were scarified and liver, kidney and brain were taken. Samples of each tissue were preserved in neutral formalin solution (10% v/v) for histopathological examination till processing as paraffin blocks and sectioning for Haematoxylin and Eosin staining according to (Culling, 1963). Histopathological technique and examination were perfomed in pathology department, faculty of veterinary medicine, Cairo University.

RESULTS

Histopathological results:

Liver:

Microscopically, liver sections from control, untreated GP revealed no histopathological alterations all over the experimental period. Meanwhile, examined liver of GP from treated with low dose of AFs (10 days post treatment) showed activation of Kupffer cells, hepatocellular vacuolization of centrilobular hepatocytes, sporadic cell necrosis associated with pyknosis of their nuclei as well as presence of small multiple areas of hepatic necrosis (Fig. 1) were also noticed. Moreover, at 30 days post treatment, examined sections revealed vacuolar degeneration of hepatocytes (Fig. 2), activation of epithelial lining bile duct, portal edema (Fig. 3), massive leucocytic cells infiltration in portal triad as well as sinusoidal leucocytosis (Fig. 4). Examined liver of GP from (G3) (treated with high dose) 10 days post treatment, revealed kupffer cells activation, hepatocellular vacuolization, focal areas of hepatic necrosis associated with leucocytic cells infiltration (Fig. 5). Meanwhile, at 30 days post treatment, examined liver revealed focal hepatic necrosis completely replaced by leucocytic cells infiltration, sinusoidal leucocytosis, dissociation and atrophy of hepatic cords (Fig. 6). Microscopically, liver of GPs from groups (G4), (G5), (G6) and (G7) treated with 6%, 9%, 12%, and 15% of PP powder respectively showed no histopathological changes throughout the experimental period (Fig. 7). Moreover, liver of GPs from groups (G8), (G9), (G10), (G11) which treated with both low dose of AFs and 6%, 9%, 12%, and 15% of PP powder respectively at either 10 days or 30 days post treatment revealed apparent normal hepatocytes (Figs. 8 & 9). Conversely, examined sections from (G12), treated with high dose and 6% of PP powder at 10 days post treatment showed vacuolization of hepatocytes, small focal hepatic necrosis (Fig. 10) and activation of epithelial lining bile duct. At 30 days (PT), examined sections revealed necrosis of sporadic hepatocytes, pyknosis of their nuclei and intravascular permeation with leucocytes (Fig. 11). However, liver of GP from group (G13) treated with high dose and 9% of PP powder at both 10 and 30 days showed no changes except vacuolar degeneration of hepatocytes (Fig. 12). Moreover, liver of GPs from groups (G14) and (G15) treated with high dose and 12% and 15% of PP powder respectively showed more or less similar histopathological alterations at both 10 and 30 days post treatment, those changes described as activation of kupffer cells, vacuolar degeneration of hepatocytes (Fig. 13) and hyperplasia of epithelial lining bile duct (Fig. 14).

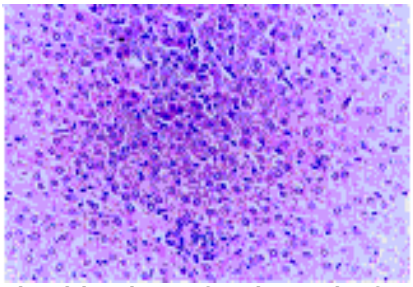

Fig. (1): Liver of guinea pig from group 2 (10 days PT) showing activation of Kupffer cells, sporadic cell necrosis associated with pyknosis of their nuclei as well as small multiple areas of hepatic necrosis. (H & E X 200).

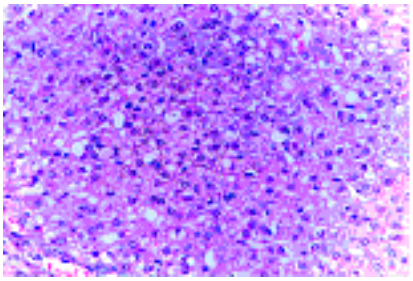

Fig. (2): Liver of guinea pig from group 2 (30 days PT) showing vacuolar degeneration of hepatocytes (H & E X 200).

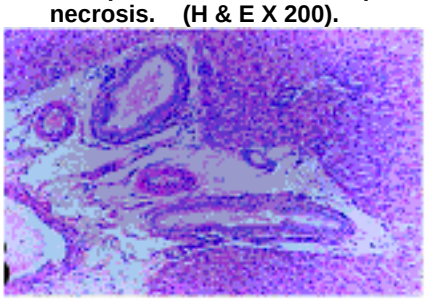

Fig. (3): Liver of guinea pig from group 2 (30 days PT) showing activation of epithelial lining bile duct and portal edema (H & E X 100).

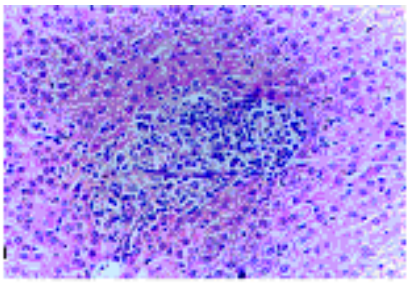

Fig. (4): Liver of guinea pig from group 2 (30 days PT) showing massive leucocytic cells infiltration in portal triad as well as sinusoidal leucocytosis (H & E X 200).

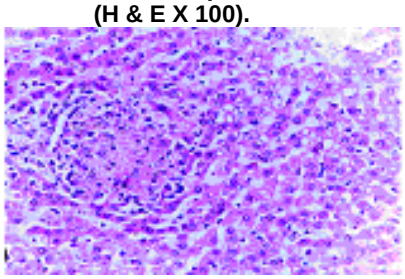

Fig. (5): Liver of guinea pig from group 3 (10 days PT) showing kupffer cells activation, hepatocellular vacuolization, focal areas of hepatic necrosis associated with leucocytic cells infiltration (H & E X 200).

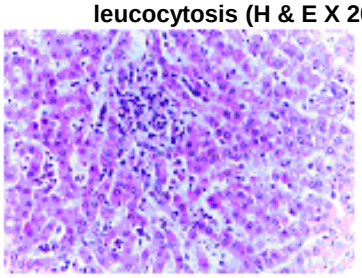

Fig. (6): Liver of guinea pig from group 3 (30 days PT) showing focal hepatic necrosis completely replaced by leucocytic cells infiltration, sinusoidal leucocytosis, dissociation and atrophy of hepatic cords (H & E X 200).

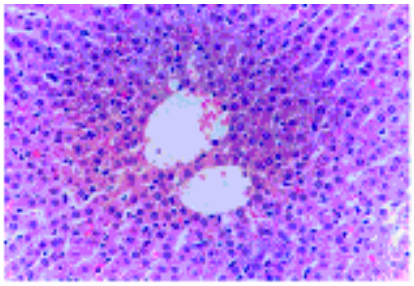

Fig. (7): Liver of guinea pig from group 7 (30 days PT) showing apparent normal hepatocytes (H & E X 200).

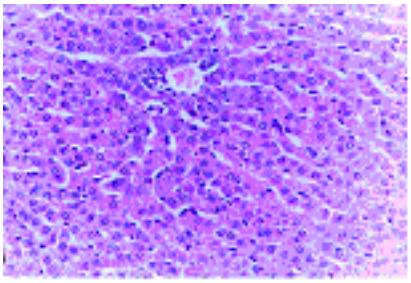

Fig. (8): Liver of guinea pig from group 8 (10 days PT) showing apparent normal hepatocytes (H & E X 200).

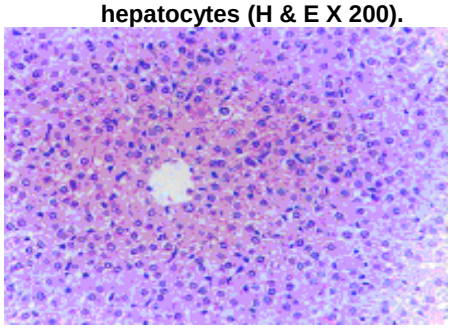

Fig.(9): Liver of guinea pig from group 11 (30 days PT) showing apparent normal hepatocytes (H & E X 200).

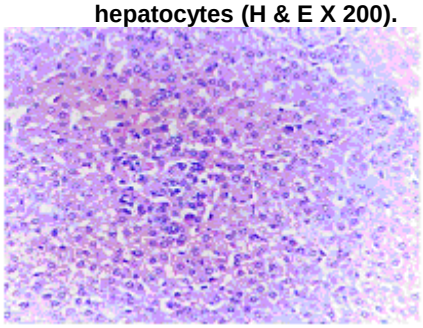

Fig.(10): Liver of guinea pig from group 12 (10 days PT) showing vacuolization of hepatocytes, small focal hepatic necrosis (H & E X 200).

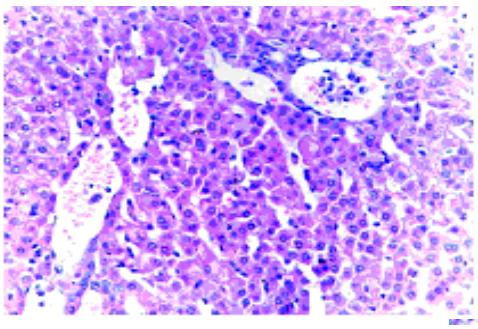

Fig.(11): Liver of guinea pig from group 12 (30 days PT) showing necrosis of sporadic hepatocytes, pyknosis of their nuclei and intravascular permeation with leucocytes (H & E X 200).

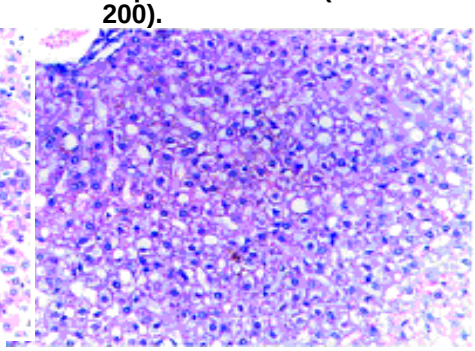

Fig.(12): Liver of guinea pig from group 13 (30 days PT) showing vacuolar degeneration of hepatocytes (H & E X 200).

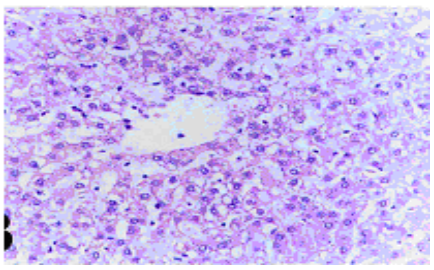

Fig.(13): Liver of guinea pig from group 15 (10 days PT) showing activation of kupffer cells, vacuolar degeneration of hepatocytes (H & E X 200).

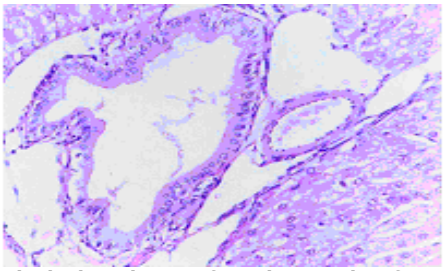

Fig.(14): Liver of guinea pig from group 15 (30 days PT) showing hyperplasia of epithelial lining bile duct (H & E X 200).

Kidneys:

Microscopically, kidneys of control, untreated GP revealed no histopathological alterations all over the experimental period. On the other hand, kidneys of guinea pig from (G2) (treated with low dose of aflatoxin) 10 days (PT) showed vacuolar degeneration of epithelial lining renal tubules, focal necrosis of renal tubules and cystic dilatation of other renal tubules (Fig. 15) together with hypercellularity of the glomerular tufts at 30 days (PT), (Fig. 16). However, kidneys of GP from (G3) treated with high dose of AF for 10 days showed vacuolation of epithelial lining renal tubules and focal area of tubular necrosis completely replaced by leucocytic cells infiltration (Fig. 17). Moreover, at 30 days(PT) revealed vacuolation of epithelial lining renal tubules, swelling of the endothelial lining parital layer of Bowman's capsule, distension of Bowman's space as well as periglomerular leucocytic cells infiltration (Fig. 18) together with focal tubular necrosis associated with inflammatory cells infiltration as well as intratubular cellular cast in the lumen of some renal tubules (Fig.19). Kidneys of GPs from groups (G4), (G5), (G6) and (G7) showed no histopathological changes throughout the experimental period. Moreover, kidneys of GPs from groups (G8), (G9), (G10), (G11) were treated with both low dose of AFs and 6%, 9%, 12%, and 15% of PP powder respectively at 10 days post treatment revealed no changes except vacuolar degeneration of epithelial lining renal tubules (Fig.20). More over at 30 days examined kidneys revealed vacuolar degeneration of epithelial lining renal tubules and peritubular inflammatory cells infiltration (Fig.21). Kidneys from (G12) and (G13) treated with high dose and 6% and 9% PP powder respectively at either 10 or 30 days revealed necrobiotic changes of epithelial lining renal tubules, peritubular leucocytic ells infiltration, hypercellularity of glomerular tuft (Fig. 22) and focal tubular necrosis. Additionally kidneys of GPs from (G14) revealed hypercellularity of glomerular tuft after 10 days and presence of intratubular cellular casts in the lumen of some renal tubules (Fig.23) after 30 days (PT).While kidneys of GPs of (G15) which were treated with high dose of AFs and 15 % of pomegranate peel powder either at 10 or 30 days (PT) revealed focal area of tubular necrosis associated with leucocytic cells infiltration (Fig. 24).

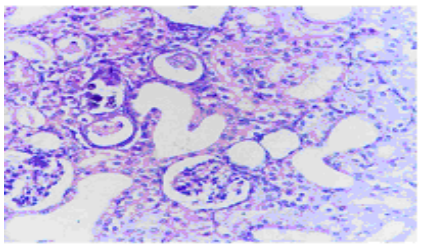

Fig.(15): Kidney of guinea pig from group 2 (10 days PT) showing vacuolar degeneration of epithelial lining renal tubules, focal necrosis of renal tubules and cystic dilatation of other renal tubules (H & E X 200).

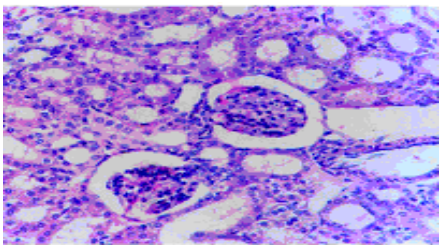

Fig.(16): Kidney of guinea pig from group 2 (30 days PT) showing hypercellularity of the glomerular tufts (H & E X 200).

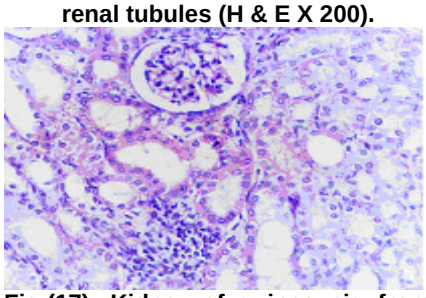

Fig.(17): Kidney of guinea pig from group 3 (10 days PT) showing vacuolation of epithelial lining renal tubules and focal area of tubular necrosis completely replaced by leucocytic cells infiltration (H & E X 200).

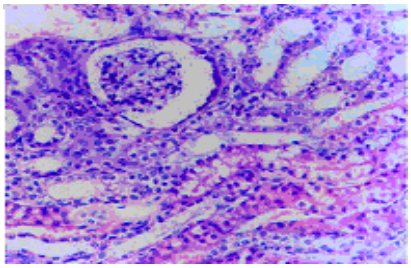

Fig.(18): Kidney of guinea pig from group 3 (30 days PT) showing vacuolation of epithelial lining renal tubules, swelling of the endothelial lining parital layer of Bowman's capsule, distension of Bowman's space as well as periglomerular leucocytic cells infiltration (H & E X 200).

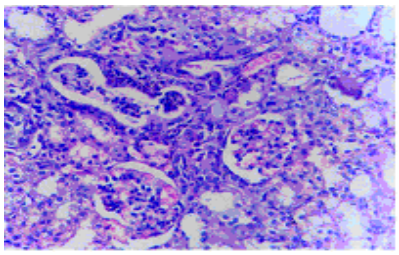

Fig.(19): Kidney of guinea pig from group3 (30 days PT) showing focal tubular necrosis associated with inflammatory cells infiltration as well as intratubular cellular cast in the lumen of some renal tubules (H & E X 200).

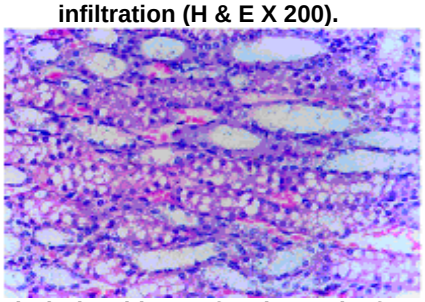

Fig.(20): Kidney of guinea pig from group 8 (10 days PT) showing vacuolar degeneration of epithelial lining renal tubules (H & E X 200).

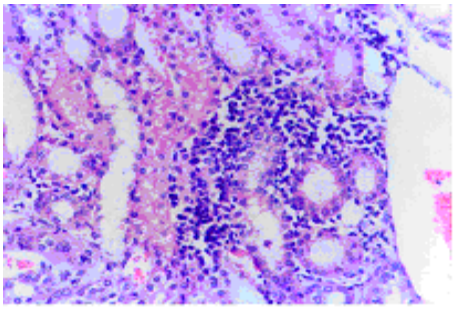

Fig.(21): Kidney of guinea pig from group 8 (30 days PT) showing vacuolar degeneration of epithelial lining renal tubules and peritubular inflammatory cells infiltration (H & E X 200).

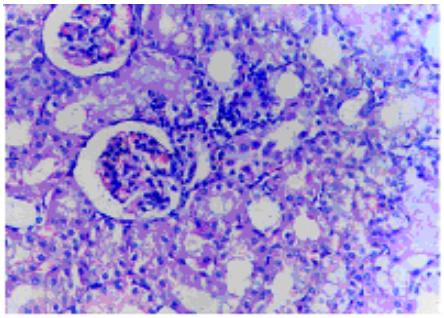

Fig.(22): Kidney of guinea pig from group12 (30 days PT) showing necrobiotic changes of epithelial lining renal tubules, peritubular leucocytic ells infiltration and hypercellularity of glomerular tuft (H & E X 200).

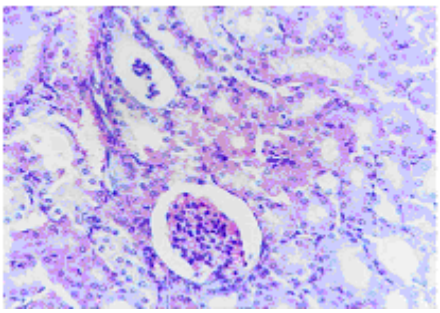

Fig.(23): Kidney of guinea pig from group 14 (30 days PT) showing hypercellularity of the glomerular tufts, presence of intratubular cellular casts in the lumen of some renal tubules (H & E X 200).

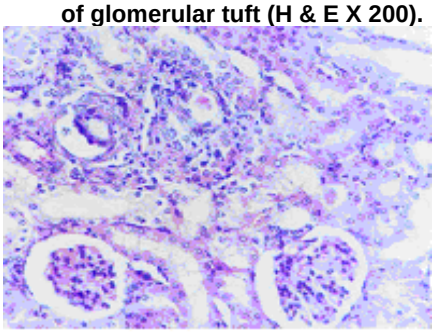

Fig.(24): Kidney of guinea pig from group 15 (10 days PT) showing focal area of tubular necrosis associated with leucocytic cells infiltration (H & E X 200).

Brain:

Microscopically, brain sections from control, untreated GP (G1) revealed no histopathological alterations all over the experimental period.

Conversely, examined brain of GP from (G2) (treated with low dose) (10 days post treatment) showed neuronal degeneration and neuronophagia (Fig. 25). Moreover, at 30 days post treatment examined sections revealed pyknosis of neurons, focal gliosis (Fig. 26) and intracellular edema. Examined brain of GP from (G3) (treated with high dose) 10 days post treatment, showed neuronal degeneration, neuronophagia, focal gliosis and necrosis of Purkinje cells of the cerebellum (Fig. 27). Meanwhile, at 30 days post treatment, examined liver revealed the previous described changes together with focal cerebral hemorrhages (Fig. 28). Microscopically, brain of GPs from

groups (G4), (G5), (G6) and (G7) treated with6%, 9%, 12%, and 15% of PP powder respectively showed no histopathological changes all over the collected samples. However, brain of GPs from groups (G8), (G9), (G10), (G11) which treated with both low dose of AFs and 6%, 9%, 12%, and 15% of PP powder respectively at either 10 days or 30 days post treatment revealed central chromatolysis of some neurons (Fig. 29) as well as neuronal edema.

Examined sections from (G12), treated with high dose and 6% PP at 10 or 30 days post treatment showed neuronal degeneration and neuronophagia. However, brain of GP from group (G13) at both 10 and 30 days showed central chromatolysis of some neurons associated with focal cerebral hemorrhage (Fig. 30). Moreover, brain of GPs from groups (G14) and (G15) showed more or less similar histopathological alterations at both 10 and 30 days post treatment, which mentioned as neuronal degeneration, neuronophagia, focal gliosis and intracellular edema (Fig. 31).

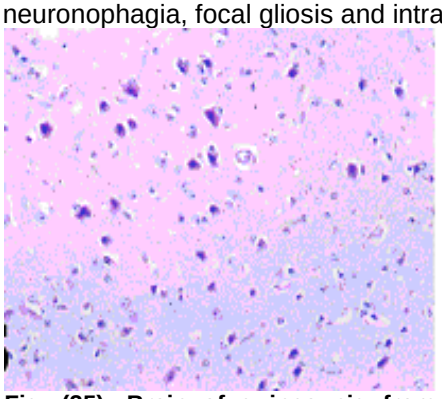

Fig. (25): Brain of guinea pig from group 2 (10 days PT) showing neuronal degeneration and neuronophagia. (H & E X 200).

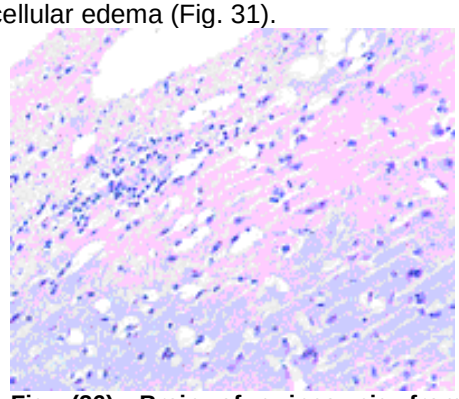

Fig. (26): Brain of guinea pig from group 2 (30 days PT) showing focal gliosis. (H & E X 200).

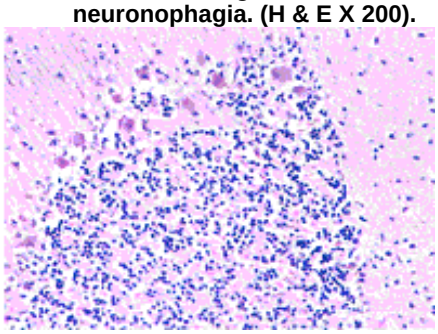

Fig. (27): Brain of guinea pig from group 3 (10 days PT) showing necrosis of Purkinje cells of the cerebellum. (H & E X 200).

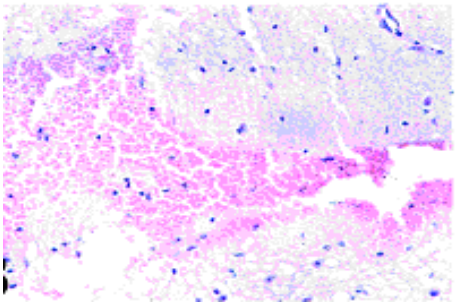

Fig. (28): Brain of guinea pig from group 3 (30 days PT) showing focal cerebral hemorrhages. (H & E X 200).

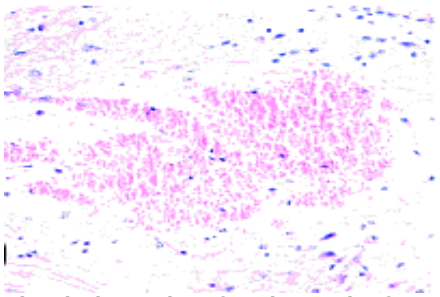

Fig. (29): Brain of guinea pig from group 8 (10 days PT) showing central chromatolysis of neurons. (H & E X 200).

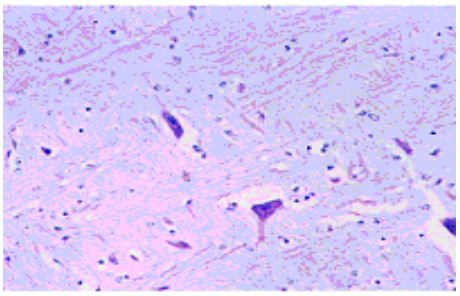

Fig. (30): Brain of guinea pig from group 13 (10 days PT) showing focal cerebral hemorrhage. (H & E X 200).

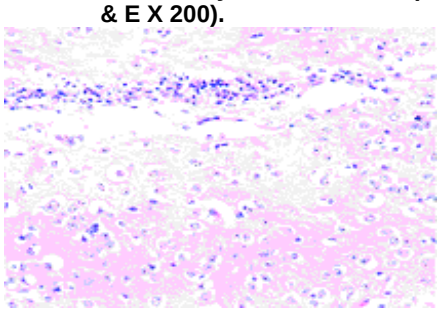

Fig. (31): Brain of guinea pig from group 15 (30days PT) showing focal gliosis and intracellular edema (neuronal edema). (H & E X 200).

DISCUSSION

In the present investigation, treatment of GPs by low and high dose of AFs 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 injury by aflatoxicosis was recorded in various study by Kandil *et al.* (1991) in chicken; Abdelhamid *et al.* (1995) in chicks; Dwiveddi *et al.* (1993); Seawright *et al.* (1993); Souza *et al.* (1999) and Abdeen *et al.* (2004) in rats; EL Zahar *et al.* (1996) and Hassanein and Abdel Gawad (2001) in rabbits and Shank *et al.* (1971) and Newbrene (1983) in human.

Kidney of GPs was also affected by aflatoxicosis in time and dose dependent manner. The severity of lesions was gradually increased by increase dose and time of treatment.

Such results were previously recorded by Balachadran and Ramarkrishnan, (1987) and Abdelhamid *et al.* (1995) in broiler and Abdeen *et al.* (2004) in rats.

The brain of GPs received AFs at low dose showed neural degeneration and neurophagia at 10 day post treatment. More over at 30 day it showed pyknosis of neurons, focal gliosis, and intracellular oedema. Beside previous changes brain of group treated by high dose of AFs showed focal gliosis, necrosis of purkinje cells of cerebellum after 10 day of treatment and focal cerebral hemorrhages after 30 days of treatment.

Toxic effect of AFs on brain was previously mentioned by Shank *et al.* (1971); Madhusudhanan *et al*. (2004); Wangikar *et al.* (2004) and Cometa *et al.* (2005).

The toxic effect of AFs on organs was explained by Gutteridge and Halliwell, (1990) who mentioned that, AF 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. And Galvano *et al.* (2001) who reported that AFs produce membrane damage through increased lipid peroxidation. More over Madhusudhanan *et al.* (2004) reported that aflatoxin B_1 (AF B_1) induced significant increase in conjugated diene formation, and lipid peroxidation not only in liver but also in kidney and brain of fish. While Gesing and Karbawink-Lewinska (2008) reported that AF B1 increased lipid peroxidation in liver, lung, brain and testis but not the kidney of male Wister rats injected by AF B_1.

Dietary intake of natural antioxidants could be an important factor in the body's defense mechanism against reactive oxygen species; also many antioxidants are being identified as anticarcinogens (Ames, 1983).

Histopathological examination revealed that the four concentrations of PP which were added to diet did not produce any toxic effect on tested organs.

Results revealed that, addition of PP powder in different concentration to diet were able to prevent the toxic effect of low dose of AFs on liver in all experimental periods. While it succeeded to reduce toxic effect of high dose of AFs on liver in dose and time dependant manner when compared by groups received high AFs dose alone.

While histopathological examination of kidney revealed that addition of PP powder in different concentrations to diet were able to reduce toxic effect of either low or high dose of AFs on kidney in all experimental periods in dose and concentration dependant manner.

Histopathological examination revealed that addition of PP powder in different concentration to diet can reduce toxic effect of AFs on brain, especially at low dose of AFs. But there is no difference between different PP concentrations in ability of protection of brain Whereas brain of groups treated by different concentration of PP showed less degenerative changes than that noticed in groups treated by AFs alone or treated by high dose of AFs plus PP.

The beneficial influence of PP noted in the present study may be attributed to the fact that PP have adsorption properities as EL-Ashtoukhy *et al.* (2008) indicting that PP powder was effective in removal of lead (II) and copper (II) from aqueous solutions.

Also Toklu *et al.* (2007) indicating that PP with its antioxidant and antifibrotic prosperities may be of potential therapeutic value in protecting the liver from fibrosis and oxidative injury due to billiary obstruction. More over Flohe *et al.* (1997) reported that pomegranate peel had antioxidant and protect activities against neurological damage, ulcers, high cholesterol, cancer and arterial plaques. Also protect neonatal rat brain from hypoxia (Loren *et al.,* 2005).

The antioxidant activity shown by the PP may be due to the presence of polyphenols, such as ellagic tannins, ellagic acid and gallic acid which are known for their properities in scavenging free radicals and inhibitory lipid oxidation in vitro (Gil et al. 2000 and Noda *et al*. 2002). 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.

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التـأثير الوقـائى لقشـر الرمـان ضـد التغيـر البـاثولوجى الحـادث بالافلاتوكسـين علـى أنسجه خنازير غينيا.

أمل عبد العزيز أبو حجر و محمد سيد مسعود
المركز الأقليمي للأغذية و الأعلاف - مركز البحوث الزراعية - الجيزة

السموم الفطرية تسبب التسمم الحاد و إحداث سرطانات و نقص المناعة لمعظم الثديات . التلوث الغذائى بالسموم الفطرية يعتبر مشكلة مستمرة .

الرمان يعتبر من أهم مصادر المركبات الحيوية المستخدمة فى الطب الشعبى لعصور عديدة .

لذا كان الهدف من هذه الدراسة هو اختبار التأثير الوقائى المحتمل لمسحوق قشر الرمان ضد التأثير الضار للسموم الفطرية على انسجة خنازير غينيا . تم إستخدام 105 ذكر خنزير غينيا و تقسيمهم إلى 15 مجموعة حيث تركت أول مجموعة كمجموعة ضابطة بينما تلقت ثانى مجموعة جرعة منخفضة من السموم الفطرية أما المجموعة الثالثة فقد عوملت بجرعة مرتفعة بالسموم الفطرية . المجموعة الرابعة و الخامسة و السادسة و السابعة عوملت ب 6% ، 9%، 12% و 15% من مسحوق قشر الرمان على التوالى بينما عوملت المجموعة الثامنة و التاسعة و العاشرة و الحادية عشر بنفس التركيزات السابقة من مسحوق قشر الرمان بجانب جرعة منخفضة من السموم الفطرية و أيضا تمت معاملة المجموعات الثانية و الثالثة و الرابعة عشر و الخامسة عشر بتفس جرعات مسحوق قشر الرمان بجانب جرعة عالية من السموم الفطرية . تم ذبح خنازير غينيا عند اليوم العاشر و الثلاثون بعد المعاملة و أخذت عينات من الكبد و الكلى و المخ بغرض الفحص الباثولوجى .

أظهرت النتائج أن السموم الفطرية تسبب ضررآ حادآ للكبد و الكلى و المخ و وجد أن شدة الاعراض تزيد مع زيادة الجرعة وقت المعاملة . أظهرت النتائج ايضا عدم وجود أى تاثير ضار لمسحوق قشر الرمان على الانسجة . أظهرت كل تركيزات قشر الرمان المستخدمة تاثير وقائى ضد التضرر الحادث فى كبد خنازير غينيا المعاملة بجرعة منخفضة من السموم الفطرية و أيضا إستطاع مسحوق قشر الرمان أن يخفض تأثير الجرعة العالية للسموم الفطرية على الكبد بشكل يتناسب مع تركيز مسحوق قشر الرمان . أيضا قشر الرمان له تأثير وقائى ضد الضرر الحادث فى الكلية و المخ خاصة فى الجرعات المنخفضة للسموم الفطرية . و وجد أيضا أن ليس هناك إختلاف بين التركيزات المختلفة من قشر الرمان فى القدرة على حماية المخ بينما أظهرت التركيزات المرتفعة من مسحوق قشر الرمان تأثير وقائى أكثر من التركيزات المنخفضة ضد تسمم الكلى بالجرعة العالية للسموم الفطرية .

Table (1) Composition of the experimental diets:

Ingredient g/kg	Diets															
	Supplemented diet															
	Basal diet	Aflatoxin contaminate diets		Basal Diet supplemented with pomegranate peel at levels				Aflatoxin contaminate basal diets at low level, supplemented with pomegranate peel at levels				Aflatoxin contaminate basal diets at high level, supplemented with pomegranate peel at levels			
		Low	High	6%	9%	12%	15%	6%	9%	12%	15%	6%	9%	12%	15%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Casein	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300
Sucrose, Granulated	50.0	46.74	43.47	50.0	50.0	50.0	50.0	46.74	46.74	46.74	46.74	43.47	43.47	43.47	43.47
Sucrose, powdered	196	196	196	156	136	116	96.0	156	136	116	96.0	156	136	116	96.0
Glucose	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150
Corn oil	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Cellulose	150	150	150	130	120	110	100	130	120	110	100	130	120	110	100
L-arginine	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral mixture	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0
Vitamin mixture	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
Choline chloride	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Myo-inositol	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
AF. contaminate corn	0.0	3.26	6. 53	0.0	0.0	0.0	0.0	3.26	3.26	3.26	3.26	6.53	6.53	6.53	6.53
pomegranate peel	0.0	0.0	0.0	60	90	120	150	60	90	120	150	60	90	120	150
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

***Minerals ingredients (g/kg diet): $CaHPO_4$, 34.92; $CaCO_3$,5.94; $KC_2O_2H_3$,2493; KCL, 7.74; NaCl, 5.76; MgO,4.96; $MgSO_4$, 4.59; Fe citrate, 0.64; MnSO4.H2O, 0.37; KIO_3, 0.015; $ZnCO_3$, 0.13; $CuSO_4$, 0.005; $KCr(SO_4)2$, $12H_2O$, 0.010; $NaMoO_4.H_2O$, 0.0005; $NiCl_2.6H_2O$,0.0002; Na_2SeO_3, 0.0002.**

****Vitamin mixture supplied (mg/kg diet):ascorbic acid, 2.000; biotin, folic acid, 10; niacinamide, 200; Ca-pantothenate, 40; pyridoxine-HCl, 16; riboflavin, thiamin-HCl, 16; vitaminB_{12}(0.1%trituration in mannitol), 50; retinal palmitate in oil, 52(5.200 IU); cholecalciferol, 0.04; DL-tocopherol acetate, 209; menadione, 2.**