

## Evaluation of radical scavenging properties and the protective role of papaya fruits extracts against oxidative stress in rats fed aflatoxin-contaminated diet

Nabila Salah Hassan<sup>1</sup>, Khaled Gmalel-din Abdel-Wahhab<sup>2</sup>, Yasser Ashery Khadrawy<sup>2</sup>  
Aziza Abdel-Salam El-Nekeety<sup>3</sup>, Fathia Abdel-Wahed Mannaa<sup>2</sup>, Mosaad Attia Abdel-Wahhab<sup>3\*</sup>

<sup>1</sup>Medical Pathology Department, National Research Center, Dokki, Cairo, Egypt

<sup>2</sup>Medical Physiology Department, National Research Center, Dokki, Cairo, Egypt

<sup>3</sup>Food Toxicology & Contaminants Departments, National Research Center, Dokki, Cairo, Egypt

\*Corresponding author, e-mail: [mosaad\\_abdelwahhab@yahoo.com](mailto:mosaad_abdelwahhab@yahoo.com)

### Abstract

This study aimed to determine the total phenols and DPPH scavenging properties of aqueous (AE) and ethanolic (EE) extracts of papaya fruits *in vitro* and to evaluate their hepatoprotective effects against aflatoxicosis *in vivo*. Sixty female Sprague-Dawley rats were divided into six groups and treated for 4 weeks including the control group, the group fed aflatoxins-contaminated diet (2 mg/kg diet); the group treated orally with EE (250 mg/kg b.w); the group treated orally with AE (250 mg/kg b.w); the groups fed AFs-contaminated diet and treated with EE or AE. At the end of the treatment period, blood and liver samples were collected for biochemical, histological and histochemical study. The results revealed that AE has higher total phenol content and DPPH scavenging activity if compared to EE. The *in vivo* results indicated that animals fed AFs-contaminated diet showed significant biochemical, histological and histochemical changes typical to those reported in the literature. Animals treated with the extracts and AFs showed a significant improvement in all biochemical parameters, histological and histochemical picture of the liver. This improvement was more pronounced in the group treated with AE. It could be concluded that papaya fruits can be used as a functional dietary ingredient to reduce hepatotoxicity.

**Keywords:** Aflatoxins, mycotoxins, oxidative stress, antioxidant, *Carica papaya*

### Avaliação das propriedades radicais de limpeza e o papel protetor de extratos de frutos de mamão contra o estresse oxidativo em ratos alimentados com dieta contaminada com aflatoxinas.

#### Resumo

Este estudo teve como objetivo determinar o teor de fenóis e propriedades de remoção de DPPH em extratos aquosos (AE) e etanólicos (EE) de frutos de mamão *in vitro* e avaliar seus efeitos hepatoprotetores contra aflatoxicose *in vivo*. Sessenta fêmeas Sprague-Dawley foram divididas em seis grupos e foram tratadas durante 4 semanas, sendo o grupo de controle, o grupo alimentado com a dieta contaminada com aflatoxinas (2 mg / kg de dieta), o grupo tratado oralmente com EE (250 mg / kg de peso corporal); o grupo tratado por via oral com AE (250 mg / kg de peso corporal), os grupos alimentados com dieta AFS contaminado e tratado com EE ou AE. No final do período de tratamento, as amostras de sangue e do fígado foram colhidas para estudos bioquímicos, histológicos e histoquímicos. Os resultados revelaram que AE tem maior teor de fenóis totais e atividade DPPH se comparado a EE. Os resultados *in vivo* indicaram que os animais alimentados com a dieta AFS contaminado mostraram significativas alterações bioquímicas, histológicas e histoquímicas, típicas dos relatos na literatura. Os animais tratados com os extratos e AFS apresentaram uma melhora significativa em todos os parâmetros bioquímicos, quadro histológico e histoquímico do fígado. Esta melhoria foi mais pronunciada no grupo tratado com AE. Pode-se concluir que os frutos de mamoeiro podem ser usados como um ingrediente funcional na dieta para reduzir a hepatotoxicidade.

**Palavras-chave:** aflatoxinas, micotoxinas, estresse oxidativo, antioxidantes, *Carica papaya*

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## Introduction

As ubiquitously natural mycotoxins, aflatoxins (AFs) are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (Cary et al., 2005; Whitlow & Hagler 2004; Wu et al., 2009). Contamination of food and feed by AFs is a global problem. Consumption of food or feed contaminated with AFs was reported to cause many severe health problems including hepatotoxicity, teratogenicity, immunotoxicity, and cancers (Trail et al., 1995; Abdel-Wahhab et al., 2005, 2007, 2010). AFB<sub>1</sub> is the most potent and hazardous feed/food-originated mycotoxin (Meki et al. 2004; Wu et al. 2009). IARC accepts AFB<sub>1</sub> as a group I carcinogen, as it is very commonly found as a carcinogenic and oxidative agent (Cotty & Bhatnagar, 1994).

AFs are considered to be one of the major risk factors of human hepatocellular carcinoma (Mcglynn et al., 1995). It was also reported that dietary and inhalation exposure to AFB<sub>1</sub> increases the risk of lung cancer (Dvorackova & Polster, 1984; Massey et al., 2000). AFs are also considered to be biological weapons which can ensure direct exposure to aflatoxins or poison the foodstuff (Massey et al., 1995). Moreover, recent findings have demonstrated that oxidative damage is one of the underlining mechanisms for AFB<sub>1</sub>-induced cytotoxicity and carcinogenicity (Abdel-Wahhab et al., 2010; El-Nekkety et al., 2011; Hathout et al., 2011). Avoid of AFs is not an only health problem but it has also economic impacts such as causing loss of billions of dollars in farming which turns to be a big burden on a country's economy. Occurrence of aflatoxins contaminated crops varies depending on the country's geographical location in the world. It was shown that the highest levels of AFs exist in the countries which are in tropical and subtropical regions (Verma, 2004) and a high frequency of human liver cancer in many developing countries has been established (Bababunmi et al., 1978; Bhat et al., 1997).

The papaya (*Carica papaya* L.) is a tropical fruit that is widely cultivated and consumed, both for its agreeable flavor as well as for its many pharmacological properties (de Oliveira & Vitória, 2011). Papaya have a biological activities as immunostimulating and antioxidant

activity (Aruoma et al., 2006; Mehdipour et al., 2006); abortifacient activity (Cherian, 2000; Sarma & Mahanta 2000); post-testicular anti-fertility drug (Lohiya et al., 2000); treating wounds and burns (Mahmood et al., 2005); anthelmintic activity (Steppek et al., 2004); and bacteriostatic activity (Osato et al., 1993). These benefits have been attributed, at least in part, to the amount of antioxidant compounds present in these foods, which reduce the oxidative stress produced by free radicals, and in consequence, cellular damage (Dosil-Díaz et al., 2008). Some of the most important antioxidant compounds present in fruits and vegetables include polyphenols, carotenoids, and vitamin C (Yahia, 2010).

Phenolic compounds are aromatic metabolites of plants secondary metabolism that have a common structure with an aromatic ring with at least one hydroxyl group, which provides the ability to neutralize reactive species, helping the body to protect itself from oxidative stress (Wojdyło et al., 2009). Additionally, phenols contribute to fruits' color and taste and they have been described as possessing anticarcinogenic and antimutagenic activity (Al-Duais, 2009; Gorinstein et al., 2009). Various studies have shown that phenolic compounds have high antioxidant potential, resulting in a beneficial effect to human health (Reddy et al., 2010). The aims of the current study were to evaluate the total phenolic content and DPPH scavenging activity of the aqueous and ethanolic extract of papaya and to evaluate the protective effects against aflatoxins-induced liver damage and oxidative stress in rats.

## Material and Methods

### Chemicals and kits

AFs standards and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Luis, Mo, USA). Kits of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were obtained from Randox Laboratories LTD Co. (UK.). Total antioxidant capacity (TAC) kit was obtained from Biodiagnostic Co. (Giza, Egypt). Kits for cholesterol (Cho), triglycerides (TriG), high-density lipoprotein (HDL-cho) and low-density lipoprotein (LDL-cho) were

purchased from DiaSys Diagnostic System GmbH, (Germany). Other chemicals were of the highest purity commercially available.

#### *Plant materials*

*Carica papaya* fruits were collected from a private plantation located in Qalubia region (Egypt), during June 2010. The fruits were identified in the Fruits Department, National Research Centre and the voucher kept in the herbarium of NRC. The amount of plant used was 500 g.

#### *Preparation of C. papaya extracts*

Fresh fruits of *C. papaya* were cut into small pieces, dried and finally ground with a blender into powder form. A crude ethanolic extract was prepared by soaking and stirring the powder in absolute ethanol (200 g/500 ml ethanol) for 3 days. The extract was filtered and the residue re-extracted twice, using ethanol. The pooled extract was vacuum-dried at 40 °C. Aqueous extract was prepared by maceration process. The filtrate was subjected to lyophilization process using Freeze Dryer system (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS system, Inc., Stone Ridge, NY, USA) under pressure, 0.1 to 0.5 mbar and temperature -35° to -41°C conditions. Both the dry ethanolic and aqueous extracts were stored at -20 °C until analysis. These procedures resulted in an approximate yield of 13% (w/w) of ethanolic extract (EE) and 27 % (w/w) of aqueous extract (AE) based on the dry weight.

#### *Determination of total phenolic compounds*

Five mg of the extract were dissolved in a 10 ml mixture of acetone and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10- fold diluted Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using V-530 UV/visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents was carried out using standard curve of catechin (Jayaprakasha et al., 2003).

#### *Determination of radical scavenging activity by DPPH assay*

Certain of crude extracts were dissolved

in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol and 1 ml DPPH solution ( $6.09 \times 10^{-5}$  mol/L), in the same solvent, was then added. The absorbance of the mixture was measured at 516 nm after 10 min standing. The reference sample (blank) was 1 ml of DPPH solution and 4 ml methanol. Triplicate measurements were made and the antioxidant activity was calculated by the percentage of DPPH that was scavenged according to Nogala-Kalucka et al. (2005).

#### *Aflatoxins production*

The aflatoxins (AFs) was produced through the fermentation of maize by *Aspergillus parasiticus* NRRL 2999 as described by Stubblefield et al. (1967). The fermented maize was autoclaved; ground to a fine meal, and the AFs content was measured by the use of HPLC (Hustchins & Hagler 1983). The AFs within the maize meal consisted of 45% B<sub>1</sub>, 12% B<sub>2</sub>, 30% G<sub>1</sub>, and 13% G<sub>2</sub> based on total AFs in the maize powder. The maize meal was incorporated into the basal diet to provide the desired level of 2 mg of total AFs/Kg diet. The diet containing AFs was analyzed and the presence of parent AFs was confirmed by HPLC. The safety measures recommended by WHO (1998) were taken when handling the AFs-contaminated diet.

#### *Experimental animals*

Three months old Sprague-Dawley female rats (100-120 g) were purchased from the Animal House Colony, Giza, Egypt, and they were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy 12.08 MJ) in artificial illumination and in a temperature controlled room free from any other source of chemical contamination at the Animal House Laboratory, National Research Center, Dokki, Cairo, Egypt. After an acclimatization period of 1 week, the animals were divided into six groups (10 rats/group) and housed in filter-top polycarbonate cages. All animals have received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt.

### *Experimental design*

Animals within treatment groups were maintained on their respective diets for 4 weeks, as follows: group 1, untreated control; group 2, fed AFs-contaminated diet (2 mg/kg diet); group 3, treated orally with EE (250 mg/kg b. w.); group 4, treated orally with AE (250 mg/kg b.w.); group 5 and 6, fed AFs-contaminated diet and treated orally with EE or AE.

The animals were observed daily as for signs of toxicity. At the end of the experimental period, fasting blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia. The blood samples were left to clot and then centrifuged at 3000 rpm for 15 minutes to separate blood sera, which was used for the determination of different biochemical parameters.

After the collection of blood samples, all animals were rapidly killed and the liver's left lobe of each animal was dissected into two parts, one was placed immediately in 10% formalin-saline buffer for the histopathological examination and the other one was washed with saline and immediately homogenized in ice-cold buffer containing 50 mM tris-HCl and 300 mM sucrose (pH 7.4) (Tsakiris et al., 2000) to give 10% w/v homogenate. The homogenate was centrifuged at 3000 rpm at 0°C for 10 minutes and the supernatant was stored at -20 °C to the second day until analysis. The supernatant (10%) was used for the determination of malondialdehyde (MDA) and total antioxidant capacity levels and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity.

### *Biochemical analysis*

The sera were used for the determination of AST, ALT, ALP activities, triglycerides, total cholesterol, HDL- cholesterol and LDL-cholesterol levels according to the kits instructions. Lipid peroxidation in liver homogenate was estimated manually by measurement of MDA by the spectrophotometric method described by Ruiz-Larrea et al. (1994). Determination of total antioxidant capacity (TAC) in liver homogenate was carried out as described by Koracevic et al. (2001). Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in liver homogenate was assayed according to the modified chemical method that described by Tsakiris et al. (2000).

### *Histological study*

Samples of liver were excised and fixed in natural formalin and were hydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections (5 µm thick) were cut and stained with hematoxylin and eosin (HX & E) for the histological examination (Drury & Wallington 1980). Another liver section from all groups was stained with Bromophenol blue technique to demonstrate total protein contents (Mazia et al., 1953).

### *Statistical analysis*

All data for biochemical analyses were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller & Duncan 1969). All statements of significance were based on probability of  $P \leq 0.05$ .

## **Results**

### *The In vitro study*

The results of the current study revealed that total phenolic compounds determined were 403 and 285 g/kg extract for the aqueous and ethanolic extract, respectively. The DPPH radical scavenging activity (SA) of the aqueous extracts was 26.5 % and 18.87% for ethanolic extract.

### *The biological assay*

The effects of different treatments on liver function are depicted in Table 1. The results indicated that animals fed AFs-contaminated diet showed a significant increase in ALT, AST and ALP if compared to the control group. The aqueous extract alone did not induce any significant change in ALT or AST, however; it decreased significantly the ALP activity if compared to the control group. Animals treated with the ethanolic extract alone have showed a significant decrease in ALT and ALP accompanied with a significant increase in AST. Animals fed AFs-contaminated diet and treated with the extracts have showed a significant improvement in liver enzyme activities although they were still higher than the control except ALP in the group treated with the water extract which was comparable

to the control level. This improvement was more pronounced in the group fed AFs-contaminated diet and treated with the aqueous extract.

**Table 1.** Effect of aqueous and ethanolic extract of papaya fruits on liver function in rats fed with AFs-contaminated diet.

Groups Parameters	Control	AFs	Aqueous extract	Ethanolic extract	AFs + Aqueous extract	AFS + Ethanol extract
ALT (IU/L)	70.15 ± 0.99 <sup>a</sup>	99.68 ± 5.24 <sup>b</sup>	68.15 ± 1.12 <sup>a</sup>	66.0176 ± 1.34 <sup>c</sup>	81.44 ± 2.08 <sup>d</sup>	85.59 ± 2.04 <sup>e</sup>
AST (IU/L)	70.37 ± 2.48 <sup>a</sup>	151.92 ± 3.27 <sup>b</sup>	71.35 ± 2.61 <sup>a</sup>	108.17 ± 4.12 <sup>c</sup>	136.75 ± 6.16 <sup>d</sup>	179.27 ± 3.31 <sup>e</sup>
ALP (IU/L)	117.04 ± 0.57 <sup>a</sup>	144.78 ± 2.16 <sup>b</sup>	95.88 ± 3.81 <sup>c</sup>	97.54 ± 3.24 <sup>c</sup>	119.75 ± 0.25 <sup>a</sup>	131.33 ± 3.22 <sup>d</sup>

Within each row, means superscript with different letters are significantly different (P≥0.05)

The results of the current study revealed that aflatoxin induce significant effects on lipid profile in rats. Data presented in Table 2 indicated that animals fed AFs-contaminated diet have showed a significant increase in total cholesterol, triglycerides and LDL-cholesterol accompanied with a significant decrease in HDL-cholesterol. The aqueous extract succeeded to induce a significant decrease in total cholesterol, triglycerides and LDL-cholesterol and increased HDL-cholesterol insignificantly. The ethanolic extract alone induced a significant decrease in total cholesterol and a significant increase in triglycerides, however; it did not induce any

significant changes in HDL and LDL. Animals fed AFs-contaminated diet and treated with the aqueous extract have showed a significant improvement in lipid profile and this treatment succeeded to normalize total cholesterol and HDL. On the other hand, treatment with the ethanolic extract during the ingestion of AFs-contaminated diet resulted in a significant improvement in lipid profile but did not normalize them. Generally, the improvement resulted from the treatment with the extracts revealed that aqueous was more pronounced than the ethanolic extract.

**Table 2.** Effect of aqueous and ethanolic extract of papaya fruits on serum lipid profile in rats fed with AFs-contaminated diet.

Groups Parameters	Control	AFs	Aqueous extract	Ethanolic extract	AFs + Aqueous extract	AFS + Ethanol extract
Ch (mg/dl)	89.80 ± 1.49 <sup>a</sup>	149.09 ± 3.19 <sup>b</sup>	80.56 ± 3.43 <sup>c</sup>	82.88 ± 3.25 <sup>c</sup>	84.25 ± 2.01 <sup>a</sup>	119.34 ± 1.76 <sup>c</sup>
TriG (mg/dl)	81.21 ± 2.46 <sup>a</sup>	115.69 ± 1.39 <sup>b</sup>	75.43 ± 4.96 <sup>c</sup>	92.02 ± 3.19 <sup>d</sup>	105.21 ± 0.72 <sup>d</sup>	111.89 ± 2.96 <sup>b</sup>
HDL-Ch (mg/dl)	20.20 ± 1.21 <sup>a</sup>	8.5 ± 0.59 <sup>b</sup>	22.36 ± 1.02 <sup>a</sup>	19.26 ± 1.71 <sup>a</sup>	19.39 ± 2.06 <sup>a</sup>	16.33 ± 2.33 <sup>c</sup>
LDL-Ch (mg/dl)	44.93 ± 1.49 <sup>a</sup>	88.81 ± 3.61 <sup>b</sup>	40.21 ± 3.55 <sup>b</sup>	43.87 ± 5.29 <sup>a</sup>	47.35 ± 3.57 <sup>c</sup>	49.96 ± 3.37 <sup>c</sup>

Within each row, means superscript with different letters are significantly different (P≥0.05)

The effect of different treatments on oxidative stress markers are presented in Table 3. The results indicated that animals treated with AFs have showed a significant decrease in total antioxidant capacity level and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity accompanied with a significant increase in MDA. TAC level and Na<sup>+</sup>/K<sup>+</sup>-ATPase level in the groups treated with the two extracts alone were

comparable to the control group, however; MDA level was decreased significantly in these groups. The combined treatment with AFs and the aqueous or ethanolic extract could normalize TAC and improved MDA and N<sup>+</sup>/K<sup>+</sup>-ATPase and this resultant improvement was more pronounced in the group treated with the aqueous extract.

**Table 3.** Effect of aqueous and ethanolic extract on total antioxidant capacity (TAC), lipid peroxidation level (MDA) and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in liver of rats fed with AFs-contaminated diet.

Groups Parameters	Control	AFs	Aqueous extract	Ethanolic extract	AFs + Aqueous extract	AFS + Ethanol extract
TAC (μmol/g liver)	27.83 ± 0.4 <sup>a</sup>	16.67 ± 0.15 <sup>b</sup>	27.36 ± 0.32 <sup>a</sup>	26.93 ± 0.19 <sup>a</sup>	27.52 ± 0.51 <sup>a</sup>	26.05 ± 0.22 <sup>a</sup>
MDA (nmol/g liver)	107.23 ± 5.02 <sup>a</sup>	186.59 ± 7.09 <sup>b</sup>	77.88 ± 4.42 <sup>c</sup>	86.67 ± 1.15 <sup>d</sup>	91.43 ± 2.33 <sup>e</sup>	115.01 ± 4.9 <sup>f</sup>
Na <sup>+</sup> /K <sup>+</sup> -ATPase (μmol pi/hr/g liver)	259.05 ± 5.06 <sup>a</sup>	230.83 ± 2.77 <sup>b</sup>	252.58 ± 4.31 <sup>a</sup>	259.38 ± 2.02 <sup>a</sup>	243.30 ± 2.49 <sup>c</sup>	245.34 ± 3.42 <sup>c</sup>

Within each row, means superscript with different letters are significantly different (p≥0.05)



The biological results were further confirmed by the histological examinations. The microscopic examination of the liver sections of the control group and those treated with the aqueous or ethanolic extracts have showed normal hepatocytes and central vein (Figure 1 a, b, c). The liver section of the animals in the AFs group showed fatty degeneration and focal necrosis in the hepatocytes with fibrous tissues deposited in the portal tract (Figure 1d). The same sections showed large fatty droplets, necrosis and vacuolar degeneration in the hepatocytes in the central area (Figure 2a). The histological examination of the liver in the group fed AFs-contaminated diet and treated with the aqueous extract have showed prominent improvement in liver cells around the central veins. However, few hepatocytes in the portal area are still showing vacuolar degeneration and pyknotic nuclei (Figure 2b). The liver of the animals fed with the AFs-contaminated diet and treated with the ethanolic extract have showed a decrease in fatty droplets and prominent improvement in liver cells (Figure 2c) accompanied with prominent improvement in liver cells around the portal area with few eosinophilic cells (Figure 2d).

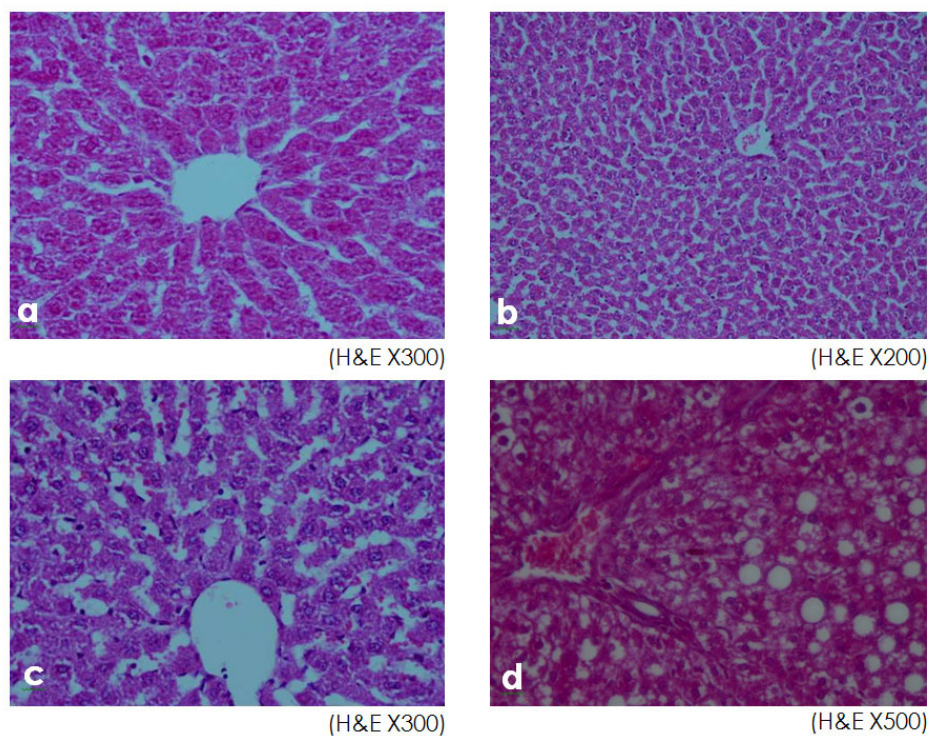
The histochemical inspection of total protein content in the liver tissue revealed that the control liver has showed normal distribution of protein reaction in the cytoplasm of hepatic cells (Figure 3a). The liver sections in the animals treated with the aqueous extract have showed an increase in protein reaction in the cytoplasm of hepatic cells (Figure 3b). However, those treated with the ethanolic extract have showed a decrease in protein reaction in the cytoplasm of hepatic cells (Figure 3c). The histochemical examination of the liver section in the group fed with AFs-contaminated diet have showed a marked decrease in protein reaction in the cytoplasm of hepatic cells (Figure 3d). The liver sections in the group fed the AFs-contaminated diet and treated with the aqueous extract of papaya fruits have showed a marked improvement in protein blue reaction around the central vein (Figure 3e). Moreover, the liver sections in the group fed with the AFs-contaminated diet and treated with the ethanolic extract have showed a marked improvement in protein contents (Figure 3f).

## Discussion

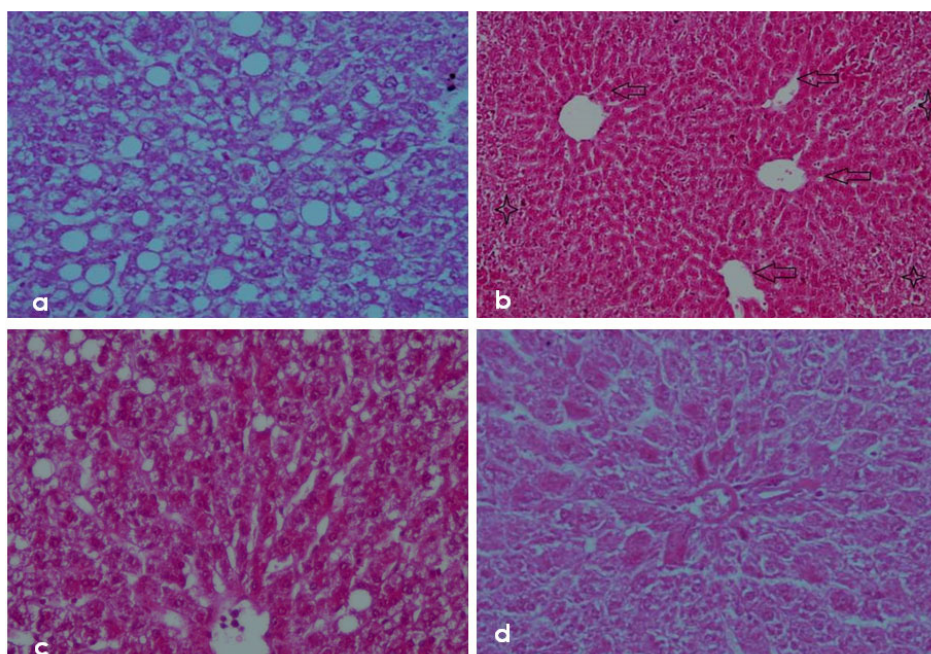
Hepatocarcinogenesis is a multistage, multifactorial process, involving viral, chemical and several other factors, including aflatoxins, alcohol and tobacco consumption, and familiar tendencies (Williams et al., 2004). Nevertheless, special emphasis has been placed on aflatoxin, due to the frequency with which they occur as food contaminants, together with their potency as liver carcinogens in numerous animal species (Abel-Wahhab et al., 2010; Abdel-Azim et al., 2011). Previously, we have reported that aflatoxin administration resulted in excessive lipid peroxidation (El-Nekkety et al., 2011; Hathout et al., 2011) with concomitant decrease in reduced glutathione (GSH) (Abdel-Wahhab & Aly, 2003, 2005; Abdel-Wahhab et al., 2010), increased protein oxidation and DNA damage (Abdel-Azim et al., 2011) in rat liver.

In the current study, we have determined the total phenols and the DPPH radical scavenging activity of the aqueous and ethanol extract of papaya fruits *in vitro* and we have evaluated the protective role of these extracts against aflatoxins induced liver damage and oxidative stress *in vivo*. The *in vitro* results indicated that both aqueous and ethanolic extracts of papaya were rich in total phenols although these phenols were higher in the aqueous extract than in the ethanolic extract. Both extracts have showed a high DPPH radical scavenging activity and this activity was more pronounced in the aqueous extract. The DPPH radical can be scavenged by carotenoids (Jimenez-Escrig et al., 2000) and the differences in phenolic composition in the two extracts may be more associated with the higher level of DPPH in papaya aqueous extract (Babu et al., 2010; Sancho et al., 2011).

Previous reports have indicated that papaya contain  $\alpha$ -tocopherol (Ching & Mohamed, 2001), lycopene (van Breemen & Pajkovic, 2008), benzylisothiocyanate (Basu & Haldar, 2008), proteolytic enzymes such as papain and chymopapain (Seigler et al., 2002), alkaloids such as carpain and carpasemine (Iyer et al., 2011), triterpenes, organic acids (Cowan, 1999; Osuna-Torres et al., 2005), cystatin, ascorbic acid, cyanogenic glucosides and glucosinolates (Seigler et al., 2002) and flavonoids (Miean &

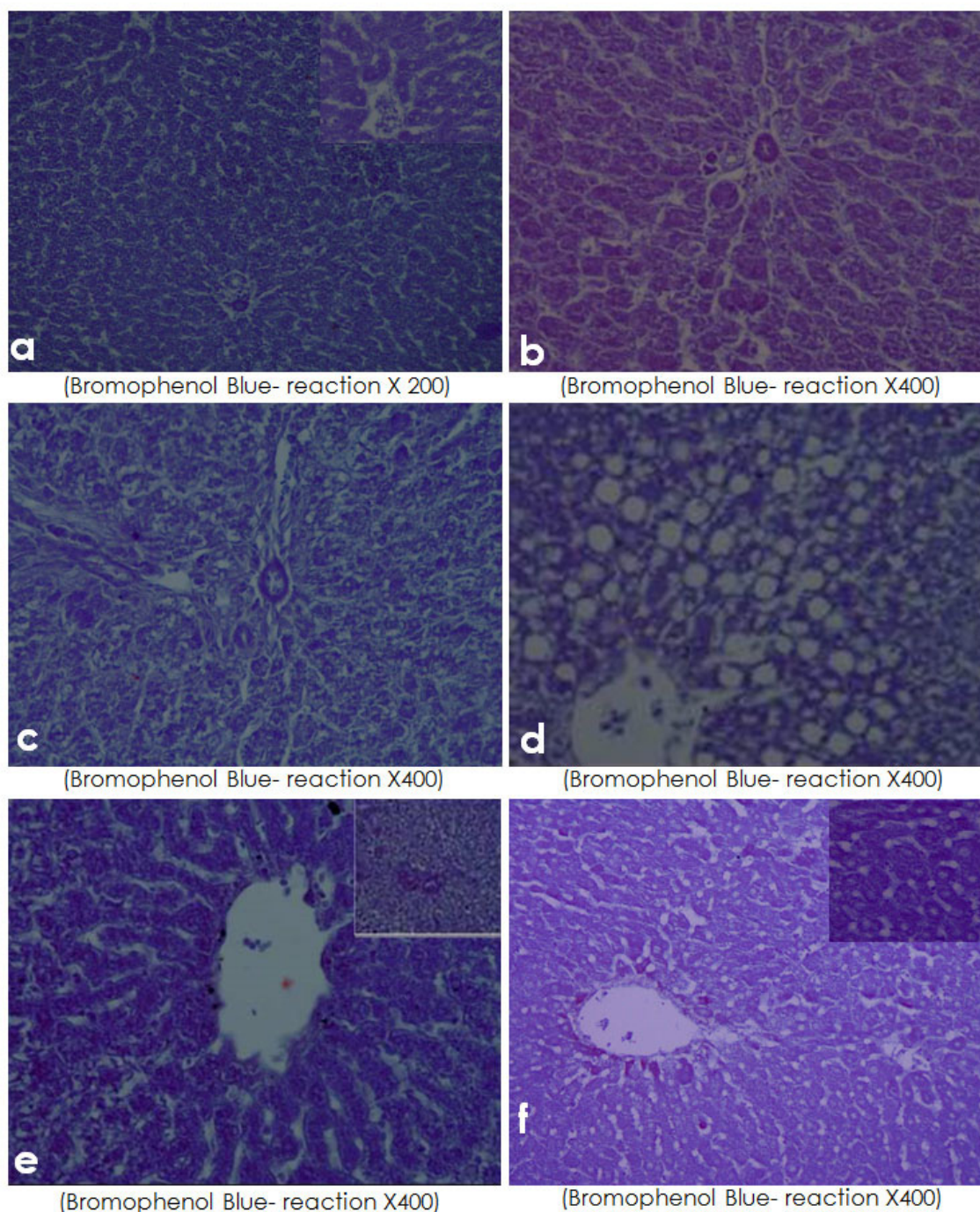


**Figure 1.** A photomicrograph of liver section from (a) control rat showing normal hepatocytes and central vein, (b) rats treated with the aqueous extract showing normal hepatocytes and central vein, (c) rats treated with the ethanol extract showing normal hepatocytes and central vein and (d) rats fed with AFS-contaminated diet showing hepatocytes fatty degeneration and focal necrosis and the fibrous tissues are deposited in the portal tract.



**Figure 2.** A photomicrograph of liver section from (a) rats fed with AFS-contaminated diet showing large fatty droplets, necrosis and vacuolar degeneration in the hepatocytes in the central area, (b) rats fed with AFS-contaminated diet and treated with the aqueous extract showing prominent improvement in liver cells around the central veins (arrow). Few hepatocytes in the portal area are still showing vacuolar degeneration and pyknotic nuclei (Stars), (c) rats fed with AFS-contaminated diet and treated with the ethanol extract showing decreased in fatty droplets and prominent improvement in liver cells and (d) rats fed with AFS-contaminated diet and treated with the ethanol extract showing prominent improvement in liver cells around the portal area with a few eosinophilic cells (H&E X500).





**Figure 3.** Photomicrographs in a section of liver from: (a) control rat showing the normal distribution of protein reaction in the cytoplasm of hepatic cells. The inset showing magnified cells, (b) rat treated with the aqueous extract showing increase in protein reaction in the cytoplasm of hepatic cells, (c) rat treated with the ethanol extract showing decrease in protein reaction in the cytoplasm of hepatic cells, (d) rat fed with AFs-contaminated diet showing marked decrease in protein reaction in the cytoplasm of hepatic cells, (e) rat fed with AFs and treated with the aqueous extract showing marked improvement in protein blue reaction around the central vein. The inset revealed marked decrease in protein reaction in the cytoplasm of hepatic cells around the portal tract and (f) rat fed with AFs and treated with the ethanol extract showing marked improvement in protein contents. The inset revealed high power of hepatic cells demonstrates the distribution of reaction.



Mohamed, 2001). Moreover, the ethanolic extract is known to contain sulfurous compounds (benzyl isothiocyanate). Beside the total soluble phenols, Mahattanatawee et al. (2006) have reported that papaya is rich in total ascorbic acid, total dietary fiber, and pectin. These components provide a protection against cellular damage caused by exposure to high levels of free radicals (Ames et al., 1993; Dillard & German, 2000; Prior & Cao 2000), while also aids digestion (Weisburger et al., 1993; AACC, 2001). Moreover, Otsukia et al. (2010) have reported that Papaya is a good source of vitamins A, C, E and K as well as folate and fiber, in addition it is fat-free, cholesterol-free and low in sodium, what suggest its potential benefit effects.

In the *in vivo* study, the selected dose of aflatoxins was based on our previous work (Abdel-Wahhab and Aly 2005); however, the selected doses of the aqueous and ethanolic extract were based on the work of Raj Kapoor et al. (2002). The results of the current study have revealed that animals fed with AFs-contaminated diet have showed severe biochemical, histological and histochemical changes, typical to those reported in the literature of aflatoxicosis. The increased activities of ALT, AST and ALP reported herein have been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and they are released into plasma as result of autolytic breakdown or cellular necrosis into circulation after cellular damage (Recknagel et al., 1989; Abdel-Wahhab et al., 2006, 2007, 2010). The elevation in Cho, TriG and LDL-Cho with the reduction in the level of HDL-Cho in AFs-treated group are coincided with those reported previously in AFs-ingested animals (Obasi et al., 1996; Abdel-Wahhab et al., 2005, 2006, 2007). This elevation in serum Cho, TriG and LDL-Cho are probably associated with biliary obstruction and acute hepatic injury (Obasi et al., 1996; Hathout et al., 2011; El-Nekeety et al., 2011).

It is well documented that the biotransformation of AFs in the liver by microsomal enzymes results in the generation of toxic metabolites, including AFB<sub>1</sub>-8,9-epoxide. The toxic effects of AFs mostly arise from the binding of this particular epoxide derivative to DNA

(Chan et al., 2003; Rawal et al., 2010; Abdel-Azim et al., 2011). Reactive oxygen species such as the hydroxyl radical, superoxide anion and hydrogen peroxide, which are generated as a result of this metabolism, are also involved in the toxic mechanism of AFs (Choi et al., 2010; El-Agamy, 2010). Further, one of the toxic mechanisms of aflatoxin include its consuming, to a great extent, the stocks of vitamin A and glutathione, which are strong radical scavengers; and its inhibiting the synthesis of protein (Adedara et al., 2010; Abdel-Wahhab et al., 2010). The decrease in total antioxidant capacity (TAC) and the increase in MDA level in the liver of the AFs-treated rats might indirectly lead to an increase in oxidative DNA damage (Abdel-Wahhab et al., 2010; Choi et al., 2010). Moreover, the reduced level of TAC may be explained by the association of glutathione peroxidase (GPX) with AFs or its metabolites (Abdel-Wahhab et al., 2010). These results are in agreement with those reported previously by Meki et al. (2004) and Verma & Mathuria (2009), who have stated that the increased MDA level with the concomitant decreased in antioxidant enzyme activities were the most pronounced markers for AFB<sub>1</sub> toxicity and carcinogenicity. In the same concern, several studies on the mechanisms of AFs-induced liver injury have demonstrated that glutathione plays an important role in the detoxification of the reactive and toxic metabolites of AFs, and the liver necrosis begins when the glutathione stores are almost exhausted (Mitchell et al., 1973; Abdel-Wahhab et al., 2005, 2006, 2010), what may explain the decrease in TAC and the increase in MDA level.

The sodium-potassium adenosine triphosphatase (Na<sup>+</sup>-K<sup>+</sup>-ATPase) is an integral membrane enzyme found in all cells and it is responsible for the ATP-dependent transport of sodium and potassium across the cell membrane (Kulkarni et al., 2002). Na<sup>+</sup>-K<sup>+</sup>-ATPase drives the transport of amino acids and sugars. Emphasis on the early pathogenesis of hepatotoxicity was established with respect to lowered Na<sup>+</sup>-K<sup>+</sup>-ATPase activity. In the present study, animals fed with AFs-contaminated diet have showed a significant decrease in Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, which indicated the damage of cell membrane and hepatic cell necrosis due to the oxidative

stress. Similar to these observations, Pascale et al. (1989) have reported a reduction in Na<sup>+</sup>-K<sup>+</sup>-ATPase in the liver of rat treated with ethanol. These finding indicated that Na<sup>+</sup>-K<sup>+</sup>-ATPase may be considered as a marker for assessing hepatocellular damage induced by AFs.

The histological and histochemical results reported herein indicated that aflatoxin has induced hepatocytes fatty degeneration, focal necrosis and depletion of protein content. These results are supported the biochemical findings and they are in agreement with those reported earlier (Mayura et al., 1998, Abdel-Wahhab et al., 1998, 2010; Yener et al., 2009; Ilic et al., 2010; El-Nekeety et al., 2011; Hathout et al., 2011).

The results of the *in vitro* study were confirmed by the *in vivo* results. Animals treated with the aqueous extract alone have showed a significant decrease in ALP, Cho, TriG, LDL-Cho and MDA, whereas, AST, ALT, HDL-Cho, Na<sup>+</sup>-K<sup>+</sup>-ATPase and TAC were comparable to the control. On the other hand, treatment with the ethanolic extract alone resulted in a significant decrease in ALT, AST, ALP, Cho, TriG, HDL-Cho and MDA, whereas, LDL-Cho, Na<sup>+</sup>-K<sup>+</sup>-ATPase and TAC were comparable to the control group. These results suggested that the aqueous extract was better than the ethanol extract. In this concern, Raj Kapoor et al. (2002) have reported that the LD<sub>50</sub> value of ethanolic and aqueous extracts of papaya were 2426.37 and 2516.53 mg/kg, respectively, what indicates that the aqueous extract is safer than the ethanol one. Although, both extracts induced a significant decrease in MDA, the changes in most of the other parameters were within the normal clinical ranges reported in the literature (Lillie et al., 1996; Kohn & Clifford, 2002). Similar to the current results, Raj Kapoor et al. (2002) reported that papaya extract improve liver function through the production of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells. Moreover, Agarwal et al. (1992) have reported a decrease in the levels of serum total lipids, serum total cholesterol, LDL-C, VLDL-C, HDL-C, phospholipids and triglycerides in normal rabbits suggesting a remarkable protective and hypolipidemic effects of papaya fruit. Generally, animals fed with AFs-contaminated diet and

treated with the two extracts have showed a significant improvement in liver function indices, lipid profile, antioxidant status, lipid peroxidation, and the histological and histochemical picture of the liver. This improvement was more pronounced in the group treated with the aqueous extract.

It is well documented that flavonoids represent the common and widely distributed group of plant phenolics. They are free radical scavengers and super antioxidants, which prevent oxidative cell damage and have strong anticancer activity (Salah et al., 1995). Flavonoids also provide anti-inflammatory action (Okwu, 2001a,b) and high potential to hydroxyl groups (Olabinri et al., 2010). Similar to the current observations, Srikanth et al. (2010) have reported that the aqueous extract of papaya has showed antioxidant activity. Moreover, Imaga et al. (2010) have indicated that the aqueous extract of papaya has showed anti-tumor effect and inhibited the proliferative responses of solid and haematopoietic tumor cell lines derived from cervical carcinoma, breast adenocarcinoma, hepatocellular carcinoma, lung adenocarcinoma, pancreatic epithelioid carcinoma and mesothelioma in a dose-dependent manner.

Another mechanism for the protective role of papaya extracts against AFs-induce liver damage may be through the enhanced production of Th1 type cytokines such as IL-12, IFN- $\alpha$  and TNF- $\alpha$  or through inducing a shift from Th2 to Th1 type immune response (Basu & Haldar 2008). Furthermore, Santiago-Silva et al. (2011) have reported that papaya is a good source of serotonin, which has been associated with enabling the gut to mediate reflex activity and also with decreasing the risk of thrombosis. These authors did not detect histamine, a peripheral vasodilator, and the vasoconstrictors tyramine, tryptamine and phenylethylamine, as well as cadaverine in the fruits. Consequently they have suggested that these fruits can provide different functional properties and can be used for different nutritional needs due to their diversity of amines content.

## Conclusions

The current study has revealed that both aqueous and ethanolic extract of papaya fruits are rich in total phenols and has showed a potential DPPH radical scavenging activity. Both extract succeeded to induce a hepatoprotective effect against AFs-induced liver damage and oxidative stress. This protection was more pronounced in the animals treated with the aqueous extract suggesting the potential role of total phenolic content consequently; papaya fruits can be used as a functional dietary ingredient to reduce hepatotoxicity in the high incidence area.

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## Ethical approval

All animals have received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt.

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