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Original Article

Panax ginseng C.A. Meyer extract counteracts the oxidative stress in rats fed multi-mycotoxins-contaminated diet

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Abstract

The current study was conducted to evaluate the protective effects of *Panax ginseng* extract (PGE) against the toxicity and oxidative stress in rats fed aflatoxin (AFs) and/or fumonisin (FB)-contaminated diet. Eighty female Sprague-Dawley rats were divided into eight experimental groups included the control group, the group treated orally with PGE (0.5 mg/kg b.w.) and the groups fed AFs (1.4 mg/kg diet) and/or FB (20 mg/kg b.w.) contaminated diet alone or plus PGE for 11 weeks. Blood, liver and kidney tissue samples were collected at the end of treatment period for biochemical and histological studies. The results indicated that PGE increased super oxide dismutase (SOD) level in liver; however, the other parameters were comparable to controls. Animals fed AFs and/or FB-contaminated diet showed a significant increase in serum biochemical parameters and oxidative stress markers accompanied with a significant decrease in antioxidant parameters levels and a severe histological changes in the liver tissue. These changes were more pronounced in the group fed AFs plus FB. PGE succeeded to induce a significant improvement in all biochemical parameters and the histological picture towards the control although it did not normalize them. It could be concluded that PGE is a promise candidate against the exposure to multi-mycotoxins in food.

Keywords: Aflatoxins, fumonisin, ginseng, oxidative stress, mycotoxins, histopathology

Extrato de *Panax ginseng* C.A. Meyer neutraliza o stress oxidativo em ratos alimentados com dieta contaminada com multi-micotoxinas

Resumo

O presente estudo foi conduzido para avaliar os efeitos protetores do extrato de *Panax ginseng* (PGE) contra a toxicidade e estresse oxidativo em ratos alimentados com aflatoxinas (AFs) e/ou dieta contaminada por fumonisina (FB). Oitenta fêmeas de ratos Sprague-Dawley foram divididas em oito grupos experimentais, incluindo o grupo controle, o grupo tratado com PGE oralmente (0,5 mg/kg de peso corporal) e os grupos alimentados com as dietas contaminadas de AFs (1,4 dieta mg/kg) e/ou FB (20 mg/kg de peso corporal) isoladas ou com mais PGE durante 11 semanas. Amostras de sangue, do fígado e do tecido do rim foram recolhidas no final do período de tratamento para estudos bioquímicos e histológicos. Os resultados indicaram que a PGE aumenta o nível de superóxido dismutase (SOD) no fígado, no entanto, os outros parâmetros foram comparáveis aos do grupo controle. Animais alimentados com dieta contaminada por AF e/ou FB mostraram um aumento significativo nos parâmetros bioquímicos séricos e marcadores de estresse oxidativo, acompanhados de uma diminuição significativa nos níveis de antioxidantes e nos parâmetros de alterações histológicas graves no tecido do fígado. Essas alterações foram mais pronunciadas no grupo alimentado com AFs mais FB. PGE obteve sucesso quanto à introdução de uma melhoria significativa em todos os parâmetros bioquímicos e histológicos com relação ao grupo controle, embora não os tenha normalizado. Pode-se concluir que a PGE é uma promissora candidata contra a exposição às multimicotoxinas em alimentos.

Palavras-chave: aflatoxinas, fumonisinas, ginseng, estresse oxidativo, micotoxinas, histopatologia

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Introduction

Mycotoxins constitute a group of secondary metabolites of moulds, especially of *Penicillium, Aspergillus* and *Fusarium* genera (Moss, 1997), Among them, aflatoxins (AFs) and fumonisins B (FB) are a matter of concern due to their widespread contamination of cereal grain commodities, corn, in particular, and their adverse effects on human and animal health (Bernabucci et al., 2011). AFs are the most potent carcinogenic substance naturally produced by *Aspergillus* species and AFB_1 was classified by the International Agency of Research on Cancer as Group 1 human carcinogen (IARC, 1993). In all species and tissues tested to date, mutagenicity, carcinogenicity and DNA-binding activity of AFB_1 appear to result from its activation by cytochrome P450 enzymes to produce AFB_1- 8,9-epoxide (WHO, 2002).

FB is produced by toxigenic fungi belonging to the genus *Fusarium* and has been associated with various diseases in animals, such as equine leucoencephalomalacia, immunosuppression, porcine pulmonary oedema, liver and kidney toxicity and liver cancer, as well as human oesophageal carcinoma in some African and Chinese populations (Gelderblom et al., 1997). FB_1 is hepatocarcinogenic in rats while other studies reported the nephrocarcinogenicity and cancer promoting activity in rats (El-Nekeety et al., 2007). FB can disrupt sphingolipid metabolism, which plays a role in membrane and lipoprotein structure and in cell regulation as second messengers for growth factors, differentiation factors, and cytokines (Abdel-Wahhab et al., 2004). Individual mycotoxicoses occur seasonally on certain areas that hinder an implementation of an effective prophylactic measure (Pfohl-Leszkowicz et al., 2002). However, interactions between given mycotoxins are still unclear. The presence of a mixture of these toxins may present a problem in terms of determining clinical symptoms of an individual mycotoxicosis.

To date, mechanisms of action through which mycotoxins can cause toxicity are not completely clarified. However, a possible mechanism is the induction of oxidative stress (El-Nekeety et al., 2007). It is well known that some mycotoxins may induce the production of free radicals and/or the reduction of antioxidant defenses. Since during toxicity, no oxidative damages are often observed (Bernabucci et al., 2011) , a first question is to establish if the onset of oxidative stress *per se* has to be regarded as a cause or as a consequence of the action of toxicants on the cellular system.

Panax ginseng C.A. Meyer is one of the most widely used medicinal plants, particularly in traditional oriental medicine, and it has a wide range of pharmacological and physiological actions (Slifman et al., 1998). Previous studies have shown that ginseng enhances immune response (Kim et al., 1999), antioxidant (Abdel-Wahhab et al., 2004) and anti-tumor activity in humans and in laboratory animals (Surh, 2001). Moreover, recent epidemiological studies have demonstrated that ginseng intake is associated with a reduced risk of environmentally related cancers (Xiaoguang et al., 1998). However, few studies have examined its preventive or therapeutic potential against the toxic effects of environmental contaminants such as mycotoxins (Abdel-Wahhab et al., 2011).

The aims of the current study were to elucidate the synergistic toxic effects resulted from the consumption of food contaminated with aflatoxins and fumonisin and to evaluate the protective role of Korean *Panax ginseng* extract in rats.

Material and Methods

Chemicals and Kits

Aflatoxins (AFs) and fumonisin B_1 (FB) standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits of Transaminase (ALT, AST), cholesterol (Cho), triglycerides (TriG), total lipids (TL), Nitric oxide (NO), Malondialdehyde (MDA), Total antioxidant capacity (TAC), Super Oxide Dismutase (SOD) and Uric acid were Obtained from Biodiagnostic (Giza, Egypt). Kits of alkaline phosphatase (ALP) and Creatinine were obtained from Quimica Clinica Aplicada (SA, Spain). Kits of alpha fetoprotein (AFP) were obtained from Immunospec Corporation (Canoga Park, CA, USA). Other chemicals were of the highest purity commercially available.

Plant materials

Panax ginseng was provided by Korean Society of ginseng, Seoul, Korea.

Preparation of Panax ginseng extracts (PGE)

Water extract of PGE was prepared according to the method described by Gum et al. (2007). The plant was extracted with 10 volumes of distilled water at 85 °C for 8 hours five times. The aqueous extracts were combined and concentrated under reduced pressure to give darkish brown syrup with moisture content of 37.21%. The ginsenosides content in the PGE was determined by HPLC, according to Ko et al. (1989), as follows: An aliquot of PGE dissolved in distilled water was passed through Sep-Pak C_{18} cartridge and the cartridge was washed with distilled water. Subsequently, ginsenosides were eluted with 90% methanol and then analyzed by HPLC. The concentrations of ginsenosides in PGE in mg/g were Rg1 (0.54), Rg2 (3.16), Rg3 (4.04), Rh1 (0.88), Rh2 (0.11), Rb1 (3.72), Rb2 (1.71), Re (0.95), Rf (1.02), Rc (1.89), Rd (1.32) with total ginsenosides 19.3 mg/g (Abdel-Wahhab et al., 2010).

Mycotoxins production

AFs were produced through the fermentation of corn by *Aspergillus parasiticus* NRRL 2999, according to Stubblefield et al.

(1967). However, FB was produced through the fermentation of corn by *Fusarium* verticillioides (=*F. moniliforme*), according to Voss et al. (1993).

Experimental Animals

Three-months old Sprague-Dawley female rats (100-120 g) were purchased from Animal House Colony Giza, Egypt and were maintained on standard lab diet (protein: 160.4; fat: 36.3 and fiber 41g/kg). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., National Research Centre, Dokki, Cairo, Egypt. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Dokki, Cairo, Egypt.

Experimental Design

Animals within different treatment groups (10 animals/ group) were maintained on their respective diets for 11 weeks, as follows: group 1, untreated control; group 2, treated orally with PGE (0.5 mg/kg b.w.); group 3, fed AFs-contaminated diet (1.4 mg/kg diet); group 4, fed FB-contaminated diet (20 mg/kg diet); group 5, fed on mixture of AFs and FB-contaminated diet (1.4 mg/kg and 20 mg/kg diet respectively); group 6, fed on AFs- contaminated diet and treated orally with PGE; group 7, fed on FB-contaminated diet and treated orally with PGE and group 8, fed on mixture of AFs and FB -contaminated diet and treated orally with PGE.

The animals were observed daily for any signs of toxicity. Body weight and feed intake were recorded daily throughout the experimental period. At the end of the experimentation period (i.e. day 78), blood samples were collected from all animals within different treatment groups from retro-orbital venous plexus for the determination of ALT, AST, ALP, TriG, Cho, TL, creatinine, uric acid, NO and AFP, according to the kits instructions.

After blood samples were collected, all animals were killed and samples of liver and kidney tissues of each animal were dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate. This homogenate was centrifuged at 1700 rpm and 4 °C for 10 min and the supernatant was stored at -70 °C for the determination of lipid peroxidation (LP) by measuring the formed malondialdehyde (MDA) using thiobarbituric acid reactive substances method, according to the kits instructions. The level of lipid peroxidation was expressed as nmol MDA per gram tissue. The liver homogenate was further diluted to give 5% homogenate (w/v), centrifuged at 3000 rpm for 5 min at 0°C and used for the determination of SOD and TAC. Other 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 (H & E) for the histological examination. Feulgen stain was used to demonstrate DNA contents. The optical densities (OD) of Feulgen stain were measured using a computerized microscopic image analyzer and Image Pro Plus software.

Statistical analysis

All data 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 the probability of $p \leq 0.05$.

Results

No animal mortality was observed in any of the treatment groups except for two died rats in the group fed AFs plus FB-contaminated diet. No significant differences in food intake were observed between the control animals and those treated with PGE alone. Whereas, animals fed AFs and/or FB-contaminated have shown a significant decrease in food intake compared to the control or the PGE alone-treated group. This decrease was pronounced in the group fed AFs plus FB-contaminated diet followed by those fed AFs-contaminated diet alone. Animals fed on the mycotoxins contaminated diet singly or in combinations and treated with PGE have shown a significant improvement in food intake. Interestingly, food intake was higher in animals fed FB alone-contaminated diet and treated with PGE compared to the control group (Figure 1). Animals fed AFs and/or FB-contaminated diet or those fed AFs and treated with PGE have shown a significant decrease in body weight gain if compared to the control group (Figure 2). Whereas, animals fed FB alone or AFs plus FB-contaminated and treated with PGE have shown significant improvement in body weight although they were still lower than the control group. The body weight was started to increase significantly in the group fed FB-contaminated diet and treated with PGE at the 8th week and continued till the end of treatment period.

The current results indicated that animals treated with PGE alone have shown a significant decrease in ALT, ALP activity, Creatinine and uric acid levels (Table 1). Animals fed AFs and/or FB-contaminated diet showed severe stress on both liver and kidney functions as indicated by significant increase in ALT, ALP, AST, creatinine and uric acid if compared to the control group. PGE succeeded to induce a significant improvement in these parameters although ALP, creatinine still higher than control. On the other hand, animals treated with PGE alone have shown a significant decrease in Cho, TriG and TL. However, animals fed AFs and/or FB-contaminated diet have shown significant increase in lipid profile (Table 2). PGE succeeded to induce a significant improvement in lipid profile.

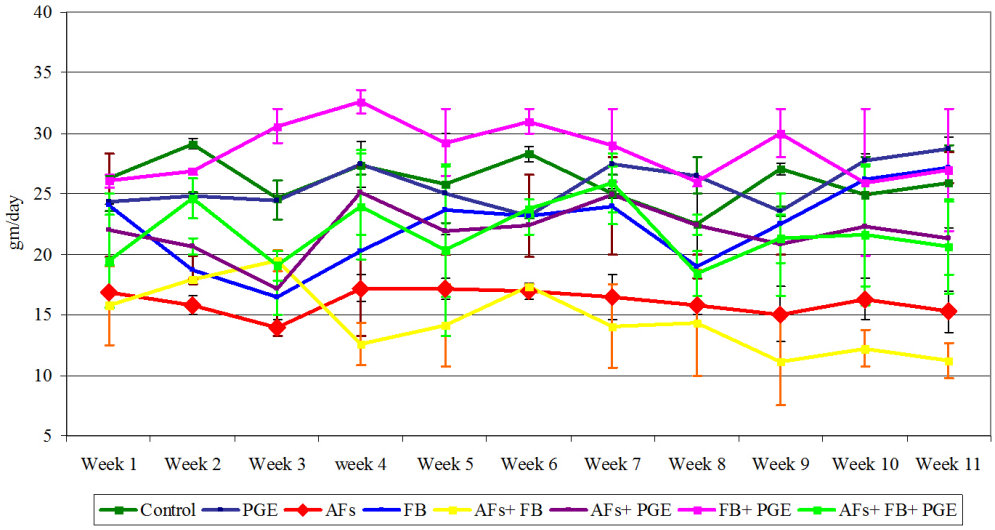

Figure 1. Changes in food intake during the experimental period in rats fed AFs and/or FB-contaminated diet for 11 weeks with or without PGE.

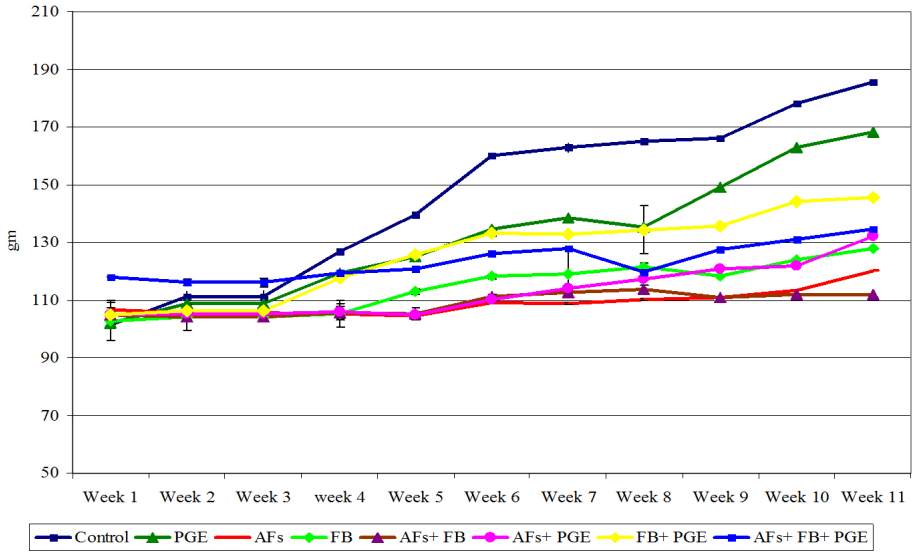

Figure 2. Changes in body weight gain in rats fed AFs and/or FB-contaminated diet for 11 weeks with or without PGE.

Table 1. Effects of PGE treatment on serum biochemical parameters in rats fed AFs and/or FB-contaminated diet. Within each column, means superscript with different letters are significantly different ($p \leq 0.05$).

Groups Parameters	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Createnine (mg/dl)	Uric acid (mg/dl)
Control	32.2 ± 0.8^{a}	59.4 ± 4.23^{a}	70 ± 3.74^{a}	0.71 ± 0.15^{a}	0.99 ± 0.25^{a}
PGE	25.2 ± 1.88^{b}	57.2 ± 3.89^{a}	67.16 ± 1.58^{b}	0.62 ± 0.13^{b}	0.77 ± 0.13^{b}
AFs	61.8 ± 3.9^{c}	93 ± 3.70^{b}	102.76 ± 4.13^{c}	1.38 ± 0.11^{c}	7.02 ± 0.3^{c}
FB	45.4 ± 1.16^{d}	78.2 ± 3.2^{c}	87.24 ± 2.40^{d}	1.02 ± 0.05^{d}	5.7 ± 0.34^{d}
AFs + FB	73.4 ± 1.12^{e}	117.4 ± 8.63^{d}	131.68 ± 4.97^{e}	1.9 ± 0.19^{e}	10.59 ± 1.02^{e}
AFs + PGE	44.8 ± 1.56^{f}	71.4 ± 0.93^{e}	79.7 ± 0.71^{f}	0.98 ± 0.02^{f}	4.42 ± 0.23^{f}
FB + PGE	35.75 ± 2.09^{a}	60.4 ± 3.00^{a}	70.38 ± 1.85^{a}	0.82 ± 0.03^{g}	2.5 ± 0.46^{g}
AFs + FB + PGE	48.4 ± 2.18^{d}	78.8 ± 2.26^{c}	84.62 ± 1.56^{d}	1.0 ± 0.04^{h}	5.41 ± 0.24^{d}

Table 2. Effects of PGE treatment on lipid profile of rats fed AFs and/or FB-contaminated diet. Within each column, means superscript with different letters are significantly different (p≤ 0.05).

Groups Parameters	Total lipid (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	437.75 ± 24.69[a]	292.8 ± 31.68[a]	146.6± 14.03[a]
PGE	378.52 ± 14.86[b]	221.4 ± 13.6[b]	146.5 ± 8.78[a]
AFs	946.39 ± 28.77[c]	592.82 ± 24.39[c]	499.9 ± 24.76[b]
FB	582.1 ± 12.18[d]	471.22 ± 37.77[d]	253.31 ± 21.46[c]
AFs + FB	1364.05 ± 18.8[e]	1014.24 ± 48.7[e]	602.37 ± 25.31[d]
AFs + PGE	610.68 ± 28.80[f]	456.92 ± 26.00[d]	318.16 ± 12.01[e]
FB + PGE	382.1 ± 21.33[b]	349.95 ± 42.66[f]	177.72 ± 9.60[f]
AFs + FB + PGE	635.68 ± 34.24[g]	749.95 ± 50.01[g]	330.85 ± 21.16[e]

The effects of different treatments on NO, AFP, SOD and TAC are presented in Table (3). The data have shown that animal treated with PGE alone were comparable to the control regarding the levels of NO, AFP, TAC in liver tissue; whereas, SOD level in liver has shown a significant increase. Animals fed AFs and/or FB-contaminated diet have shown a significant increase in serum NO and AFP, accompanied with a significant decrease in SOD and TAC in liver if compared to controls. Moreover, animals fed AFs and/or FB-contaminated diets have shown a significant increase in MDA in liver and kidney (Table 4). This increase was much higher in the group fed AFs plus FB-contaminated diet followed by those fed AFs alone than the group fed FB-contaminated diet. PGE administration succeeded to induce a significant improvement in MDA in both liver and kidney of animals treated with the mycotoxins singly or in combination. This improvement was much higher in liver tissue of the group fed AFs plus FB followed by the group fed AFs than that fed FB-contaminated diet.

Table 3. Effect of PGE treatment on alpha-fetoprotein (AFP) and Nitric oxide (NO) in serum and antioxidant parameters in liver of rats fed AFs and/or FB-contaminated diet. Within each column, means superscript with different letters are significantly different (p≤ 0.05).

Groups Parameters	NO (µmol/L)	AFP (ng/ml)	SOD (u/mg protein)	TAC (mol/g protein)
Control	21.36 ± 3.11[a]	4.3 ± 0.28[a]	324.84± 12.54[a]	0.09 ± 0.002[a]
PGE	20.71 ± 2.88[a]	4.42 ± 0.18[a]	349.92 ± 15.35[b]	0.0954 ± 0.003[a]
AFs	79.08 ± 3.49[b]	12.2 ± 0.14[b]	87.27 ± 15.32[c]	0.0458 ± 0.002[b]
FB	46 ± 1.48[c]	9.3 ± 0.091[c]	174.96 ± 12.54[d]	0.0632 ± 0.001[c]
AFs + FB	97.5 ± 3.96[d]	14.55 ± 0.28[d]	74.76 ± 12.51[e]	0.0264 ± 0.001[d]
AFs + PGE	48.56 ± 2.65[c]	6.65 ± 0.52[e]	212.4 ± 15.24[f]	0.065 ± 0.0021[c]
FB + PGE	25.18 ± 1.44[a]	5.9 ± 0.21[e]	274.77 ± 15.32[g]	0.0734 ± 0.001[e]
AFs + FB + PGE	50.46 ± 1.92[c]	8.5 ± 0.33[c]	199.95 ± 12.45[f]	0.0454 ± 0.002[b]

Table 4. Effect of PGE on MDA in liver and kidney of rats fed AFs and/or FB-contaminated diet. Within each column, means superscript with different letters are significantly difference (p≤ 0.05).

Groups Parameters	MDA (mol/mg protein) Liver	 Kidney
Control	68.93 ± 1.56[a]	7.18 ± 0.28[a]
PGE	67.73 ± 2.54[a]	8.22 ± 0.80[a]
AFs	139.09 ± 10.8 2[b]	76.28 ± 3.47[b]
FB	106.61 ± 7.72[c]	64.28 ± 3.47[c]
AFs + FB	186.94 ± 9.48[d]	100.51 ± 4.00[d]
AFs + PGE	79.72 ± 3.99[e]	39.78 ± 3.86[e]
FB + PGE	78.4 ± 4.87[e]	20.16 ± 2.85[f]
AFs + FB + PGE	98 ± 2.75[f]	49.9 ± 2.50[g]

The biochemical results were confirmed by the histological and histochemical study of the liver. The histological examination of the control liver has showen a normal central vein and normal hepatocytes (Figure 3a). The liver sections in the rats treated with PGE have shown normal parenchyma and focal aggregation of lymphocytes (Figure 3b). The histological examination of the liver of the rats fed AFs-contaminated diet has shown massive distribution of fatty degenerative changes in all hepatic tissues (Figure 3c) accompanied with large and small lipid droplets, nuclear pleomorphism, bile ducts dilatation and prominent fibrosis around the blood vessels. The microscopic examination of the liver sections in the rats fed FB-contaminated diet has shown hepatocellular fatty degeneration and prominent fibrosis around the dilated and congested portal tract (Figure 3d). The histological examination of the liver sections of

the rats fed AFs plus FB-contaminated diet has shown prominent increased in hepatocellular fatty degenerative changes and fibrotic blood vessels (Figure 3e). On the other hand, the liver of animals fed AFs-contaminated diet and treated with PGE has shown marked improvement in hepatocytes (Figure 3f). However, the liver of animals fed FB-contaminated diet and treated with PGE has shown marked improvement in hepatocytes architecture (Figure 3g). The liver sections of rats fed AFs plus FB-contaminated diet and treated with PGE has shown the same picture of fatty degenerative changes and fibrosis in the hepatocytes (Figure 3h).

The histochemical results of the liver revealed that the optical density (O.D) values of DNA content in the animals treated with AFs and/or FB have shown a significant decrease in DNA content of hepatocytes. This decrease was more pronounced in the group which received the combined mycotoxins (Figure 4). Animals treated with PGE alone showed insignificant increase in DNA content in the liver cell nuclei. However, DNA content in hepatocytes of the groups fed AFs-contaminated diet and treated with PGE has shown a significant improvement towards the control values.

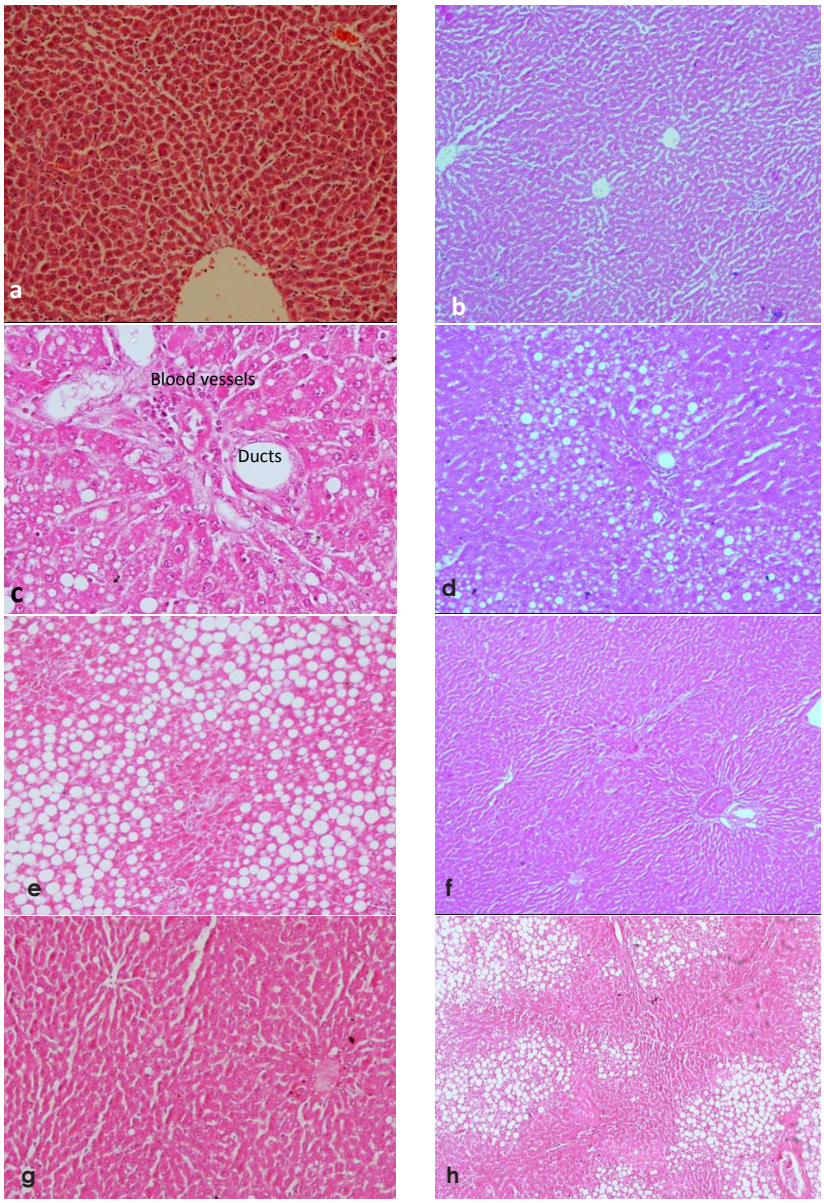

Figure 3. A photomicrograph of liver sections: (a) (HX & E X 300) control rats showing normal central vein and normal hepatocytes, (b) (HX & E X 100) rat treated with PGE showing normal parenchyma and focal aggregation of lymphocytes, (c) (HX & E X 400) rats fed AFs-contaminated diet showing the large and small lipid droplets, nuclear polymorphism, bile ducts dilatation and prominent fibrosis around the blood vessels, (d) (HX & E X 300) rat in the group fed FB-contaminated diet showing hepatocellular fatty degeneration and prominent fibrosis around the dilated and congested portal tract, (e) (HX & E X 100) rat fed AFs plus FB-contaminated diet showing the fatty degenerative changes and fibrotic blood vessels, (f) (HX & E X 100) rat fed AFs-contaminated diet and treated with PGE showing marked improvement in hepatocytes, (g) (HX & E X 200) rat fed FB-contaminated diet and treated with PGE showing marked improvement in hepatocytes architecture, (h) (HX & E X 100) rat fed AFs plus FB-contaminated diet and treated with PGE showing the hepatocytes have the same picture of fatty degenerative changes and fibrosis.

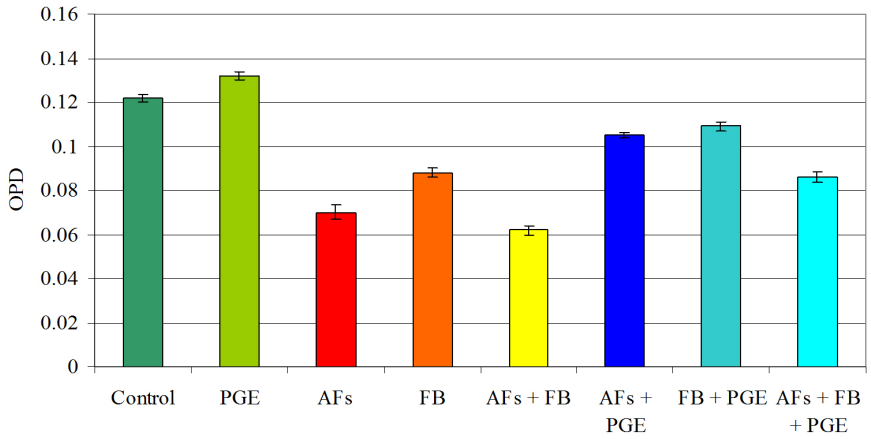

Figure 4. Effect of PGE on the mean optical density (O.D) values of DNA content of hepatocytes of rats fed AFs and/or FB.

Discussion

In the current study, the protective effects of PGE against the toxicity resulted from the exposure to AFs and/or FB in rat model was evaluated. The selected doses of AFs, FB and PGE were based on our previous work (Abdel-Wahhab et al., 2010).

The current results indicated that ingestion of AFs and/or FB resulted in a significant decrease in food intake and consequently the body weight gain was also reduced. Previous work have reported similar decrease in food consumption and body weight in rats fed AFB_1-contaminated diet (Abdel-Wahhab et al., 2006) or FB- contaminated diet. This decrease in feed intake may indicate protein catabolism, consequently contributing to the observed kidney injury and causing impaired glomerular filtration (Abdel-Wahhab et al., 2006).

On the other hand, the decrease in body weight in the animals treated with the mycotoxins alone may be due to the synergistic effects of these mycotoxins on the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation, leading to cachexia (Rastog et al., 2001). Rats treated with AFB_1 have shown a significant decrease in leptin, what is usually associated with high levels of cortisol and IL-6 and together act to influence the feeding response (Barber et al., 2004). This correlation may explain the recorded decrease in body weight reported here.

The current results indicated that PGE alone had no significant effects on serum biochemical parameters of liver and kidney function in rats. Animals received the two mycotoxins singly or in combination have shown a significant increase in ALT, AST and ALP. This increase in transaminases in mycotoxins-treated animals is indicative of changes in the hepatic tissues and biliary system (Abdel-Wahhab & Ahmed, 2004). On the other hand, the significant increase in uric acid and creatinine observed in the animals treated with the two mycotoxins may indicate protein catabolism and/or renal dysfunction (Abdel-Wahhab et al., 2006).

These results clearly indicated that AFs has stressful effects on the hepatic and renal tissues, consistent with those reported in the literature of mycotoxicosis. Moreover, El-Nekeety et al. (2007) have reported that rats fed FB-contaminated diet have shown a significant increase in serum transaminases, what indicated necrosis in the liver. In this regard, AST in animal fed FB-contaminated diet was present in high concentrations in muscle, liver, and brain, and serum elevation indicates necrosis or disease of one or more of these tissues (Abdel-Wahhab & Ahmed, 2004).

The significant increase in Cho, TriG and TL in the group which has received mycotoxins alone are coincided with those reported previously in AFB_1 or FB-ingested animals (Abdel-Wahhab et al., 2010). Similar to these observations, El-Nekeety et al. (2007) have reported that cholesterol and triglycerides were significantly higher in rats fed FB-contaminated diet. The present study has also revealed that animals fed AFs and/or FB contaminated-diet have shown a significance increase in AFP level in serum, which is considered an specific biomarker for liver cancer and is synthesized mainly in the fetal stage; practically no production of this marker occurs in normal adult. Similar elevation in serum AFP was reported in rats treated with AFs (Abdel-Wahhab et al., 2006).

In the current study, NO was found to be increased significantly in the animals treated with AFs and/or FB. Although the role of NO in cell death is complex, the increased in NO level reported herein in the animals fed mycotoxins-contaminated diet has suggested that these mycotoxins preferentially affect macrophage functions. On the other hand, the decrease in TAC and SOD in the liver of AFs and/or FB-treated rats might indirectly lead to an increase in oxidative DNA damage (El-Nekeety et al., 2011). Moreover, the reduced level of TAC may be explained by the association of glutathione peroxidase (GPX) with FB or its metabolites. Some studies on the mechanisms of mycotoxins-induced liver injury have demonstrated that glutathione and SOD play an important role in the detoxification of the reactive and toxic metabolites of these mycotoxins, and that the liver necrosis begins

when the glutathione stores are almost exhausted (Abdel-Wahhab et al., 2010).

LP is one of the main manifestations of oxidative damage and it has been found to play an important role in the toxicity and carcinogenicity. However, the antioxidant enzymes represent the major defense system against liver injury and carcinogenesis. Several reports indicated that exposure to either AFB_1 or FB increased LP in liver. In here, AFs and/or FB administration enhanced LP as indicated by the significant increase in MDA level, which directly results of free radical-mediated toxicity (El-Nekeety et al., 2007; Abdel-Wahhab et al., 2010; Gad et al., 2011). Abdel-Wahhab et al. (2006) & Abdel-Azim et al. (2011) have reported that free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce LP, which is considered a key process in many pathological events and is one of the reactions induced by oxidative stress. Another mechanism for FB-induced injury was suggested by Yin et al. (1998), who have stated that FB induced a down-regulation of cytoplasmic phospholipase A_2 ($cPLA_2$) activity and arachidonic acid (AA) metabolism by a mechanism involving prostaglandin production, cAMP synthesis and protein kinase activation (PKA).

The treatment with AFs and/or FB resulted in cirrhotic livers with numerous regenerative and dysplastic nodules encircled extensively by ballooning and fatty degeneration cells and decrease in DNA content. Therefore, AFs and/or FB extensively enhanced the susceptibility of the liver to the toxicity and the induction of dysplastic nodules. It is evident that, in studies of the synergistic interaction of different compounds with respect to certain biological effects, the time of administration of one compound relative to the other will have an important impact on the outcome of the study. Despite the fact that AFs and FB were administrated simultaneously, they seem to act synergistically with respect to cancer initiation as the number of hepatocytes nodular cirrhosis and foci was significantly increased. The underlying mechanism that resulted in the significant increase in the size of damaged area nodular cirrhosis during AFs plus FB treatment could be ascribed to the potent cancer promoting potential of FB (Abdel-Wahhab et al., 2010).

Administration of PGE to rats that received AFs and/or FB-contaminated diet resulted in a significant improvement in food intake, body weight gain and all other biochemical parameters. It was suggested that PGE displays a pronounced hepatoprotective effect, assessed through the transaminases (ALT, AST) activities following hepatotoxicity in rats treated with carbon tetrachloride (Jeong et al., 1997). Treatment of the intoxicated rats with PGE resulted in significant improvement in kidney function as indicated by the marked decrease in serum in uric acid and creatinine levels. These results were in conformity with those reported by Yokozawa & Liu (2000), who have demonstrated that ginsenoside could decrease the severity of renal injury induced by cisplatin.

The current results indicated that treatment with PGE resulted in a significant improvement in lipid profile in mycotoxin-treated animals. Similar results were suggested by Abdel-Wahhab et al. (2010), who have reported an increase in the serum HDL and simultaneously decreases in total serum Cho, LDL and TriG levels in rats caused by ginseng. Therefore, it is suggested that the protective effect of PGE is attributed to its free radical scavenging activity (Surh et al., 2001). It was reported that the non-saponin components of red ginseng suppressed the harmful effects of free oxygen radicals (O_2, H_2O_2, and OH_2), which exercise an important role in tissue degeneration. Zhang et al. (1996) have shown that hydroxyl radical formed by the Fenton reaction were completely inhibited by ginseng extract. This antioxidant effect of ginseng may be responsible for its wide pharmacological actions in clinical practice by a free radical reaction-inhibition mechanism. Therefore, the protective effects of PGE may be related to the antioxidant properties consequently decreased risk for most cancers including carcinomas of the esophagus, stomach, colon, pancreas, lung and liver (Xiaoguang et al., 1998). Recently, Abdel-Azim et al. (2011) have postulated that Ginsenoside Rg1, cinnamic acid, and tanshinone IIA isolated from ginseng could serve as protective agents in cancer prevention and treatment, suppress FAS gene expression and reduce the DNA-fragmentation percentage in AFs-exposed rats.

Animals treated with PGE during the mycotoxins treatment have shown various degree of protection on the histological and histochemical changes in the liver. PGE was also found to decrease the fibrosis area in the liver tissue (Jeong et al., 1997). As suggested previously, the major components of *Panax ginseng* are ginsenosides (Yokozawa & Liu, 2000), which have been shown to have a variety of beneficial effects, including anti-inflammatory, antioxidant, and anticancer effects. More than 95% of protopanaxadiol ginsenosides in natural *P. ginseng* are converted into ginsenoside Rg3 and Rg5 (Panwar et al., 2005); of course, other protopanaxatriol ginsenosides are also converted into their congeners.

The anticancer activities of ginsenoside after oral administration are probably attributable to these metabolites and fatty acid esterification. Bae et al. (2002) have isolated and identified this novel ginseng saponin metabolite after giving ginseng extract to human beings and rats. This novel ginseng saponin was 20-O-(b-D-glucopyranosyl)-20(S)-protopanaxadiol (IH-901, compound K, or M1). Since IH-901 was detected as one of the major metabolites in urine and blood after the oral administration, it is speculated that IH-901 is most likely the major

form of protopanaxadiol saponin absorbed from the intestine (Akao et al., 1998). This compound inhibits glucose uptake by tumor cells and exhibits an anti-metastatic effect *in vivo* (Hasegawa et al., 1997). Moreover, Konoshima et al. (1999) have isolated an ocotillol-type saponin, majonoside-R2 (MR2) from the rhizome and root of ginseng and they have reported that this active constituent exhibited potent anti-tumor-promoting activity on two-stage carcinogenesis test of mouse hepatic tumor. Furthermore, Kim et al. (1999) have suggested that G-Rs4 induces apoptosis through the down regulation of both cyclins E- and A-dependent kinase activity as a consequence of selectively elevating protein levels of p53 and p21WAF1 in SK-HEP-1 cells.

In the same concern, Zeng & Tu (2004) have stated that G-Rh2 may effectively reduce telomerase activity and arresting cell cycle progression. Oh et al. (1999) have suggested that GR-h2 inhibited the growth of MCF-7 cells, by inducing protein expression of p21 and reducing the protein levels of cyclin D which resulted in the down-regulation of cyclin/Cdk complex kinase activity, decreasing phosphorylation of pRb, and inhibiting E2F release. GS-Rh2 was also found to arrest Eca-109 cells at G0/G1 phase and induce cell differentiation tending to normal. Another mechanism was suggested by Chang et al. (1999), who have stated that the panaxadiol fraction and its ginsenosides could induce the antioxidant enzymes, which are important for maintaining cell viability, by lowering the level of oxygen radical generated from intracellular metabolites.

Conclusions

It can be concluded that exposure to AFs and FB induced severe synergistic effects have resulted in the induction of oxidative stress and severe toxicological effects in rats. PGE alone did not induce any toxicity and it succeeded to counteract the toxicity of both mycotoxins and it may be effective as food supplement in the high risk area.

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References

Abdel-Azim, S.H., Hassan, A.M., Abdel-Wahhab, M.A. 2011. Dietary supplementation with whey protein and ginseng extract counteract the oxidative stress and DNA damage in rats fed aflatoxins-contaminated diet. *Mutation Research* 723:65-71.

Abdel-Wahhab, M.A., Ahmed, H.H. 2004. Protective effects of Korean *Panax ginseng* against chromium VI toxicity and free radical generation in rats. *Journal of Ginseng Research* 28: 11-17.

Abdel-Wahhab, M.A., Ahmed, H.H., Hagazi, M.M. 2006. Prevention of aflatoxin B_1-initiated hepatotoxicity in rat by marine algae extracts. *Journal of Applied Toxicology* 26 (3): 229-238.

Abdel-Wahhab, M.A., Hassan, A.M., Amer, H.A., Naguib, K.M. 2004. Prevention of fumonisin-induced maternal and developmental toxicity in rats by certain plant extracts. *Journal of Applied Toxicology* 24: 469 -474.

Abdel-Wahhab, M.A., Hassan, N.S., El-Kady, A.A., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Sharaf, H.A., Mannaa, F.A. 2010. Red ginseng extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats. *Food and Chemical Toxicology* 48: 733-742.

Abdel-Wahhab, M.A.,Gamil, K., El-Kady, A.A., El-Nekeety, A.A., Naguib, K.M. 2011. Therapeutic effects of Korean red ginseng extract in Egyptian patients with chronic liver diseases. *Journal of Ginseng Research* 35: 69-79.

Akao, T., Kida, H., Kanaoka, M., Hattori, M., Kobashi, K. 1998. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. *Journal of Pharmacology and Pharmacotherapeutics* 50:1155-1160.

Bae, E.A., Choo, M.K., Park, E.K., Park, S.Y., Shin, H.Y., Kim, D.H. 2002. Metabolism of ginsenoside Rc by human intestinal bacteria and its related antiallergic activity. *Biological and Pharmaceutical Bulletin* 25:743-747.

Barber, M.D., McMillan, D.C., Wallace, A.M., Ross, J.A., Preston, T., Fearon, C. 2004. The response of leptin, interleukin-6 and fat oxidation to feeding in weight-losing patients with pancreatic cancer. *British Journal of Cancer* 90: 1129-1132.

Bernabucci, U., Colavecchia, L., Danieli, P.P., Basiric, L., Lacetera, N., Nardone, A., Ronchi, B. 2011. Aflatoxin B_1 and fumonisin B_1 affect the oxidative status of bovine peripheral blood mononuclear cells. *Toxicology In Vitro* 25: 684-691.

Chang, M.S., Lee, S.G., Rho, H.M. 1999. Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenoside extracted from *Panax ginseng*. *Phytotherapy Research* 8: 641-644.

El-Nekeety, A.A., El-Kholy, W., Abbas, N.F., Ebaid, A., Amra, H.A., Abdel-Wahhab, M.A. 2007. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. *Toxicon* 50: 256-269.

El-Nekeety, A.A., Mohamed, S.R., Hathout, A.S., Hassan, N.S., Aly, S.A., Abdel- Wahhab, M.A. 2011. Antioxidants properties of *Thymus vulgaris*

oil against aflatoxin- induced oxidative stress on male rats. *Toxicon* 57: 984-991.

Gad, A.S., Khadrawy, Y.M., El-Nekeety, A.A., Mohamed, S.R., Hassan, N.S., Abdel-Wahhab, M.A. 2011. Antioxidant activity and hepatoprotective effects of whey protein and *spirulina* in rats. *Nutrition* 27:582-5.

Gelderblom, W.C.A., Smuts, C.M., Abel, S., Snyman, S.D., Van Der, Westhuizen, L., Huber, W.W., Swanevelderll, S. 1997. Effect of Fumonisin B_1 on the levels and fatty acid composition of selected lipids in rat liver *in vivo*. *Food and Chemical Toxicology* 35: 647-656.

Gum, S.I., Jo, S.J., Ahn, S.H., Kim, S.G., Kim, J.J., Shin, H.M., Cho, M.K. 2007. The potent protective effect of wild ginseng (*Panax ginseng* C.A. Meyer) against benzo (alpha) pyrene-induced toxicity through metabolic regulation of CYPIAI and GSTs. *Journal of Ethnopharmacology* 112 (3): 568-576.

Hasegawa, H., Sung, J.H., Huh, J.D. 1997. Ginseng intestinal bacteria metabolite IH-901 as a new antimetastatic agent. *Archives of Pharmacal Research* 20: 539-544.

IARC. International Agency for Research on Cancer. 1993. Some Naturally Occurring Substances: Food Items, Constituents, Heterocyclic Aromatic Amines, Mycotoxins. In: IARC. *IARC Monographs on the evaluation of carcinogenic risks to humans*. IARC, Lyon, France. p. 249-395.

Jeong, T.C., Kim, H.J., Park, J.I., Ha, C.S., Kim, S.I., Rho, J.K. 1997. Protective effects of red ginseng saponins against carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats. *Planta Medica* 63: 136-140.

Kim, S.E., Lee, Y.H., Park, J.H., Lee, S.K. 1999. Ginsenoside-Rs4, a new type of ginseng saponin concurrently induces apoptosis and selectively elevates protein levels of p53 and p21WAF1 in human hepatoma SK-HEP-1 cells. *European Journal of Cancer* 35: 507-511.

Ko, S.R., Choi, K.J., Kim, S.C., Kim, M.W. 1989. Contents of crude saponin and ginsenosides in white ginsengs. *Korean Journal of Pharmacology* 20: 170-174.

Konoshima, T., Takasaki, M., Ichiishi, E., Murakami, T., Tokuda, H., Nishino, H., Duc, N.M., Kasai, R.K., Yamasaki, K. 1999. Cancer chemopreventive activity of majonoside-R2 from *Vietnamese ginseng, Panax vietnamensis*. *Cancer Letters* 147:11-16.

Moss, M.O. 1997. Economic importance of mycotoxins-recent incidence. *International Biodeterioration and Biodegradation* 27:195-204.

Oh, M., Choi, Y.H., Choi, S., Chung, H., Kim, K., Kim, S.I., Kim, D.K., Kim, N.D. 1999. Anti-proliferating effects of ginsenoside Rh2 on MCF-7 human breast cancer rcells. *International Journal of Oncology*14: 869-875.

Panwar, M., Kumar, M., Samarth, R., Kumar, A. 2005. Evaluation of chemopreventive action and antimutagenic effect of the standardized *panax ginseng* extract, EFLA400, in swiss albino mice. *Phytotherapy Research* 19: 65-71.

Pfohl-Leszkowicz, A., Petk-Bocharova, T., Cherozemsky, I.N., Castegnaro, M. 2002. Balkan endemic nephropathy and associated urinary tract tumors: a review on etiological causes and the potential role of mycotoxins. *Food Additives and Contaminants* 19:282-302.

Rastog, R., Srivastava, A.K., Rastogi, A.K. 2001. Biochemical changes induced in liver and serum of aflatoxin B_1-treated male Wister rats: preventive effect of picroliv. *Pharmacology and Toxicology* 88:53-58.

SAS. 1982. *SAS User's Guide: Statistics*. SAS Institute Inc., Cary, USA.

Slifman, N.R., Obermeyer, W.R., Aloi, B.K., Musser, S.M., Correll, W.A., Cichowicz, S.M., Betz, J.M., Love, L.A. 1998. Contamination of botanical dietary supplements by *Digitalis lanata*. *New England Journal of Medicine* 339 (12): 806-81.

Stubblefield, R.D., Shotwell, O.L., Hesseltine, C.W., Smith, M.L., Hall, H.H. 1967. Production of aflatoxin on wheat and oats: measurement with a recording densitometer. *Applied Microbiology* 15: 186-190.

Surh, Y.J. 2001. Molecular mechanisms underlying anti-tumor promoting activities of heat-processed ginseng. *Journal of Korean Medical Science* 16: S38-S41.

Voss, K.A., Chamberlain, W.J., Bacon, C.W., Norred, W.P. 1993. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B_1. *Natural Toxins* 1: 222-228.

Waller, R.A., Duncan, D.B. 1969. A Bayes rule for the symmetric multiple comparison problems. *Journal of American Statistical Association* 64: 1484-1503.

WHO. *World Health Organization*. 2002. Evaluation of Certain Mycotoxins in Food. *WHO, Technical Report* 906: 16-27.

Xiaoguang, C., Hongyan, L., Xiaohong, L., Zhaodi, F., Yan, L., Lihua, T., Rui, H. 1998. Cancer chemoprevention and therapeutic activities of red ginseng. *Journal of Ethnopharmacology* 60: 71-78.

Yin, J.J., Smith, M.J., Eppley, R.M., Page, S.W., Sphon, J.A. 1998. Effects of fumonisin B_1 on lipid

peroxidation in membranes. *Biochimica et Biophysica Acta* 1371: 134-142.

Yokozawa, T., Liu, Z.W. 2000. The role of ginsenoside-Rd in cisplatin-induced acute renal failure. *Renal Failure* 22: 115-127.

Zeng, X.L., Tu, Z.G. 2004. Effect of telomerase on ginsenoside Rh2-induced differentiation of hepatocarcinoma cell line SMMC-7721. *Ai Zheng Chinese Journal of Cancer* 12:1655-1659.

Zhang, D., Yasuda, T., Yu, Y., Sheng, P., Kawabata, T., Ma, Y., Okada, S.1.1996. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron mediated lipid peroxidation. *Free Radical Biology and Medicine* 1: 145-150.