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Full Length Research Paper

Protective effect of milk thistle and grape seed extracts on fumonisin B1 induced hepato- and nephro-toxicity in rats

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Fumonisin B1 (FB1) is a mold metabolite produced by *Fusarium* species that is frequently found in corn worldwide, it is toxic to both liver and kidney. Hepato- and nephro-toxicity were induced in rats by feeding them with FB1 contaminated corn. Evidence of those toxicities were observed after 60 days by an increase in the serum activity alanine aminotransferase (ALT) to 78%, creatinine to 65% and urea to 30%, in comparison with control group (p = 0.000). Pretreatment with silymarin (S), or grape seeds (G) extracts or both (S+G) was found to return the ALT activity to normal. In case of creatinine, S and S+G lowered the level down to 22 and 24%, respectively and G could successfully return it to normal. The pretreatment S, G, and S+G could significantly reduce urea level to 52, 37 and 46%, respectively. FB1 drastically depleted glutathione peroxidase (GPx) to 48%, while pretreatment with S, G, and S+G could elevate the GPx by 30, 31 and 50%, respectively. Lipid peroxidation represented by malondialdehyde (MDA) was elevated significantly to 137% and the pretreatment with S, G, and S+G altered the levels down to 38, 37, and 44%, respectively. Significant improvement in lipid profile was also observed in all pretreated groups. These improvements might be due to the free radical scavenging properties of S and G and its ability to enhance endogenous antioxidant defenses.

Key words: Fumonisin B1, grape seed extract, silymarin, liver, oxidative stress.

INTRODUCTION

Fumonisins are recently discovered mycotoxins in 1988, their chemical structure and biological activity were elucidated in South Africa. Fumonisins are produced by several *Fusarium* species (Marasas, 2001); amongst these, *Fusarium verticillioides* and *Fusarium proliferatum* are by far the most prolific fumonisin producers (Shephard et al., 1990). Fumonisin B1 is a mycotoxin produced by the fungus *F. verticillioides* known to be the causative agent of several diseases in animals (Marasas, 1996), it causes equine leukoence-phalomalacia (Marasas, 2001) and porcine pulmonary edema (Haschek et al., 2001; Marasas, 2001). In all species tested thus far, the liver and kidney are the main target organs for FB1 (Carlson et al., 2001). Consumption of food containing *F. verticillioides* and or fumonisins has been linked epidemiologically to the high incidence of cancer and other health conditions in some areas of the world where corn is a dietary staple (Rheeder et al., 1992).

Most of the toxicities resulting from exposure to fumonisins can be explained by the ability of the toxins to alter sphingolipid metabolism by inhibiting the enzyme ceramide synthase, an enzyme responsible for the acylation of sphinganine and sphingosine. Structurally, fumonisin resembles sphinganine and sphingosine, free sphingoid bases plays critical roles in cell communication and signal transduction (Desai et al., 2002). The disruption

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Abbreviations: (MDA), Malondialdehyde; **(ALT),** alanine aminotransferase; **(GPx),** glutathione peroxidase; **(G),** grape seed extract; **(S),** silymarin; **SOD,** superoxide dismutase.

of the sphingolipid biosynthetic pathway leads to increased levels of sphingolipid precursors and decreased levels of complex sphingolipids, since fumonisins disrupt sphingolipid biosynthesis, the resulting elevation in the sphinganine or sphingosine ratio in serum, plasma, or urine has been used as a biomarker for estimating dietary exposure to fumonisins in animals (Marasas, 2001).

Silymarin (S), an extract from milk thistle seeds, is a mixture of flavonoid isomers such as silibinin, isosilibinin, silidianin, and silichristin. The seeds of this plant have been used in Europe for many centuries for the treatment of liver and gall bladder dysfunctions (Blumenthal, 2003; Wichtl, 2004). Silymarin is used primarily to treat various liver diseases and dysfunctions including alcoholic cirrhosis, hepatitis (due to viral infections or drug-induced), as well as hepatic problems related to diabetes (Lieber et al., 2003; Gruenwald, 2004).

Silymarin has liver regenerative effects by stimulating the enzyme known as RNA polymerase in the nucleus of liver cells. This results in an increase of ribosomal protein synthesis helps to regenerate hepatocytes (Gruenwald, 2004). The ability of silymarin to protect against oxidative stress-induced hepatocellular damage (such as lipid peroxidation of membranes and subsequent membrane degradation) is associated with its free radical scavenging properties and its ability to enhance endogenous antioxidant defences, such as those mediated by superoxide dismutase SOD or the glutathione system (Kiruthiga et al., 2007).

Grape (*Vitis vinifera*) is one of the most widely consumed fruits in the world, it has been reported that grape has important role in controlling of some liver diseases, high blood pressure and anemia (Celik et al., 1998). Grape seed extract (G) is a medical herb used primarily for its high proanthocyanidin content; it contains catechins, epicatechin, and gallic acid. It was hypothesized that novel grape seed proanthocyanidin extract (GSPE) might be useful since it has been shown to serve as a free radical scavenger and antioxidant both *in vitro* and *in vivo* models (Bagchi et al., 1997, 1998a). G proanthocyanidins are powerful antioxidants that have been shown in research studies to promote cardio-vascular (Karthikeyan et al., 2009), and brain activities (Deshane et al., 2004); and has antibacterial (Palma et al., 1999), and anti-inflammatory effect (Salah et al., 1995; Rice-Evans et al., 1996), they have been reported to inhibit lipid peroxidation (Feng et al., 2007). Grape polyphenols relax the blood vessels and inhibit platelet aggregation, leading to improved blood flow (Vitseva et al., 2005). Grape seed extracts have been reported to possess a broad spectrum of pharmacological and therapeutic effects including anti-inflammatory activity and reduced apoptotic cell death (Yassa et al., 2008). In the present study, we have investigated the effect of milk thistle extract (S) and grape seed extract (G) on the prevention of FB1 induced toxicity in experimental Sprague Dawley rats.

MATERIALS AND METHODS

Chemicals

Ascorbic acid and linoleic acid were purchased from Sigma-Aldrich Co., Germany. Four ammonium antipyrene, ammonium thiocyanate was purchased from Nice Co., India. ALT, albumin, total protein, uric acid, urea, lipid peroxide and GPx kits were purchased from Bio-Diagnostic Co., Cairo-Egypt, creatinine and lipid profile kits were purchased from Diamond Co., Cairo-Egypt.

Animals and experimental design

Preparation of contaminated corn: The corn kernels were autoclaved and then inoculated with 1 ml of spore suspension of Fusarium moniliforme, cultures were incubated in the dark for 28 days at 26°C. After the incubation time, the corn cultures were autoclaved to kill the fungus and the corn kernels were dried overnight at 50°C using the oven and were finely grind with blender and stored at 4°C until FB1 analysis (Visconti and Doko, 1994).

HPLC method was used for the determination of FB1 in corn samples according to Shephard et al. (1990), where, the FB1 content in corn sample was calculated from chromatographic peak areas using the standard curve.

Silymarin and GSE doses

The dose of phenolics-rich extracts has been selected according to the previously observed efficiency of both extracts (1% S and 3% G w/w) to improve the lipoprotein profile in rat (Škottová et al., 2003 and El-Adawi et al., 2006).

Rats were selected based on their utility as animal models and because FB1 induces spontaneous disease in this species. A total of 50 adult male Sprague-Dawley rats (100 to 120 g) were obtained from the experimental animals breeding center of the Holding Company for Biological Products and Vaccines (Helwan, Cairo, Egypt). The study was carried out and approved by the Animal Ethics Committee of Faculty of Medicine-Alexandria, Egypt. The rats were divided into 5 groups by a computerized random sort program.

Housing and Environment: pairs of rats were housed in elevated stainless-steel wire mesh cages during the experimental period. Diets were dispensed in cage cups which were refilled everyday. Water, was likewise available without restriction via an automated watering system. There were no contaminants in the food or water which could interfere with the results of the study. The animal room was maintained on 12 h light or dark cycle controlled via an automatic timer. Temperature and humidity were monitored for maintenance within specified ranges of 18 to 26°C and 30 to 7 relative humidity. The animals were acclimatized to the laboratory environment for one week before beginning the experiment.

The first group

Ten rats served as control group (C-gp), where they were fed on un-contaminated corn, the second group were fed on contaminated corn with at least 250 mg/ kg FB1 (T). In the third and fourth groups, rats were fed on contaminated corn with at least 250 mg/ kg FB1, supplemented with either silymarin extract (S 750 mg/kg b.wt.) or grape seed extract (G 3 g/kg b.wt.) as a protective herbal plant, in the fifth group, ten rats were fed on contaminated corn with at least 250 mg/ kg FB1, supplemented with both extracts (S+G). During the experiment, the body weight was recorded weekly. At the end of experimental period, blood samples were collected from animals in clean sterilized test tubes and serum samples were separated and

used for analysis parameters. The animals were sacrificed and examined by necropsy.

Extraction of grape and milk thistle seeds

Pre-extraction sample preparation

Red grape (Vitis vinifera) seeds were obtained from Ganaclise Veinyard factory (Abu-Elmatameer) and milk thistle (Silybum marianum) seeds were obtained from a local market at Alexandria, Egypt. The seeds were washed with tap water and then left to dry in open air away from direct sunlight. Seeds were crushed in a coffee grinder for two min, but at 15 s intervals. The process was stopped for 15 s to avoid heating of the sample. The crushed seeds were wrapped and stored at 18°C until the extractions were performed.

Extraction process

The crushed samples (grape and milk thistle) seeds were subjected to preliminary treatment (defatting); crushed seeds were soaked in suitable volume of hexane overnight. Defatted seeds were extracted by pressurized hot water and lyophilized using lyophilizer (Telstar, Spain).

Determination of phenol content

The classic technique employed in phenol analysis is the 4 aminoantipyrine colorimetric procedure (Ettinger et al., 1951). The absorbance of the samples was read against blank at 500 nm using spectrophotometer (PerkinElmer Lambda EZ 201, USA). The concentration of the sample was calculated from the standard curve prepared previously.

Determination of antioxidant activity

The antioxidant activities of the herbs water extracts were determined using the ferric thiocyanate (FTC) method (Osawa and Namiki, 1981) with slight modification. Sample (20 mg) was dissolved in 4 ml of 95% (w/v) ethanol, it was then mixed with 4.1 ml of linoleic acid [2.51% (v/v) in 99.5% (w/v) ethanol], 8 ml of 0.05 M phosphate buffer pH 7.0 and 3.9 ml distilled water and kept in screw-cap containers at 40°C in the dark. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was measured every 24 h until the time when the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid peroxidation was calculated as:

(%) inhibition = 100 - [(absorbance increase of the sample/absorbance increase of the control) × 100].

All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

HPLC analysis

The analysis for milk thistle and grape seed extracts was done using a Beckman-C18 column (100 × 4.6 mm, 5 µm particle size), equipped with an autosampler, pump and UV/visible multi wavelength detector.

Serum analysis

Blood samples for liver function, kidney function and lipid profile assays were centrifuged at 3000 rpm for 15 min, using centrifuge (Heraeus, Germany). The resultant serum samples were stored at -20°C till analysis (Oser, 1965).

Preparation of crude liver homogenate

Liver tissue homogenate was prepared for total glutathione peroxidase (GPx) determination according to Paglia and Valentine (1967), the sample was homogenized in 4 to 8 volumes (per weight tissue) of cold buffer (for example, 50 mM phosphate buffer, pH 7.0, containing 5 mM EDTA and 1 mM 2 mercaptoethanol) using Teflon glass homogenizer. The sample was centrifuged to 4000 rpm for 10 to 20 min at 2 to 8°C. The supernatant fluid containing the enzyme was removed, then freezed at 70°C before use. The sample could be incubated on ice if the determination will be run on the same day. The protein concentration of the clarified homogenate was calculated to determine the required volume which should be added on this assay.

Measurement of liver function markers

Total proteins were determined by means of the burette reaction as described by Gornall et al. (1949). In the presence of an alkaline cupric sulfate, the protein produces a violet color, the intensity of which is proportional to their concentration.

Albumin was assayed according to the method of Doumas et al. (1971) where, a green complex of albumin/bromcresol was formed at pH 4.1 and measured spectrophotometerically at 630 nm.

Alanine aminotanseferase was determined following the method of Reitman and Frankel (1957). ALT catalyzes the transfer of the amino group from alanine to 2 oxoglutarate, forming pyruvate glutamate; the catalytic activity was measured by spectrophotometery at 505 nm.

Measurement of kidney function markers

Creatinine level was determined by colorimetric kinetic method as described by Larsen (1972), where Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Urea in the serum originated, by means of the coupled reactions described by Fawcett and Scott (1960).The blue dye indophenol product reaction absorbs light between 530 nm and 560 nm proportional to initial urea concentration.

Uric acid level was determined as described by Barham and Trinder (1972), where the uric acid was hydrolyzed enzymatically to release hydrogen peroxide which reacts with 4 aminoantipyrine in the presence of 3, 5, Dichloro-2- hydroxybenzensulphonate to form a quinoneimine.

Lipid profile

The Total cholesterol was determined after enzymatic hydrolysis and oxidation according to Allain et al. (1974). The quinoneimine is formed from hydrogen peroxide and 4 aminoantipyrine in the presence of phenol and peroxidase. The HDL-cholesterol was determined by enzymatic colorimetric method as described by Lopez–Virella et al. (1977) where phosphotungstic acid and magnesium ions selectively precipitating all lipoproteins except the HDL fraction – cholesterol present in the supernatant can be determined

Table 1. Determination of antioxidant activities of G and S extracts by the Ferric Thio-cyanate (FTC) method.

Time (h)	Inhibition %		
	Ascorbic	GSE	Silymarin
0	100	100	100
24	90.9	95	92.3
48	80.6	89.2	87
72	73.4	81.1	79.6
96	28.7	46.9	34.1

by the same method used for total cholesterol.

LDL-cholesterol was computed mathematically according to Friedwald's equation (Friedwald, 1972): LDL = TC - (HDL + TG/5).

Measurement of malondialdehyde (MDA) and glutathione peroxidase (GPx)

Malondialdehyde level was determined by the method described by Ohkawa (1979). Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product, the absorbance of the resultant pink product can be measured at 534 nm. The GPx assay is an indirect measure of the activity of c-GPx. Oxidized glutathione (GSSG) produced upon reduction of organic peroxide by c-GPx is recycled to its reduced state by the enzyme reductase (GR).

The assay is an indirect measure of the activity of c-GPx (Paglia and Valentine, 1967). The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A340 is directly proportional to the GPx activity in the sample.

Determination of superoxide dismutase SOD

The Cu/Zn SOD was detected quantitatively by the enzyme-linked immunosorbent assay (ELISA) using (Anthos 2010 with Anthos ADAP software, Anthos labtec instruments GmbH, Austria) according to the method of Yao and Rarey (1996). A coloured product is formed in proportion to the amount of Cu/ZnSOD present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 Cu/ZnSOD standard dilutions and Cu/ZnSOD concentration determined.

Histological examination

After the animals were sacrificed, the abdomen was opened; the rat livers were removed and immediately fixed in 10% formalin saline solution (pH 7.4) and processed by successive dehydration with a sequence of ethanol solution and embedded in paraffin.

The serial sections were cut 5 µm thick and stained with haematoxylin-eosin (HE) stain using standard procedures (Drury and Willington, 1980).

Stained liver sections were examined for structure and architecture changes photomicroscope. The liver sections were examined for the type of the fatty change (macrovesicular: large droplets or microvesicular: small droplets) and the acinar zone involvement considering the three zones (peripheral/periportal, middle and central/Perivenular zones) of the hepatic lobule, also assessment for presence of complications such as steatohepatitis, steatohepatitis with cirrhosis or hepatic fibrosis was examined (Scheuer and Lefkowitch, 2005).

Statistical analysis

The data were given as individual values and as mean ± standard error. Comparisons between the means of various treatment groups were analyzed using one way ANOVA. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using the statistical software SPSS, version 10.

RESULTS

We adopted the HPLC method for analyzing the isolated compounds as well as screening the extracts. Our results (Figure 1) were highly sensitive and reproducible, which agree with the standard chromatograms produced by NSF.

The resulting chromatograms showed that the four major compounds in G are gallic acid, catechin, epicatechin and epicatechin gallate. On the other side, there were two major compounds taxifolin and silychristin in silymarin extract. Taxifolin derivative is also present in a significant amount, as well, low amount of both silybinin A and B were also recorded. All compounds were identified by their retention times against standard samples. Other peaks in all chromatogram were not identified due to the lack of standards, they are most probably, phenollic compounds in a sense contributed significantly to the total phenolics in the extract because total phenolics measured were much higher than the sum of the individual phenolic concentration identified and quantified by HPLC.

Total antioxidant activity

Table 1 shows the inhibition of linoleic peroxidation by G and silymarin extracts in comparison with ascorbic acid as a standard.

Serum analysis

FB1 was characterized as a non-genotoxic liver cancer promoter and shown to be hepato-and nephrocarcinogenic in rats (Gelderblom et al., 1991; Voss et al., 1995). The present study confirmed the FB1-induced hepatotoxic and nephrotoxic effect, which were manifested by a significant ($P \Re 0.05$) increase in the biochemical parameters associated with Liver and kidney functions (Figures 2 and 3, respectively). Diet supplemented with milk thistle(S) or grape seeds (G)extracts or both (S+G) could return the elevation of ALT level (78%) to normal. There was no significant difference between the control animal group (C) and the FB1 group at the total protein and albumin levels. Significant elevation in total protein was recorded in S and S+G group by 35 and

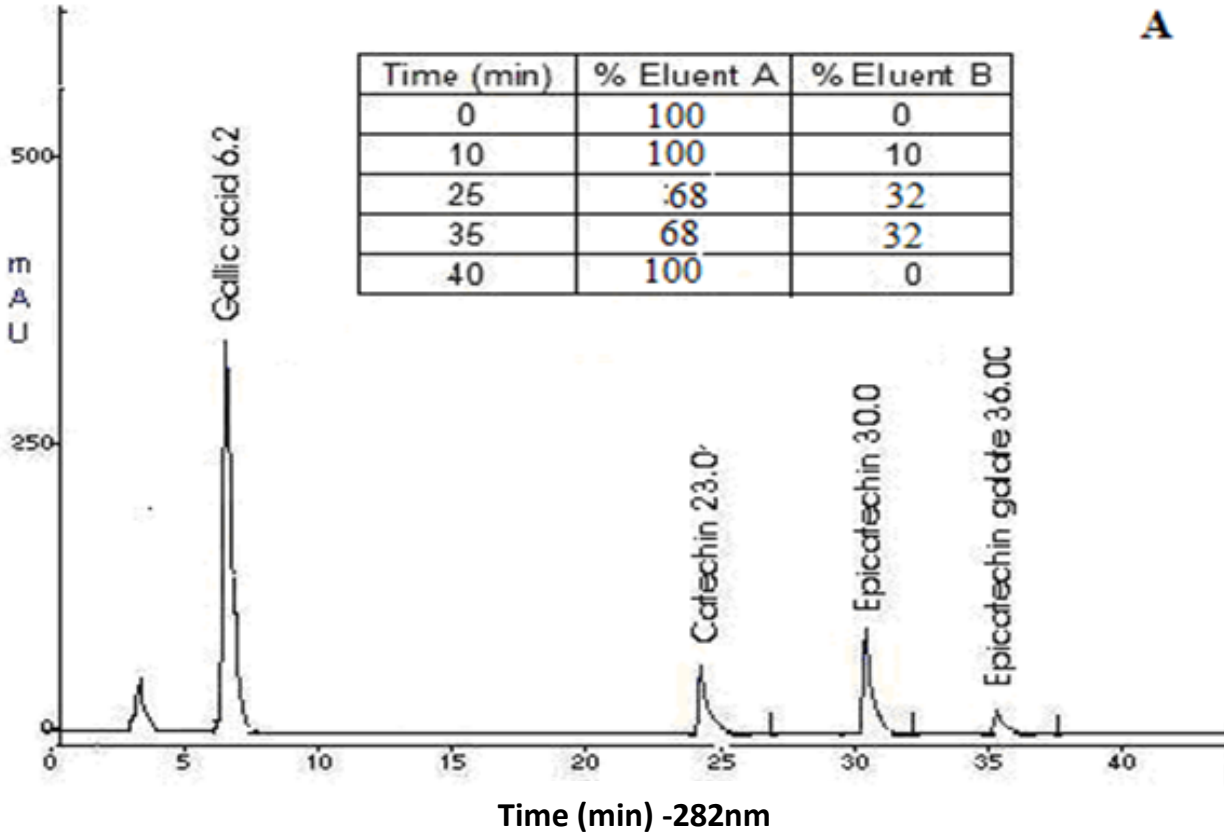

Figure 1. Resulting chromatogram and gradient elution schedule for HPLC-UV analysis of (A): Grape seed extract and (B): Milk thistle extract.

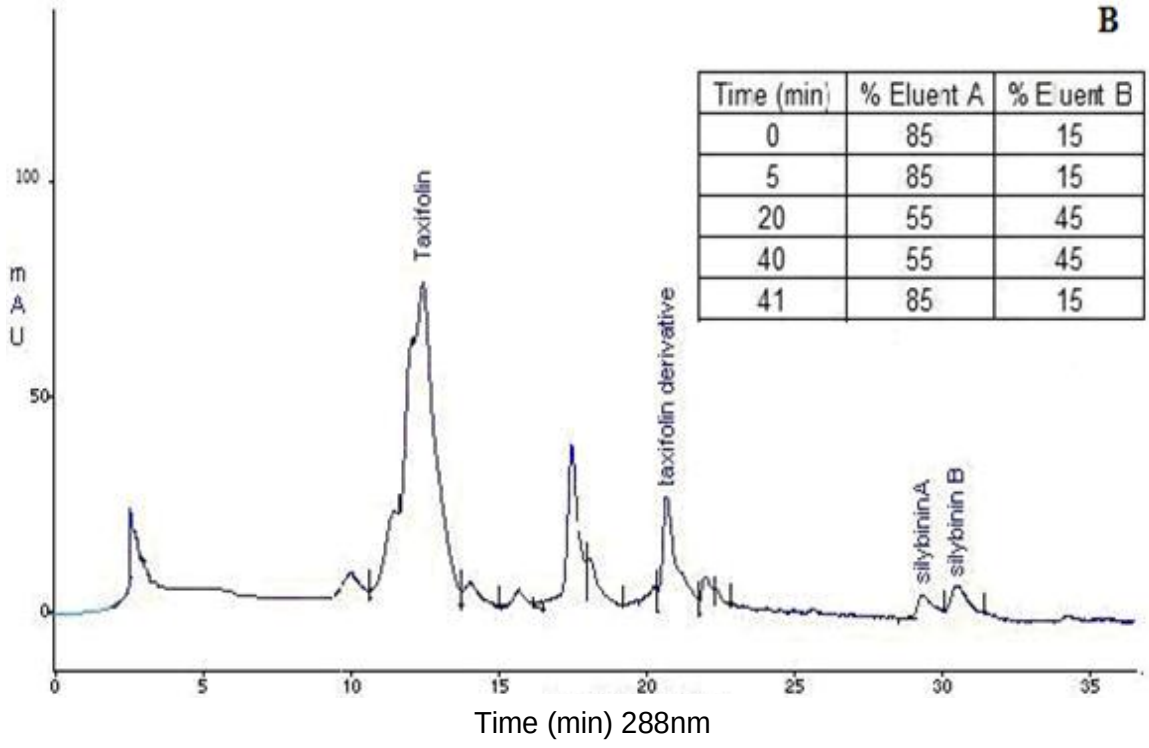

Figure 2. Serum levels of ALT, albumin, and total protein in protection groups.

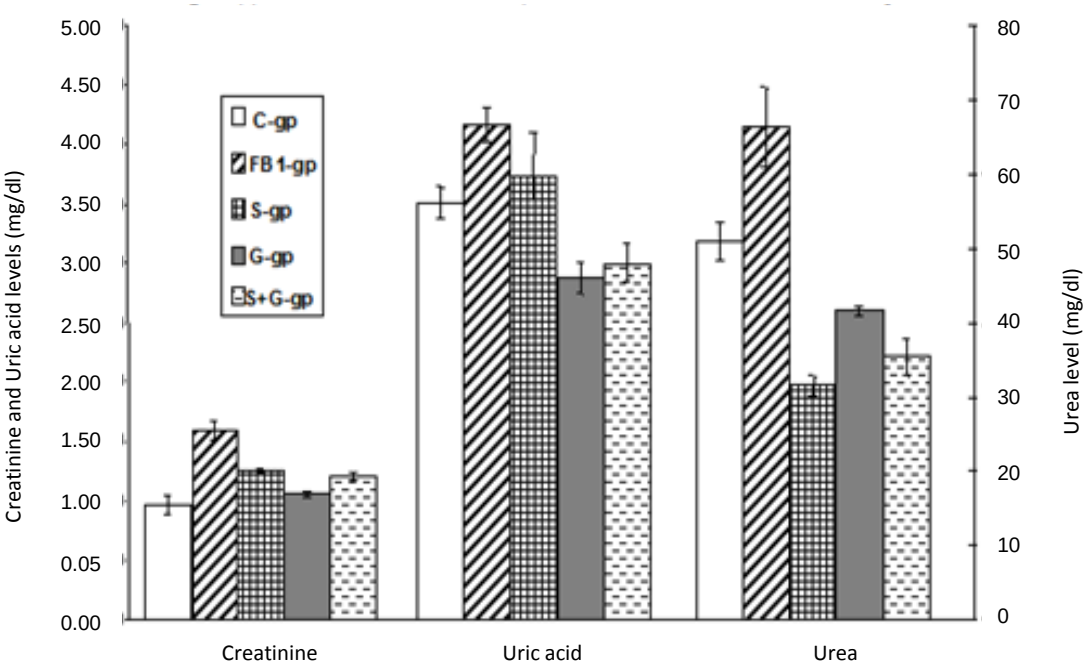

Figure 3. Serum Levels of creatinine, urea and uric acid in protection groups.

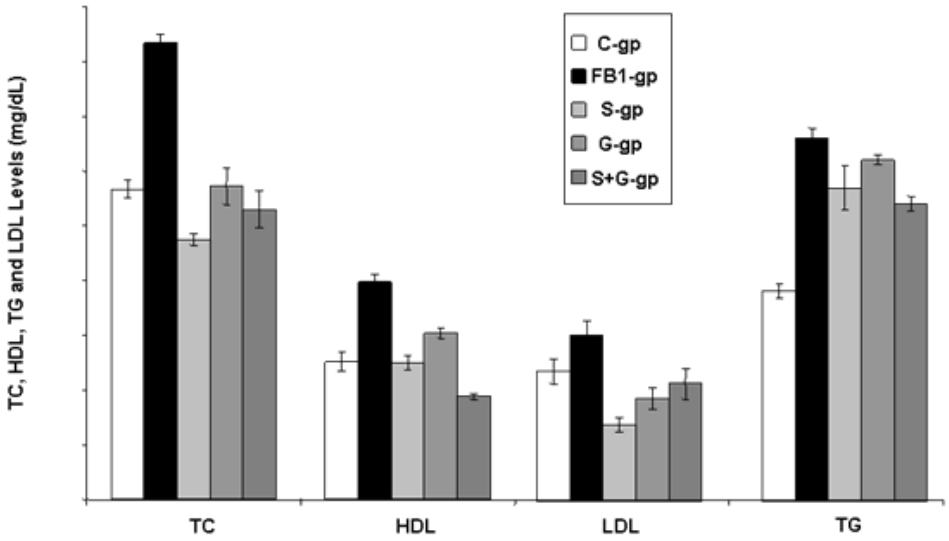

Figure 4. Serum levels of TC, HDL, TG and LDL in protection groups.

23%, respectively in addition to significant elevation in albumin level was recorded in S+G group (Figure 2).

On the other side, Creatinine, uric acid and urea as biomarkers for kidney function were adversely affected by FB1 where, elevation reached 65, 63 and 15%, respectively. Prior treatment with S or G or combined treatment with both (S+G) could significantly reduce those levels as shown on Figure 3, suggesting protective effect against FB1-induced nephrotoxicity. The tested herbal extracts were also protected against FB1- induced accumulation of serum total cholesterol (TC). The lipid profile has been significantly elevated in FB1 group, and the prior treatment of S, G and S+G group resulted in significant reduction in lipids including TC, HDL, TG and LDL (Figure 4). Figure 5 illustrates the significant elevation (137 %) in MDA level of FB1group. In the S, G and S+G groups this elevation was reduced by 38, 37 and 44%, respectively. In contrast, FB1 significantly

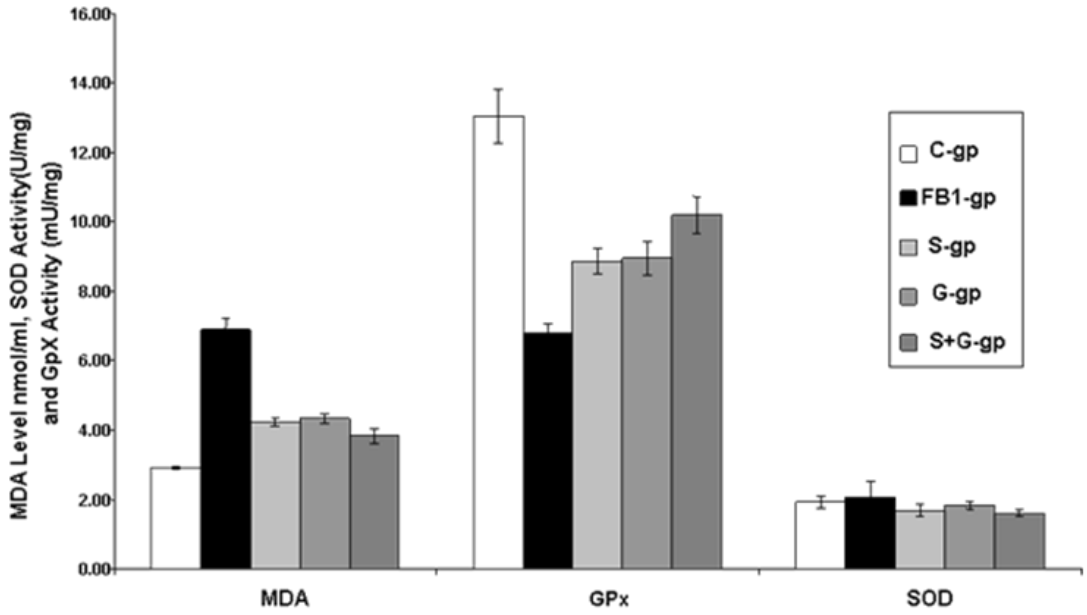

Figure 5. Serum levels of MDA, GPx, and SOD in protection groups.

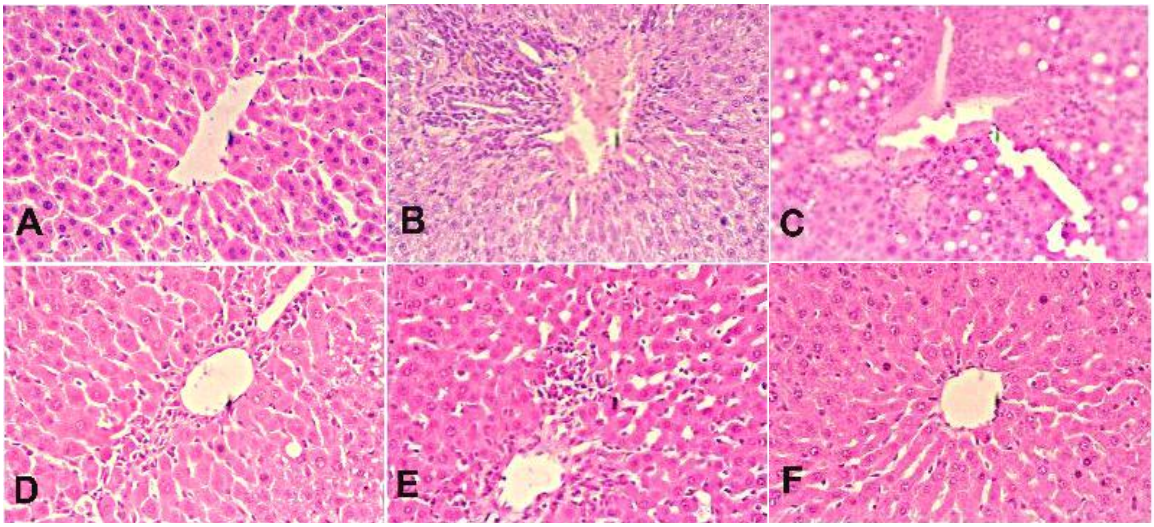

Figure 6. H and E staining on liver tissue of (A): control group, showing normal architecture of hepatocytes, (B): FB1group without treatment, showing increase of lymphocyte infiltration in portal area (blue arrow), apoptotic bodies (black arrow), pyknotic nuclei and vascular congestion, (C): FB1-group without treatment, showing lymphocyte infiltration, moderate and large sized vacuoles(black arrow), (D): G-group, showing less infiltration of lymphocytes, larger sized hepatocytes and cloudy swell cells, (E): S-group, showing ameliorated cell size and cytoplasmic inclusions, less infiltration than FB1 group but sinusoidal dilation is evident (black arrow) , and (F): S+G group, showing improvement of hepatocytes architecture, decrease of lymphocyte infiltration and prominent kuppfer cells (black arrows) (magnification ×400).

reduced the GPx level (48%), while pre- supplementation of S, G and S+G could significantly elevate the GPx by 30, 31 and 50%, respectively. The activity of SOD was not affected in all groups as shown in Figure 5.

Histopathological results

Control animals showed the normal liver lobular architecture which is formed of radiating strands of cells (hepatocytes) around a central vein, hepatocytes are polyhedral in shape with relatively large sizes with prominent centrally located nuclei (Figure 6A). No abnormal changes were seen in any of the livers of the control rats. In the FB1-gp, feeding of FB1 contaminated corn resulted in extensive hepatocellular damage, as evidenced by the presence of portal inflammation, pyknotic cells, congestion of terminal hepatic venules (central veins). Moreover,

changes of cell size with less cytoplasmic inclusions and appearance of large vacuoles were observed. Signs of necrosis in some hepatic areas were also marked in FB1 treated animals (Figure 6B and C). These histopathological changes were ameliorated by feeding the animals with G (0.3 g/100 g b.wt.), silymarin (750 mg/kg b.wt), and the both of them together before administration of FB1. Less inflammatory cell infiltration, large sized hepatocytes and sometimes cloudy swell cells were found, although these findings were evidenced by mild inflammation, decrease of irregular or ruptured boundaries of central veins and minimal hepatocelluar necrosis (Figure 6; D, E and F).

DISCUSSION

Dietary polyphenols are thought to be beneficial to human health by exerting various biological effects such as free radical scavenging, metal chelation, modulation of enzymatic activity, and alteration of signal transduction pathways (Singh and Aggarwal, 1995; Stocker, 1999; Yoshioka et al., 1995).

The liver function

He et al. (2004) demonstrated that the FB1-induced elevations of serum ALT and apoptotic hepatocytes were dramatically decreased by silymarin. The ability of silymarin to stimulate the regeneration of liver tissue inFB1 intoxication could partly account for the observed hepatoprotective actions. The same study concluded that silymarin protects against FB1 toxicity through blocking the actions of free sphingoid bases. The ability of silymarin to preserve the integrity of cellular and mitochondrial membranes could partly explain its protective effects on FB1 hepatotoxicity. Ramadan et al. (2002) reported that the protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties. Silymarin was also hepatoprotective and had antioxidant effects in ethanol intoxication (Saravanan et al., 2002). Madani et al. (2008) confirmed that polyphenolic extracts have protective effects against hepatic cell injury induced by thioacetamide.

In the current study, silymarin group (S-gp), could keep the ALT activity at normal level (Figure 2). This result agrees with the previous work done by Saller et al. (2001), on which the silymarin has protected against injury from various other hepatotoxicants (carbon tetrachloride and paracetamol) through lowering the elevation on ALT level. Another study by Ahmed et al. (2003) confirmed the hepatoprotective effect of silymarin where they tested the effect of silymarin against Cisplatin. Silymarin significantly restores the change of ALT due to its antioxidant effect and its ability to act as a free radical scavenger, thereby protecting membrane permeability. In the G-gp, the G diminished the elevation of ALT activity as silymarin did. The same result was recorded by Karthikeyan et al. (2007) and Yousef et al. (2009) when they pretreated the rats with G, and induced the hepatotoxicity by isoproterenol and cisplatin, respectively. The maintenance of the levels of marker enzymes may be due to the free radical scavenging property of antioxidative polyphenolic molecules present in G.

Pretreatment with silymarin (S and S+G-gp) elevated the serum total protein significantly when compared to the control and FB1-gp. That elevation might be explained by the study of Sonnenbichler and Zetl (1986), who stated that Silymarin increases the biosynthesis of protein through stimulation of the enzymatic activity of DNA-dependent RNA polymerase 1 and the subsequent biosynthesis of RNA and protein, resulting in DNA biosynthesis and cell proliferation. Saller et al. (2001) stated that silymarin is able to stabilize cellular membrane through pharmacological properties which involve regulating cell membrane permeability and integrity, inhibiting leukotriene, scavenging reactive oxygen species, suppressing NF-kB activity, depression of protein kinases, and collagen production. Due to those previous studies, it was highly expected to record the significant elevation in total protein (35 and 23%) in S and S+G groups.

Significant elevation in albumin level was recorded in S+G group only; such result suggests a synergistic effect between G and silymarin.

The oxidative stress

Lipid peroxidation is a well-known mechanism of liver injury induced by FB1 and malondialdehyde (MDA) is one of its end products. Thus, MDA is a good indicator of the degree of lipid peroxidation (Abado-Becognee K, 1998). As seen in Figure 5, MDA in liver increased 137% above the original value by FB1. In the S-gp, pretreatment with milk thistle extract reduced the MDA concentrations, and caused an elevation of GPx activity. Many mechanisms have been suggested for the protective effects of silymarin, which include antioxidation, prevention of lipid peroxidation (Basaga et al., 1997), enhancing detoxification (Baer-Dubowska et al., 1998) and retarding glutathione depletion (Alidoost, 2006).

G significantly reduced the level of MDA and elevated the GPx activity in comparison with FB1-gp (Figure 5). It is proposed that the consumption of flavonoid-rich foods and beverages helps to limit oxidant damage in the body (Yamanaka et al., 1997). *In vitro* experimental results have demonstrated that proanthocyanidins have specificity for the hydroxylradical (Zayachkivska et al., 2006).

Tebib et al. (1997) reported that plasma tissue MDAs in rats fed polymer grape seed tannins were reduced, it can be expected that plasma LDL would be less oxidized, strengthens the beneficial effect of decreased LDL-C concentration. Recent study by Cetin et al. (2008) reported that G moderately reserved the MDA levels in

comparison with the radiation-induced oxidative stress group. G was seen to protect the cellular membrane from oxidative damage and consequently from lipid oxidation. Taken together these results show that both silymarin and G could moderately reserve the MDA and GPx levels. It was obvious that pretreatment of both extracts could keep the MDA and GPx levels from the significant alterations due to FB1 toxicity. The current work reveals that the activity of SOD was not affected suggesting that the formation of superoxide is not a prominent feature during FB1-induced hepatotoxicity as was suggested previously (Marnewick et al., 2009).

The kidney function

High values of creatinine, urea and uric acid as a result of FB1 toxicity indicate kidney damage. Pretreatment with milk thistle extract could partly recover this damage by diminishing the levels of creatinine and urea (Figure 3). Our results are in agreement with previous study that showed, protective effect of silymarin against damage to kidney from acetaminophen, cisplatin (platinol), and vincristine (oncovin) (Sonnenbichler et al., 1999) and the protective effect of silymarin against cisplatin nephrotoxicity (Karimi et al., 2005). Scavenging free radicals and increasing intracellular glutathione was the suggested mechanism. Recent evidence (Kaur et al., 2010) suggests that silymarin may be just as important for kidney health. Silymarin concentrates in kidney cells, where it aids in repair and regeneration by increasing protein and nucleic acid synthesis. The study showed that it increased cell replication by 25 to 30%.

Catechin analogues such as (-) –epicatechin 3-o-gallate (ECG) and EGCG found in G, are known to have many physiological effects, such as to exert suppressive effects on renal failure (Nakagawa et al., 2004; Yamabe et al., 2006). The reduction in uric acid, urea, and creatinine concentrations in G pretreated rats compared to the group of FB1 alone indicated a restoration of kidney function. The free radical scavenging ability of proanthocyanidins and flavonoids present in G (Sato et al., 2005) may account for the nephroprotective action of G against FB1 toxicity. Similar results were reported in a study by Yousef et al. (2009) in which renal toxicity was caused by cisplatin and G was used for protection. Thus, G indirectly corrects body homeostasis through its improvement of kidney function. In addition to those studies our lab studied the nephrotoxocity of G and reported that G has no nephrotoxicity (Abd El-wahab et al., 2008). The current study proved that G has nephroprotective effect too, where; G could return back the creatinine and urea levels to normal and significantly reduced the elevation in uric acid level.

Both extracts, S+G could reduce the levels of creatinine, urea, and uric acid. In this case we suggest that the lowering effect in uric acid was attributed to the G effect which was evident in the G-gp.

The lipid profile

Silymarin also decreased the elevation of TC, HDL-C, LDL-C, TG (Figure 4), suggesting the hepatoprotective effect of silymarin. The antihypercholesterolemic effect of silymarin was associated with liver cholesterol reduction (Krecman et al., 1998), which improves cholesterol uptake from blood (Steinberg et al., 1989). It was obvious from the histopathological results which confirm the biochemical results of the S-gp that there was less infiltration of lymphocytes and regeneration of the hepatocytes (Figure 6E).

Oral administration of proanthocyanidins from grape seed produced a hypocholesterolemic effect in a high cholesterol animal feed model; specifically it prevented an increase in total and LDL plasma cholesterol (Fine, 2000; El-Adawi et al., 2006). These findings are confirmed by Yousef et al. (2009) who showed that administration of G combined with cisplatin has reduced the level of cholesterol when compared with cisplatin group. The significant decrease in the elevated levels of cholesterol in rats receiving G prior to cisplatin indicated the ability of G to counteract cisplatin-induced toxicity. Regarding the current study, G reduced the levels of elevated TC and LDL-C in comparison with that of FB1-gp which confirms the previous results and might be explained by Tebib et al. (1994a, b) who demonstrated the protective effect of grape seed tannins against plasma cholesterol and LDL-cholesterol, then they hypo-thesized that tannins through their antioxidant properties would exert a beneficial effect against oxidant stress. That finding was supported by Natella et al. (2002) who reported that oligomeric proanthocyanidins supple-mentation resulted in decreased lipid peroxidation, increased plasma antioxidant levels, and improved resistance of LDL to oxidation in volunteers consuming a lipid-rich test meal. El-Adawi et al. (2006) reported that G-supplemented diet exhibited an obvious hypolipidemic effect in rats fed on high cholesterol diet. G could reduce the TC, LDL and TG in G pre and post treated groups. The hypolipidemic effect of G may result from increasing the rate of cholesterol catabolism by increasing the activity of hepatic cholesterol 7-a-hydroxylase enzyme. This enzyme is the rate-limiting enzyme of bile acid biosynthesis, thus suggesting that G could stimulate the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from body (Del Bas et al., 2005). The water-soluble antioxidant, proanthocyanidins in the G might trap reactive oxygen species (ROS) in aqueous series such as plasma thereby inhibiting oxidation of LDL.

As a result of S+G administration, total cholesterol, HDL-C, LDL-C and TG levels were reduced. The scavenging properties of both extracts were evident in the inhibition of lipid peroxidation by reducing the high levels of peroxidaton end product MDA and in the same time increasing the activity levels of antioxidant enzymes especially the GPx activity. That confirms the hepato-

protective effect of both extracts.

Conclusion

Carcinogenesis by FB1, the main fumonisin produced, is associated with a chronic hepatotoxic effect and the induction of oxidative damage. The role of reactive oxygen species (ROS) in carcinogenesis is well established as it regulates critical events regarding cell proliferation and apoptosis. The modulating role of antioxidants in the ROS-induced effects on the different cell survival parameters is therefore of interest, with respect to fumonisin-induced hepatocarcinogenesis. The present study investigated the modulating properties of aqueous extracts of milk thistle(S), Grape seeds (G), and both (S+G) against different biochemical parameters and oxidative stress. These modulations might be due to their ability to lower serum total cholesterol and low-density lipoprotein cholesterol levels as well as slowing the lipid peroxidation process by enhancing antioxidant enzyme activity. It is assumed too that the effect of both extracts on liver and kidney protection is related to glutathione-mediated detoxification as well as free radical suppressing activity.

These results may provide a scientific basis for progression to clinical trials of the chemoprevention of both extracts on hepato and nephrotoxicity. In addition, both seeds may be added as nutrition supplement for domestic animals farm.

Further study on the plants could be extended for the isolation and structure determination of the hepato-protective and nephroprotective principle or principles.

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