Grape seeds proanthocyanidin extract as a hepatic-reno-protective agent against gibberellic acid induced oxidative stress and cellular alterations

Hanaa A. Hassan · Maisaa M. Al-Rawi

Received: 6 June 2012 / Accepted: 1 October 2012 / Published online: 8 November 2012
© Springer Science+Business Media Dordrecht 2012

Abstract The present study aims to investigate the hepatonephro-protective effect of grape seeds proanthocyanidin extract (GSPE) against the risks induced by gibberellic acid (GA₃) in male rats. The results recorded that GA₃ caused a significant increase in total lipids, total cholesterol, triglycerides and LDL-C levels in serum, concomitant with a significant decrease in serum HDL-C. A significant increase in serum AST, ALT, urea and creatinine, while, a significant decrease in total protein content in serum was observed in rats given GA₃. Hepatic and renal lipid peroxidation product (MDA) was significantly increased, meanwhile, total antioxidant capacity (TAC), glutathione, and catalase levels were significantly decreased. In addition, there was a negative change in liver structure including dilatation in the central veins with degeneration of endothelium cells and cellular injury around the veins as well as in the kidney structure such as lesion in both glomeruli and tubules, detachment of the Malpighian corpuscles from the Bowman's capsule's epithelium, shrinkage in the glomerular capillary network. However, almost all of these adverse effects seemed to be ameliorated by oral administration of GSPE with GA₃ to rats for 2 month

indicating the protective effect of grape seeds GSPE on GA₃ induced oxidative stress in rats.

Keywords Plant growth regulators (PGRs) · Gibberellic acid (GA₃) · Antioxidants · Grape seeds proanthocyanidin extract (GSPE) · Liver · Kidney

Introduction

Plant growth regulators (PGRs) are chemicals commonly used to alter the growth of plant and increase seeds production (Ashikari et al. 2005). Some of them are endogenous plant hormones which are included in the diet of all herbivorous and omnivorous animals (Celik et al. 2002). PGRs fall into six major classes: auxins, gibberellins, cytokinins, ethylene generators, growth inhibitors and growth retardants. One of the gibberellins, gibberellic acid-3 (GA₃) accelerates and improves the yield of a wide variety of plants by increasing cell division (Silverstone and Sun 2000), promoting stem elongation, overcoming dormancy in seeds and buds, involved in parthenocarpic fruit development, flowering and mobilization of food reserves in grass seed germination (Salisbury and Ross 2009). Moreover, it is used against harmful agriculture pests to accelerate the growth of fruits and vegetables (Arous et al. 2001). Since animals and human beings consume GA₃ in their diet, it was hypothesized that metabolic events in animal cells

H. A. Hassan (✉)
Faculty of Science, Zoology Department,
Mansoura University, Mansoura, Egypt
e-mail: drhanaahassan@yahoo.com

M. M. Al-Rawi
Biology Department, Practical Science College,
Umm Al-Qura University, Mecca, Saudi Arabia

could influence such dietary ingredients (Srikumar et al. 2009). Consequently, GA₃ could pose a risk to those professionally exposed, as well as the general population by the consumption of such food products.

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones (Craig and Beck 1999). The human diet, which contains a large number of natural compounds, is essential in protecting the body against the development of diseases. Grape is one of the well known plants with remarkable anti-carcinogenic and antioxidant properties (Bagchi et al. 2000; Shan et al. 2010). Grape seeds proanthocyanidin extract (GSPE) are considered to be bioactive compounds because they influence physiological and cellular processes and, therefore, can have beneficial effects on health (Serrano et al. 2009). GSPE are safe compounds extracted from grape seeds and their basic structure unit are the catechins (Wren et al. 2002) mainly catechin monomer, dimer and trimer. These catechins are water-soluble molecules and contain a number of phenolic hydroxyls (Bagchi et al. 2002). Moreover, oligomeric GSPE is a naturally occurring antioxidant which has been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress (Bagchi et al. 2000). Besides antioxidant activity, GSPE has been described as anti-microbial, anti-cancer, anti-inflammatory and anti-fatigue agent (Shan et al. 2010) and has cardioprotective properties (Serrano et al. 2009). Therefore, the present work aims to investigate the hepatonephro protective role of GSPE against the adverse effects induced by GA₃ widely used as a plant growth regulator.

Materials and methods

Chemicals

Gibberellic acid (GA₃) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Grape seeds proanthocyanidin extract (GSPE) was purchased from the Arab Company for Gelatin and Pharmaceutical Products, Alexandria, under licence of Nulife International U.S.A.

Animal grouping and experimental design

Adult male albino rats (*Rattus rattus*) weighing about 130–140 g were used in this study and were purchased

from Experimental Animal Care Center. Animals were housed in cages with free access to drinking water and diet and maintained in the animal care facility throughout the duration of the experiment. The animals were kept at 22–24 °C with the 12 h light/dark cycle. All animals received human care according to the instruction's guidelines of the National Institutes of Health (NIH) and the protocol was approved by the local committee of the Mansoura University. After an acclimatization period of 1 week, animals were divided into four groups, 6 rats each as follow: Group 1, untreated control; Group 2, treated orally with GSPE (100 mg/kg b.w) (Karthikeyan et al. 2007); Group 3, treated with GA₃ (3.85 mg/Kg body weight) (Celik et al. 2007); Group 4, treated with GSPE and GA₃ at the same doses of group 2&3. All treatments were given daily using gastric intubation and continued for 2 months.

Sampling

At the end of the experimental period (2 months), all animals were fasted for overnight and blood samples were collected into chilled non-heparinized tubes and centrifuged at 860 G for 20 min at 4 °C. The separated sera were frozen at –20 °C for biochemical analysis. After the collection of blood samples, animals were sacrificed and samples of the liver and kidney tissues were accurately weighed and homogenized (Potter–Elvehjem) in a tenfold volume of ice-cold distilled water for later biochemical analysis. Other samples of the liver and kidney were fixed in neutral formalin solution for histopathological studies.

Biochemical analysis

The sera were used for the determination of total lipid (TL) level (Frings et al. 1972), total cholesterol (TC), triglycerides (TG) and HDL-C (Young 1995) using assay kits (Biodiagnostic for diagnostic reagents: Dokki, Giza, Egypt). LDL-C level was calculated according to Friedewald et al. (1972). Aspartate amino transferase (AST) and alanine amino transferase (ALT) activity was estimated using commercial assay kits (Biodiagnostic for diagnostic reagents: Dokki, Giza, Egypt) according to the method described by Reitman and Frankel (1957). Total protein was determined according to the method of Henry (1964). Liver and kidney lipid peroxidation product

Table 1 Serum biochemical parameters in the control and the different treated rat groups

Parameters↓	Animal groups			
	Control	GSPE	GA ₃	GSPE + GA ₃
Total lipids (mg/100 ml)	358.5 ± 1.7	351.4 ± 1.6	472.9 ± 3.7 ^a	357.7 ± 1.9 ^b
Total Cholesterol (mg/100 ml)	77.9 ± 1	76.6 ± 1	99.2 ± 1.1 ^a	71.3 ± 0.87 ^{a, b}
Triglyceride (mg/100 ml)	76.6 ± 1.7	73.7 ± 1.6	102 ± 1.3 ^a	73.2 ± 1.9 ^b
HDL-C (mg/100 ml)	43.8 ± 0.55	44.1 ± 0.40	30.1 ± 0.83 ^a	47.3 ± 0.36 ^{a, b}
LDL-C (mg/100 ml)	18.9 ± 0.65	17.8 ± 0.39	45.9 ± 0.73 ^a	10 ± 0.44 ^{a, b}
AST (U/IL)	35.9 ± 0.93	34 ± 0.71	64.9 ± 1.1 ^a	39.8 ± 0.35 ^{a, b}
ALT (U/IL)	34.6 ± 0.93	34.2 ± 0.46	63.7 ± 1.2 ^a	42.9 ± 0.33 ^{a, b}
Total protein (g/dl)	6.95 ± 0.43	6.55 ± 0.33	5.53 ± 0.5 ^a	8.44 ± 0.47 ^{a, b}
Uric acid (mg/dl)	2.95 ± 0.33	2.65 ± 0.36	3.76 ± 0.02 ^a	3.04 ± 0.09 ^b
Creatinine (mg/dl)	0.48 ± 0.04	0.46 ± 0.04	0.62 ± 0.02 ^a	0.36 ± 0.04 ^{a, b}

Data are expressed as mean ± SE of 6 animals

Low density lipoprotein- cholesterol (LDL-C), high density lipoprotein- cholesterol (HDL-C), aspartate amino transferase (AST), alanine amino transferase (ALT), grape seeds proanthocyanidin extract (GSPE), gibberellic acid (GA₃), molandialdehyde; (MDA), glutathione (GSH), total antioxidant capacity (TAC)

^a Significantly different from the control rats group at $p < 0.05$

^b Significantly different from the GA₃ treated rats group at $p < 0.05$

^{a, b} Significantly different from the control and the GA₃ treated rat groups at $p < 0.05$

Table 2 Hepatic and renal oxidative and antioxidant parameters in the control and the different treated rat groups

Parameters↓		Animal groups			
		Control	GSPE	GA ₃	GSPE + GA ₃
Liver	MDA (nmol/g)	154.7 ± 1.09	139.9 ± 0.79 ^a	210.4 ± 2.58 ^a	138.3 ± 2.07 ^{a, b}
	GSH (mg/g)	0.34 ± 0.008	0.38 ± 0.013	0.15 ± 0.009 ^a	0.32 ± 0.014 ^b
	TAC (mM/g)	0.85 ± 0.004	0.87 ± 0.005	0.50 ± 0.008 ^a	0.80 ± 0.009 ^b
	Catalase (μmol/sec/g)	29.9 ± 0.58	31.4 ± 0.38	10.6 ± 0.14 ^a	29.9 ± 0.48 ^b
Kidney	MDA (nmol/g)	144.6 ± 1.2	149.9 ± 0.71	222.4 ± 2.3 ^a	134.3 ± 2.4 ^{a, b}
	GSH (mg/g)	0.44 ± 0.007	0.49 ± 0.011	0.25 ± 0.009 ^a	0.42 ± 0.011 ^b
	TAC (mM/g)	0.112 ± 0.005	0.103 ± 0.005	0.66 ± 0.004 ^a	0.98 ± 0.007 ^b
	Catalase (μmol/sec/g)	19.9 ± 0.58	20.4 ± 0.38	10.9 ± 0.14 ^a	18.9 ± 0.48 ^b

Data are expressed as mean ± SE of 6 animals

Grape seeds proanthocyanidin extract (GSPE), gibberellic acid (GA₃), molandialdehyde (MDA), glutathione (GSH), total antioxidant capacity (TAC)

^a Significantly different from the control rats group at $p < 0.05$

^b Significantly different from the GA₃ treated rats group at $p < 0.05$

^{a, b} Significantly different from the control and the GA₃ treated rat groups at $p < 0.05$

(molandialdehyde, MDA), glutathione (GSH), catalase (CAT) and total antioxidant capacity (TAC) were estimated according to the methods of Ohkawa et al. (1982); Prins and Loose (1969); Bock et al. (1980) and Koracevic et al. (2001), respectively.

Histopathological examination

The liver and kidney samples were immediately fixed in neutral formalin solution, dehydrated in a graded series of alcohol, embedded in paraffin wax, sectioned

at 5–7 μm and stained with hematoxylin and eosin and examined under a research light microscope.

Statistical analysis

Data are presented as mean $\pm$ standard error (SE) of six animals. Significance of differences between means was calculated using one-way analysis of variance (ANOVA) and Student's *t* test as described by Snedecor and Cochran (1982).

Results

Data presented in Table 1 did not show any significant changes in rats given GSPE compared to control rats. While those treated with GA_3 in drinking water showed a significant increase in total lipids, total cholesterol, triglycerides, LDL-C in serum, accompanied with a significant decrease in HDL-C level compared in the control rats group. Animals in this group showed a significant increase in the activity of AST and ALT activity, urea and creatinine levels accompanied with a significant decrease in the total protein content in serum. Additionally, hepatic and renal MDA content was significantly increased, while GSH, TAC, CAT were significantly decreased in rats having received GA_3 compared in the control group (Table 2). Meanwhile, the administration of GSPE with GA_3 for 2 months succeeded to induce a marked improvement in these parameters (Tables 1, 2).

The histopathological examination of the liver tissue of the control rats revealed normal structure for hepatic cells, and sinusoidal spaces with Kupffer cells. The liver of animals treated with GA_3 showed dilated central vein and degeneration of endothelium cells (Fig. 1c). Another sections in the same group showed vacuolation of the cytoplasm of hepatocytes, and sinusoid filled with red blood cells (Fig. 1d). Some sections showed dilated central vein with severe lymphocytic infiltration (Fig. 1e) and others showed severe lymphocytic infiltration in the portal region (Fig. 1f). The liver section of the animals in the group treated with GSPE and GA_3 showed appearance of improvement of the central vein structure (Fig. 1g) and the portal region (Fig. 1h).

The histological examination of the kidney of the control group showed the normal structure, Bowman's capsules, glomerulus and convoluted tubules (Fig. 2a).

The kidney of rats treated with GSPE showed the normal structure of the kidney as control (Fig. 2b). Examination of the kidney in the GA_3 treated group showed detachment of two Malpighian corpuscles (Fig. 2c), a shrinkage of the glomerular capillaries leaving space between them and the wall with degeneration of the cellular epithelium of the tubules vacuoles and fat vacuoles (Fig. 2d). Some sections in the same group showed dilatation of the renal tubules with hemorrhage and lymphocytic infiltration (Fig. 2e). The kidney after treatment with GSPE plus GA_3 showed an improvement appearance of the kidney structure (Fig. 2f).

Discussion

Organ dysfunctions have been recently ascribed as one of the causes contributing to various physiological changes induced by plant growth regulators (PGRs). Such products may increase lipid peroxidation, which can be harmful to different organs including liver and kidney (Soliman et al. 2010). On the other hand, these free radicals, known to cause oxidative stress, can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products (Spranger et al. 2008; Wang et al. 2008). The present study was undertaken to determine whether GSPE can prevent and/or reduce GA_3 -induced oxidative stress by examining different biochemical parameters of oxidative damage in the liver and kidney of male rats.

Our results clearly showed that there was a significant increase in lipid profiles. The increased total lipids concentration may be due to that GA_3 activates fat metabolism (Srikumar et al. 2009; Muthuraman et al. 2010). Also, the increase in serum TC level by GA_3 may be related to the mobilization of this compound from membrane stores due to increased lipid peroxidation or due to the activation of 3-hydroxy-3-methyl-glutaryl- Coenzyme A reductase responsible for increased synthesis of cholesterol (Srikumar et al. 2009). Liver plays an essential role in lipid metabolism through several stages of lipid synthesis and transformation. Therefore, it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction (Halsted 2004), as metabolism of TG, TC and synthesis of lipoproteins predominantly occurs in the liver and various parenchymal diseases may lead to alterations in lipoproteins structure and transfer through the blood (Studenik 2000).

Fig. 1 **a** Photomicrograph of a transverse section of the liver of the control rat group showing normal structure for hepatic cells (h), sinusoidal spaces (s) with Kupffer cells (k) and central vein (CV). (X200), **b** Photomicrograph of a transverse section of the liver of the GSPE rat group showing normal structure of the liver as for the control. (X200), **c** Photomicrograph of a transverse section of the liver after treatment with GA₃, showing dilated central vein (CV) and degeneration of endothelium cells. Note vesiculation of the nuclei (arrow) and fat vacuoles (head arrows). (X250), **d** Photomicrograph of a transverse section of the liver after treatment with GA₃, showing: vacuolation (v) of the cytoplasm of hepatocytes, and sinusoid filled with red blood

cells (*). (X400), **e** Photomicrograph of a transverse section of the liver after treatment with GA₃, showing dilated central vein (CV) with severe lymphocytic infiltration. (X400), **f** Photomicrograph of a transverse section of the liver after treatment with GA₃, illustrating the portal region. Note severe lymphocytic infiltration. (X250), **g** Photomicrograph of a transverse section of the liver after treatment with GSPE + GA₃ showing appearance of improvement of the central vein (CV) structure. (X250), **h** Photomicrograph of a transverse section of the liver after treatment with GSPE + GA₃ showing improvement of the structure of the portal region. (X250)

Fig. 2 **a** Photomicrograph of a transverse section of the kidney of control group, showing the normal structure, Bowman's capsules (*arrow*), glomerulus (G), convoluted tubules (t). (X250), **b** Photomicrograph of a transverse section of the kidney of GSPE rat group, showing the normal structure of the kidney as control. (X250), **c** Photomicrograph of a transverse section of the kidney after treatment with GA_3 , showing detachment of two Malpighian corpuscles. Note vacuolated glomeruli (*arrow*). (X 250), **d** Photomicrograph of a transverse section of the kidney after treatment with GA_3 , showing the

shrinkage of the glomerular capillaries leaving space between them and the wall (*). Note the degeneration of the cellular epithelium of the tubules vacuoles (v), fat vacuoles (F). (X400), **e** Photomicrograph of a transverse section of the kidney after treatment with GA_3 , showing the dilatation of the renal tubules. Note hemorrhage (*arrow*) and lymphocytic infiltration (head arrows). (X400), **f** Photomicrograph of a transverse section of the kidney after treatment with GSPE + GA_3 . Note the appearance of improvement of the kidney structure. (X250)

In fact, the observed disturbances in liver enzymes associated with GA_3 treatment may be attributed to the harmful effect of plant growth regulators (PGRs) on tissues. However, it is conceivable that the PGRs might be interacting primarily with the liver and muscle tissue cell membranes resulting in structural damage due to increased production of ROS, enzyme

leakage in the serum and altered metabolism of the constituents (Tuluze and Celik 2006). Moreover, liver damage is correlated with alterations in TC, HDL-C and LDL-C (Ghadir et al. 2010).

The recorded changes in total protein content in rats administered with GA_3 was partially attributed to the decreased level of their synthesis in the hepatic cells

suffering from pathological changes or perhaps due to the hyperactivity of hydrolytic enzymes (Sakr et al. 2003; Srikumar et al. 2009). Other factors include kidney dysfunction which is recorded in the present data by increased urea and creatinine levels. The elevated blood urea is correlated with an increased protein catabolism in the mammalian body or more efficient conversion of ammonia to urea as a result of increased synthesis of enzymes involved in urea production (Rodwell 1979). Urea is the end-product of the protein catabolism and this is confirmed by the decrease in plasma proteins and referred to kidney dysfunction as proven by enlargement of the relative weight of kidney as suggested by Yousef et al. (2003). Additionally, the recorded oxidative stress that is indicated by the current elevation in MDA due to GA₃ administration is an agreement with various studies (De Melo et al. 1998; Soliman et al. 2010). This elevation in MDA may be due to that GA₃ is associated with generation of ROS which interacts with tissues leading to numerous pathophysiological alterations (Yu et al. 2008). ROS could damage every major cellular component, including membranes, lipids, carbohydrates and DNA (Bhalla and Dhawan 2009). So, GA₃ treatment might cause peroxidation of polyunsaturated fatty acids leading to the cellular deterioration (Troudi et al. 2011). Also, the increased content of MDA may result from an increase of hydroxyl radicals (OH[•]) (Herken et al. 2001). In this concern, it was reported that GA₃ might interact primarily with erythrocytes, liver, brain, and muscle tissues, resulting in lipid peroxidation processes by the way of increased superoxide radicals as result of stressed condition in the rats, leading to an increase in lipid peroxidation (Tuluze and Celik 2006).

Furthermore, in the present study, the revealed harmful changes in the antioxidants caused by the administration of GA₃ may be due to that PGRs may lead to inhibition of the enzyme synthesis in tissues as a result of mRNA breakdown or cellular transcription mechanisms. Also, oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to the PGRs (Celik et al. 2007; Soliman et al. 2010). Moreover, Dimitrova et al. (1994) suggested that the superoxide radicals by themselves or after their transformation into H₂O₂ cause an oxidation of cysteine in the enzymes, causing decrease in enzymatic activities. Further, the decrease in CAT activity could be due to the flux of superoxide

radicals, which have been reported to inhibit CAT activity (Regoli and Principato 1995). Regarding the antioxidant GSH, its considerable decline in the present study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from the GA₃ administration. It is possible that GA₃ might interact with the tissues, resulting in fluctuated enzyme activities by the way of increased free radicals as a result of stress condition in the rats (Celik et al. 2007).

Additionally, the obtained adverse effects of GA₃ on the histological structure of both liver and kidney may be attributed to the current oxidative stress that is evidenced by increased MDA in both liver and kidney. It was evidenced that, the reactive oxygen resulting from oxidative stress in the tissue causes a significant alteration in the cellular structure resulting in tissue injury and cell damage. This is in accordance with Troudi et al. (2009) who observed that intrahepatic blood vessels, central and portal veins are congested and their lining epithelia are eroded. This is besides an inflammatory leucocytic infiltrations, which were more pronounced in the livers of rats which were given daily doses of GA₃ in the drinking water. Moreover, oxidative stress is presumed to play an important role in hepatic fibrogenesis and several reports on liver pathology show a significant alteration of cellular structure (Sarandol et al. 2005).

On the other hand, the administration of GSPE with the plant growth regulators caused marked improvement in the lipid profile. The hypolipidemic effect of GSPE may occur through inhibition of VLDL-C secretion by the liver which is mainly due to a reduction in the bioavailability of lipids, mainly TG or the reduction in the availability of free fatty acids to the liver. Another suggestion is the delay and inhibition of dietary fat and cholesterol absorption and a reduction in chylomicron secretion by enterocytes (Blade et al. 2010; Shan et al. 2010), since GSPE inhibits the absorption of TC and bile acids by decreasing micellar cholesterol solubility. The diminished lipid concentrations by administration of GSPE may be related to the capacity of GSPE to trap reactive oxygen species in aqueous series such as plasma and interstitial fluid of the arterial wall (Yamakoshi et al. 1999). Moreover, GSPE has demonstrated the inhibition of oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules (Bagchi et al. 2000). Also, the current

increases in the measured antioxidants may be related to the protection against oxidative stress with subsequent increases in the antioxidant components which break down reactive oxygen species and attenuate their harmful effects on the lipids and other cellular structures (Joshi et al. 2001). The antioxidative properties of GSPE have been reported by several authors and thus it may be recommended for the treatment of disorders of multi-radical etiology by maintenance of cellular defense mechanisms (Kanner et al. 1994; Jiao et al. 2005).

Furthermore, the obtained enzymatic improvement by administration of GSPE may be due to protecting the cell membranes by the scavenging property of the free radical by the antioxidative phytochemicals present in GSPE such as flavonoids (Yousef et al. 2009) and/or due to the fact that GSPE improves hepatic ischemic injury (Sehirli et al. 2008). The recorded improvement of TP, urea and creatinine levels by administration of GSPE may be due to the antioxidant effects of GSPE which improved the liver cells function and consequently increased protein synthesis. Moreover, GSPE has the ability to interfere directly with free radicals and restore the balance of oxidants/antioxidants leading to improvement of liver functions (Wang et al. 2008). This is indicated by the decreased oxidative stress through the diminished MDA due to the administration of GSPE. This decrease may be related to the capacity of GSPE to trap reactive oxygen species in aqueous fluids such as plasma and interstitial fluid of the arterial wall (Yamakoshi et al. 1999). It is well documented that GSPE has the ability to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules (Bagchi et al. 2000). Additionally, GSPE contains mainly flavonoids, all involved in ameliorating the oxidative stress in vitro and in vivo by increasing the endogenous antioxidant status (Martinez-Florez et al. 2002). Besides, GSPE can clear off free radicals and protect the overoxidative damage caused by free radicals (Spranger et al. 2008). Furthermore, the observed protective action of GSPE on the antioxidants may be due to the protection of SH groups in GSH from oxidative damages via free radical quenching action of the di-OH (catechol) structure in the B ring of GSPE (Ishige et al. 2001). Additionally, grape seed extract, increased the formation of antioxidants products in hepatocytes, and this effect may be

due to the phenolic composition of GSPE and its antioxidant activity (Cetin et al. 2008) as phenolic compounds are known to act as antioxidants not only because they are hydrogen or electron donors, but also because they stabilize radical intermediates, thus preventing oxidation of various food ingredients (Sun et al. 1999). Concerning the histopathological studies of both liver and kidney, the obtained amelioration in the hepatic and renal histological changes (recovery in liver and kidney histology) and reduction of inflammatory cells after administration of GSPE indicates its hepatonephro-protective role. This finding may be attributed to the inhibitory effect of GSPE on pro-inflammatory cytokines evidencing its anti-inflammatory effect (Terra et al. 2007). The observed antioxidant properties of GSPE in the present study are more likely to explain the recovery in liver and kidney structure through their protection from damage. Furthermore, the hepatonephro-protective effect of GSPE in the development of both liver and kidney may be related to the reduction of lipid peroxidation by its antioxidative activity and the ability to scavenge reactive oxygen species (Shin et al. 2010).

In conclusion, it can be concluded that the administration of gibberellic acid (GA_3) resulted in severe biochemical and histological changes in liver and kidney resulting from the oxidative stress and antioxidant disturbances. Grape seed proanthocyanidins extract (GSPE) has an extremely beneficial role in overcoming the occurred adverse effects of gibberellic acid (GA_3) ingestion probably due to its excellent antioxidant properties and highly nutritional values.

Acknowledgments The authors of this manuscript express their deepest gratitude and appreciation to Prof. Dr Mosaad A. Abdel-Wahhab, Food Toxicology & Contaminants Department, National Research Center, Dokki, Cairo, Egypt for his financial assistance, sincere guidance in revision of this work with kindly directions.

References

- Arous S, Boussaid M, Marrakchi M (2001) Plant regeneration from zygotic embryo hypocotyls of Tunisian chilli (*Cap-sicum annuum* L.). J Appl Hortic 3:17–22
- Ashikari M, Sakakibara H, Lin S, Takashi T, Nishimura T, Angeles A, Qian ER, Matsuoka H (2005) Cytokinin oxidase regulates rice grain production. Science 309: 741–745
- Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000) Free radicals and grape seed

- proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* 148:187–197
- Bagchi D, Bagchi M, Stohs SJ, Ray SD, Sen CK, Pruess HG (2002) Cellular protection with proanthocyanidins derived from grape seeds. *Ann New York Acad Sci* 957:260–270
- Bhalla P, Dhawan DK (2009) Protective role of lithium in ameliorating the aluminium induced oxidative stress and histological changes in rat brain. *Cell Mol Neurobiol* 29:513–521
- Blade C, Arola L, Salvado MJ (2010) Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. *Mol Nutr Food Res* 54:37–59
- Bock PP, Karmner R, Paverka M (1980) A simple assay for catalase determination. *Cell Biol Monogr* 7:44–74
- Celik I, Ozbek H, Tuluce Y (2002) Effects of subchronic treatment of some plant growth regulators on serum enzyme levels in rats. *Trends J Biol* 26:73–76
- Celik I, Turker M, Tuluce Y (2007) Abscisic acid and gibberellic acid cause increased lipid peroxidation and fluctuated antioxidant defense systems of various tissues in rats. *J Hazard Mater* 148:623–629
- Cetin A, Kaynar L, Kocyigit I, Hacıoglu SK, Saraymen R, Ozturk A, Orhan O, Sagdic O (2008) The effect of grape seed extract on radiation induced oxidative stress in the rat liver. *Turk J Gastroenterol* 19:92–98
- Craig W, Beck L (1999) Phytochemicals: health protective effects. *Can J Diet Pract Res* 60:78–84
- De Melo MP, Pithon-Curi TC, Miyasaka CK, Palanch AC, Curi R (1998) Effect of indole acetic acid on oxygen metabolism in cultured rat neutrophil. *General Pharm* 31:573–578
- Dimitrova MST, Tsinova V, Velcheva V (1994) Combined effect of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (*Cyprinus carpio*). *Comp Biochem Physiol* 108:43–46
- Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502
- Frings CS, Fendley TW, Dunn RT, Queen CA (1972) Improved determination of total serum lipids by the sulfo-phosphovanillin reaction. *Clin Chem* 18:673–674
- Ghader MR, Riahi AA, Havaspour A, Nooranipour M, Habibinejad AA (2010) The relationship between lipid profile and severity of liver damage in cirrhotic patients. *Hepat Mon* 10:285–288
- Halsted CH (2004) Nutrition and alcoholic liver disease. *Semin Liver Dis* 24:289–304
- Henry RJ (1964) Determination of total protein by colorimetric method. *Clin. Chem.* Harper and Row publishers, New York, p 181
- Herken H, Uz E, Ozyurt H, Sogut S, Virit O, Akyol O (2001) Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. *Mol Psychiatry* 6:66–73
- Ishige K, Schubert D, Sagara Y (2001) Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radic Biol Med* 30:433–446
- Jiao Z, Liu J, Wang S (2005) Antioxidant activities of total pigment extract from blackberries. *Food Technol Biotechnol* 43:97–102
- Joshi SS, Kuszyński CA, Bagchi D (2001) The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. *Current Pharmacol Biotechnol* 84:187–194
- Kanner J, Frankel E, Grant R, German B, Kinsella E (1994) Natural antioxidants in grape and wines. *J Agric Food Chem* 42:64–69
- Karthikeyan K, Sarala Bai BR, Niranjali Devaraj S (2007) Cardioprotective effect of grape seed proanthocyanidins on isoproterenol induced myocardial injury in rats. *Int J Cardiol* 115:326–333
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001) Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 54:356–361
- Martinez-Florez S, Gonzalez-Gallego J, Culebras JM (2002) Flavonoids: properties and antioxidizing action. *Nutr Hosp* 17:271–278
- Muthuraman P, Ravikumar S, Vikramathithan J, Nirmalkumar K, Srikumar K (2010) Effect of phytohormones on tissue hexokinase and on some blood components in wistar rats. *Int J Drug Delivery* 2:168–172
- Ohkawa H, Wakatsuki A, Kaneda C (1982) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358
- Prins HK, Loose JA, (1969) Glutathione. Chapter 4. *Biochemical Methods in Red Cell Genetics*. Edited Academic press. N.Y.D. London, p 126–129
- Regoli F, Principato G (1995) Glutathione, glutathione dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. *Aquat Toxicol* 31:143–164
- Reitman S, Frankel S (1957) Colorimetric estimation of serum transaminases. *Am J Clin Path* 28:56–63
- Rodwell EW (1979) In: A review of physiological chemistry (Harper HA, Rodwell EW, Mayes P (Eds), 17th edn, Lange Medical Publications, California pp 401–404
- Sakr S, Okdah Y, El-Abd S (2003) Gibberellin A₃ induced histological and histochemical alterations in the liver of Albino rats. *Sci Asia* 29:327–331
- Salisbury FB, Ross CW (2009) General and applied plant physiology. 35: 146–152
- Sarandol E, Ta S, Dirican M, Serdar Z (2005) Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. *Cell Biochem Funct* 23:1–8
- Sehirli O, Ozel Y, Dulundu E (2008) Grape seed extract treatment reduces hepatic ischemia reperfusion injury in rats. *Phytother Res* 22:43–48
- Serrano J, Puupponen-Pimia R, Dauer A, Aura AM, Saura-Calixto F (2009) Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Mol Nutr Food Res* 53:310–329
- Shan Y, Ye X, Xin H (2010) Effect of the grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during the movement. *Sci Res Essay* 5:148–153
- Shin MO, Yoon S, Moon JO (2010) The proanthocyanidins inhibit dimethylnitrosamine induced liver damage in rats. *Arch Pharm Res* 33:167–173

- Silverstone AL, Sun T (2000) Gibberellins and the green revolution. *Trends Plant Sci* 5:1–2
- Snedecor GW, Cochran WG (1982) *Statistical Methods*, 7th edn. The State University Press American, Iowa, p 593
- Soliman HAE, Mantawy MM, Hassan HM (2010) Biochemical and molecular profiles of gibberellic acid exposed albino rats. *J Am Sci* 6:224–229
- Spranger I, Sun B, Mateus AM, Freitas V, Ricardo-da-Silva JM (2008) Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chem* 108:519–532
- Srikumar K, Vikramathithan J, Gautami G, Ganesh I (2009) Differences in rat tissue lactate dehydrogenase activity caused by giberellic acid and homobrassinolide. *Turk J Biochem* 34:57–61
- Studenik P (2000) Lipid disorders in liver diseases. *Vnitr Lek* 46:547–548
- Sun BS, Belchior GP, Ricardo-da-Silva JM, Spranger MI (1999) Isolation and purification of dimeric and trimeric procyanidins from grape seeds. *J Chromato A* 841:115–121
- Terra X, Valls J, Vitrac X, Mérrillon JM, Arola L, Ardèvol A, Bladé C, Fernandez-Larrea J, Pujadas G, Salvadó J, Blay M (2007) Grape-seed procyanidins act as antiinflammatory agents in endotoxin-stimulated RAW 264.7 macrophages by inhibiting NFkB signaling pathway. *J Agric Food Chem* 55:4357–4365
- Troudi A, Samet AM, Zeghal N (2009) Hepatotoxicity induced by gibberellic acid in adult rats and their progeny. *Exp Toxicol Pathol* 62:637–642
- Troudi A, Bouaziz H, Soudani N, Amara IB, Boudawara T, Touzani H, Lyoussi B, Zeghal N (2011) Neurotoxicity and oxidative stress induced by gibberellic acid in rats during late pregnancy and early postnatal periods: biochemical and histological changes. *Exper Toxicol Pathol* 37:429–438
- Tuluze Y, Celik I (2006) Influence of subacute and subchronic treatment of abscisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Pest Biochem Physiol* 86:85–92
- Wang J, Ho L, Zhao W, Ono K, Rosensweig C, Chen L, Humala NB, Teplow DM, Pasinetti G (2008) Grape derived polyphenolics prevent A β oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease. *J Neurosci* 28:6388–6392
- Wren AF, Cleary M, Frantz C, Melton S, Norris L (2002) 90-day oral toxicity study of a grape seed extract (IH636) in rats. *J Agric Food Chem* 50:2180–2192
- Yamakoshi J, Kataoka S, Koga T, Ariga T (1999) Proanthocyanidin rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* 142:139–149
- Young DS (1995) *Effects of Drugs on Clinical Laboratory Tests*, 4th Ed. AACC Press, Washington, DC, pp 4–57
- Yousef MI, El-Demerdash FM, Kamel KI, Al-Salhen KS (2003) Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. *Toxicology* 189:223–234
- Yousef MI, Saad AA, El-Shennawy LK (2009) Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. *Food Chem Toxicol* 47:1176–1183
- Yu F, Wang Z, Ju B, Wang Y, Wang J, Bai D (2008) Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamin C and E. *Exp Toxicol Pathol* 59:415–423