Effect of Grape Seed Extract on Oxidative Damages in Gamma Irradiated Rats

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Abstract

Background: Ionizing radiation is known to produce deleterious effects in the living organisms.

Aim of the work: The aim of this study is to evaluate the possible protective effect of the chemical constituents of grape seed extract on oxidative stress induced by gamma rays.

Materials & Methods: Rats were subjected to 8 Gy fractionated doses of gamma radiation, grape seed extract 100 mg/kg body weights were daily administrated before and within radiation exposure. All parameters were investigated at 1st and 14th days post last radiation exposure.

Results: The results revealed that administration of grape seed extract to irradiated rats significantly ameliorates the changes induced in antioxidant system. TBARS (lipid peroxidation index) were significantly decreased when compared with their equivalent values in irradiated rats.

In conclusion, the administration of grape seed extract to irradiated rats might provide substantial protection against oxidative damages due to its free radical scavenging and antioxidant properties of its ingredients. It could be suggested that, grape seed extract may have a potential benefits to people receiving radiotherapy.

Key words: Grape seed extract, γ-Irradiation, Oxidative Stress, Mitochondria, and Antioxidant Enzymes.

Introduction

Radiation damage, is to a large extent caused by over production of reactive oxygen species(ROS) which cause disruption of membrane lipids leading to subsequent formation of peroxide radicals (Rajapakse et al., 2007). Experimental studies have demonstrated that exposures to ionizing radiation induce oxidative stress in different tissues (Saada et al., 2003; Said et al., 2004). ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy,
experimentation, or even space flight (Fang et al., 2002).

Mitochondria are the main cellular organelles that are involved in free radical production. Mitochondria consume about 90% of the cellular oxygen and are the most susceptible organelles to oxidative damage (Khansari et al., 2009). Mitochondria are a major source of ROS, which are a by-product of mitochondrial electron transfer activity (Gustafsson et al., 2008). Since mitochondria are the major site of free radical generation, they are highly enriched with antioxidants including GSH and enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which are present on both sides of their membranes in order to minimize oxidative stress in the organelle (Cadenas and Davies, 2000). The lack of equilibrium between free radical production in mitochondria and anti-oxidant defense mechanisms in this organelle may leads to leak of these harmful reactants to cytoplasm or connective tissues (Khansari et al., 2009).

Over the past decades, researchers have become increasingly interested in polyphenolic compounds. The chief reason for this interest is recognition of their antioxidant properties and their probable role in the prevention of various degenerative-diseases associated with oxidative-stress, such as cancer and cardiovascular-diseases (Manach et al., 2005).

Grape seed extract (GSE) is a natural extract from the seeds of Vitis vinifera, rich in flavonoids, mainly flavan-3-ols and proanthocyanidins (Ferreira and Li, 2000). These flavonoids have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress (Bagchi et al., 2000). Grape seed proanthocyanidin extract (GSPE) has more powerful antioxidative activity than other well-known antioxidants, including vitamin C, vitamin E, and gallic acid (Ariga, 2004). GSPE has various biological functions such as antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory actions (Bagchi et al., 2000).

The present study was planned to elucidate the possible antioxidative and radioprotective effect of grape seed extract as a natural phytochemical compound against oxidative damage induced through extended exposure to ionizing radiation in rats.
Material and Methods

Experimental Plant:

GSE from *Vitis vinifera* was obtained from the vitamin shoppe. The product is supplied as tablets of 100 mg (standardized to 90% proanthocyanidins 90 mg). Tablets were dissolved in water and animals received by gavages the equivalent of 100 mg/Kg body weight/day (Said et al., 2005).

Experimental animals:

Forty eight adult male albino rats (100-120g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines (VACSERA); Cairo, Egypt. The Animals were kept in isolated cages, under standard laboratory condition including all hygienic measures with constant illumination and ventilation, temperature and humidity. Animals were maintained on a starter poultry pellets and water *ad libitum*. All animals received human care in compliance with institutional guidelines.

Radiation process

Irradiation processing was performed using Canadian gamma cell-40, (137Cs) at the National Centre for Radiation Research and Technology (NCCRT), Cairo, Egypt. Animals were subjected to fractionated whole body γ-radiation; delivered as 2Gy every other day up to total dose of 8 Gy. 137Cs source offers a dose rate of 0.61 Gy/min at the time of experiment.

Experimental design

The rats were randomly distributed into 4 groups (n=12, 6-rats/group for each time interval). Group (I): served as controls. Group (II): Animals received *Grape seed extract* by stomach tube in a dose of 100 mg/kg body weight for 14 successive days throughout exposure to gamma radiation. Group (III): subjected to fractionated whole body γ-radiation up to total dose of 8 Gy. Group (IV): Animals were received *Grape seed extract* as described in group II and subjected to gamma irradiation as described in group III.

Samples collection

Animals were fasted over night prior to sacrificing. Samples were collected at 1st and 14th days post irradiation Whole blood was withdrawn by means of heart puncture technique Blood samples were allowed to clot at room temperature then centrifuged at 5000 rpm for 15 min. Serum samples were aspirated, divided into two Eppendorff vials, and stored at -20°C until analysis. Part of liver tissue was dissected, weighed and homogenized in physiological saline (9g Nacl/1000 ml distilled water) (10% w/v).
homogenate was centrifuged at 9000 x g for 20 min using cooling centrifuge (Memmert, MLW, and GDR). The pellet was discarded and the supernatant was stored at -20°C until used for biochemical analysis.

Another part of Liver was removed, and homogenized in 0.25 M sucrose containing 1 mM EDTA. The homogenate was centrifuged at 3000g for 10 min to remove cell debris and the nuclear fraction. The resulting supernatant was centrifuged at 10,000g for 10 min to sediment mitochondria in the centrifuge. The mitochondrial pellets thus obtained were washed thrice with 5 mM potassium phosphate buffer pH 7.4 to remove sucrose and were suspended in the same buffer (Kamat et al., 1997).

Biochemical assays

Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substances (TBARs) level in liver and mitochondrial liver tissue homogenates according to the method described by Yoshioka et al. (1979). Superoxide dismutase (SOD) activity was determined according to the method of Kakker et al. (1984). Determination of reduced glutathione (GSH) content was performed according to Ellman (1959). Catalase (CAT) activity was determined according to the method described by Sinha (1972). Estimation of glutathione peroxidase (GSH-Px) activity was performed after the method of Gross et al. (1967). Tissues total protein content was assayed according to Lowry et al. (1951).

Statistical analysis was performed by using Duncan's multiple range tests using SAS "Statistical Analysis System" Institute, (1988). The results were presented as means ± SE over the experimental periods. The prolonged administration of Grape seed extract throughout exposure to fractionated γ- irradiation induced a decrease in TBARs contents of liver tissue compared to irradiated animals (P<0.05), while a significant decrease of SOD, GSH and CAT activity in liver tissues of animals exposed to fractionated

Results

The thiobarbituric acid reactive substances (TBARs) concentrations in liver tissues of animals exposed to fractionated γ-irradiation with or without Grape seed extract treatment are presented in Table (1). There were elevations in liver (TBARs) concentration of irradiated groups as compared to control and Grape seed extract groups all
γ-radiation in comparison with respective control groups all over the experimental periods. Administration of *Grape seed extract* attained a significant increase in SOD, GSH and CAT activity (*P* < 0.05) in comparison with γ-irradiated groups at both time intervals.

The thiobarbituric acid reactive substances (TBARs) concentrations in liver mitochondrial tissues of animals exposed to fractionated γ-irradiation with or without *Grape seed extract* treatment are presented in Table (2). There were elevations in liver mitochondrial (TBARs) concentration of irradiated groups as compared to control and *Grape seed extract* groups all over the experimental periods. The prolonged administration of *Grape seed extract* throughout exposure to fractionated γ-irradiation induced a decrease in TBARs contents of liver mitochondrial tissue compared to irradiated animals (*P* < 0.05), while a significant decrease of SOD, GSH and GSH-Px activity in liver mitochondrial tissues of animals exposed to fractionated γ-radiation in comparison with respective to control groups all over the experimental periods. Administration of *Grape seed extract* attained a significant increase in SOD, GSH and GSH-Px activity (*P* < 0.05) in comparison with γ-irradiated groups at both time intervals.
Table (1): Effect of grape seed extract on lipid peroxidation (TBARS), superoxide dismutase (SOD), reduced glutathione (GSH) content and catalase (CAT) activity in liver tissues of different animals groups subjected to fractionated γ- radiation.

Data are presented as mean ± SE. Similar characters denote insignificance difference between groups using Duncan Multiple Range Test for comparative Means at (P<0.05)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental period(days)</th>
<th>Groups(n=6)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>control</td>
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<tr>
<td>TBARS (nmole/g wet tissue)</td>
<td>1 day</td>
<td>268.85±2.17c</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>260.52±1.36c</td>
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<td>SOD (U/mg protein)</td>
<td>1 day</td>
<td>2.77±0.06b</td>
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<tr>
<td></td>
<td>14 day</td>
<td>2.87±0.06a</td>
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<td>GSH (mg/g wet tissue)</td>
<td>1 day</td>
<td>22.44±0.43a</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>23.03±0.34a</td>
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<tr>
<td>CAT (U/mg protein)</td>
<td>1 day</td>
<td>4.37±0.11a</td>
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<tr>
<td></td>
<td>14 day</td>
<td>4.29±0.08a</td>
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Table (2): Effect of grape seed extract on lipid peroxidation (TBARS), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in mitochondrial liver tissues of different animals groups subjected to fractionated γ- radiation.

Data are presented as mean ±SE

Similar characters denote insignificance difference between groups using Duncan Multiple Range Test for comparative Means at (P<0.05).

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<tr>
<td></td>
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<td>control</td>
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<tr>
<td>TBARS (nmole/g wet tissue)</td>
<td>1 day</td>
<td>97.97±0.66c</td>
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<tr>
<td></td>
<td>14 day</td>
<td>93.18±0.70c</td>
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<td>SOD (U/mg protein)</td>
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<td>2.36±0.05a</td>
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<tr>
<td></td>
<td>14 day</td>
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<td>GSH (mg/g wet tissue)</td>
<td>1 day</td>
<td>17.01±0.19a</td>
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<tr>
<td></td>
<td>14 day</td>
<td>19.15±0.20a</td>
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<tr>
<td>GSH-Px (U/mg protein)</td>
<td>1 day</td>
<td>0.51±0.01a</td>
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<td></td>
<td>14 day</td>
<td>0.53±0.00a</td>
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Discussion

The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis (Kamat et al., 2000). In this context, the potential of antioxidants to reduce the cellular damage and cytotoxic effects induced by ionizing radiation has been extensively studied in animal for the last few decades (Okunieff et al., 2008).

Numerous studies have suggested positive associations between the consumption of phenolic-rich foods or
beverages and the prevention of disease (Scalbert and Williamson, 2000). Grape seed extract (GSE), a well-known dietary supplement, contains important vitamins, minerals, and polyphenols including flavonoids, proanthocyanidins and procyanidins (Weber et al., 2007). Grape seed proanthocyanidin extract (GSPE) has more powerful antioxidative activity than other well-known antioxidants, including vitamin C, vitamin E, and gallic acid (Ariga et al., 2004). Proanthocyanidin from grape seeds have been reported to show various beneficial properties including hepatoprotective effects as well as modulatory role on age-related oxidative DNA damage (Balu et al., 2006).

The present study attains that enhancement of TBARS concentration; concomitant with depletion of antioxidant parameters (SOD, GSH, CAT and GSH-Px) in liver and liver mitochondrial tissues is a characteristic observation in γ-irradiated animals.

Lipid peroxidation, a process induced by free radicals leads to oxidative deterioration of polyunsaturated lipids (Català, 2009). Under normal physiological conditions, only low levels of lipid peroxides occur in body tissues. The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation (Joshi et al., 2007). The increase in TBARS level might result from the interaction of the highly reactive OH· produced in the cell as the result of irradiation on polyunsaturated fatty acid in the phospholipids portion of cellular membranes which initiating lipid peroxidation cell reaction (Spitz et al., 2004). The present result are in line with that obtained by (Cai et al., 2010) who suggested that Ionizing radiation resulted in a significant increase in the content of MDA, a biomarker of lipid peroxide in rats liver mitochondria causing radiation-induced mitochondrial dysfunction and oxidative damage.

GSE treatment has significantly minimized the formation of lipid peroxidation products obvious by a lower level of TBARS in liver and liver mitochondrial tissues when compared to their corresponding values in irradiated rats. The results are consistent with Hüseyin et al. (2007) who reported that GSE enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation when administered to rats before whole-body irradiation. Furthermore, experimental studies have demonstrated that GSE protects against oxidative stress by doubling the intracellular synthesis of
anti-oxidative enzymes (Puiggros et al., 2005). Also (Lu et al., 2004) stated that treatment with procyanidins from grape seeds in hepatic mitochondrial tissues markedly decreased the MDA levels which agree with our results.

SOD catalyses the dismutation of the highly reactive O$_2$ to oxygen and to the less reactive species, H$_2$O$_2$ (Shijun et al., 2000 and Matsumoto and Fridovich, 2001). Thus the decrease in the activity of SOD observed in the present study could be due to feed back inhibition or oxidative inactivation of SOD due to excess ROS generation. Also (Kamat et al., 2000) stated that the inactivation of superoxide dismutase (SOD), one of the antioxidant enzymes together with protein thiols, the efficient scavengers of ROS involved in the maintenance of the integrity of the membranes confirmed the oxidative damage following exposure to γ-radiation in rat liver mitochondria.

The present study indicated that GSE pre-treatment increase SOD activities in liver and liver mitochondrial tissues. This may be due to that the chemical properties of proanthocyanidins in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers and singlet oxygen quenchers predicts their antioxidant activity (Bagchi et al., 2000).

Glutathione is the most abundant nonprotein sulfhydryl containing compound and constitutes the largest component of the endogenous thiol buffer (Holmgren et al., 2005).

The depletion of GSH recorded in irradiated animals in liver and liver mitochondrial tissues are in agreement with Kamat et al. (2000), which may be due to their utilization in large amount to combat the radiation induced, Free-radical damage, as glutathione is a major non-enzymatic antioxidant, where reduced glutathione (GSH) participate in the cellular system of defense against oxidative damage directly as a free radical scavenger or indirectly by repairing initial damage to macromolecules and could maintain protein and non-protein SH group in reduced form (Ross, 1988; Scibior et al., 2008). The results obtained by this study are in line with (Rajendra et al., 2005) who stated that the decrease in the activities of GSH in hepatic tissues of mitochondria may be due to their utilization by the enhanced production of ROS.

The present study indicated that GSE pre-treatment increase GSH, activities in liver and liver mitochondrial tissues; a possible mechanism explains the results that GSE contains mainly flavonoids. Numerous flavonoids have been shown to
alleviate the oxidative stress by increasing the endogenous antioxidant status, protecting cells against free-radical damage by increasing resistance to oxidative stress (Zwart et al., 1999; Perez et al., 2002).

CAT is one of the most efficient enzymes known. CAT protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Hunt et al., 1998; Oral et al., 2000). The recorded depletion of enzymatic activity of CAT may be due to the increased utilization of this antioxidant to counteract lipid peroxidation production, however CAT removing H2O2 which occurred (Kalpana and Menon, 2004).

The present study indicated that GSE pre-treatment increase CAT activities in liver. The results are in line with that obtained by (Cetin et al., 2008) who suggested that GSE treatment considerably increased the formation of antioxidants products in hepatocytes which represented by increase in SOD, CAT activities and this effect may be due to the phenolic composition of GSE and its antioxidant activity.

GSH-Px has a well-established role in protecting cells against oxidative injury. GSH-Px utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and H2O2 (Ray and Husain, 2002). Li et al. (2007) found that exposure of rat liver mitochondria to γ-irradiation lead to decrease in SOD and GSH-Px activity which may be due to that irradiation-induced ROS markedly alters the physical, chemical and immunologic properties of endogenetic antioxidant enzymes (SOD, CAT, and GSHPx), which further increase oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells.

The present study indicated that GSE pre-treatment increase GSH-Px activities in liver mitochondrial tissues (Alía et al., 2003) reported that glutathione peroxidase activity increased after consumption of grape seeds and grape skins. These results were supposedly caused by polyphenols in the grape seeds. It has been generally recognized that polyphenols have numerous important beneficial effects on oxidative stress, including inhibition of inflammation, inhibition of LDL oxidation, and protection of cells and tissues from oxidative damage (Quettier-Deleu et al., 2003 and Schreckinger et al., 2010).
In conclusion, the present results suggest that grape seed extract constituents controlled the excess production of free radicals produced by gamma irradiation and has a protective effect against oxidative stress by decreasing liver and liver mitochondrial lipid peroxide concentrations and increasing the antioxidant system. It would protect the liver and liver mitochondrial tissues from oxidative damage; preserve the integrity of tissue functions.

References


Effect of Grape Seed Extract on Oxidative Damages….


تأثير مستخلص بذور العنب علي أضرار التأكسد في الجرذان المعرضة لأشعة جاما

عبد الجواد علي فهمى, محمد علي الدسوقي, نعمة محمد الفاتح, وسام عبد الحميد محمد

هيئة الطاقة الذرية – كلية العلوم – قسم الكيمياء, جامعة القاهرة, 2. المركز القومي لبحث وتطوير الأشعة. هيئة الطاقة الذرية

الهدف من هذه الدراسة هو تقييم التأثير الوقائي الممكن لمستخلص بذور العنب على أضرار الأكسدة الناجمة عن أشعة جاما، وقد تعرضت الفئران إلى جرعات مجزأة من أشعة غاما (8 جراة كل بضعة أيام)، وتم إعطاء جرعة يومية من مستخلص بذور العنب مقدارها 100 وزن الجسم ملغم / كغم من وزن الجسم قبل وخلال التعرض للإشعاع.

وتم التضحية بحيوانات التجارب في اليوم الأول والرابع عشر بعد التعرض لأخر جرعة من الأشعاع الجامى.

أظهرت النتائج أن إعطاء مستخلص بذور العنب للفرسان المشعة أدى إلى التقليل بشكل كبير من التغيرات الناجمة في المنظومة المضادة للأكسدة. وانخفاض بشكل ملحوظ تركيز (حمض الثيوببتورك) مقارنة مع التركيز في الفئران المعرضة للإشعاع.

الاستنتاج: في ضوء النتائج التي تم الحصول عليها من هذه الدراسة من الواضح أن تناول مستخلص بذور العنب للفرسان المعرضة للإشعاع يمكن أن يوفر حماية كبيرة ضد أضرار الأكسدة بسبب قدرة مكوناته علي الحد من الشقائق الحرة والخصائص المضادة للأكسدة.

ويمكن أن أشير إلى أن مستخلص بذور العنب يمكن أن يكون له فوائد محتملة بالنسبة إلى الأشخاص الذين يتلقون العلاج الإشعاعي.

الكلمات الدالة: مستخلص بذور العنب, أشعة جاما, الجهاد التأكسدي, الميتوكوندريا, أنزيمات مضادة للأكسدة