Attenuation of γ-Rays Hazards on Mitochondrial Level by Cranberry Extract in Rats

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ABSTRACT

Current study deals with attenuation of gamma-irradiation induced damages in rats using Cranberry. Rats weighting (100-120g) were subjected to 8 Gy fractionated doses of gamma rays (2 Gy every other day up to 8 Gy). Cranberry extract 100 mg/kg body weight was daily administrated before and within radiation exposure period form 14 days. All parameters were investigated at 1st and 14th days post last radiation exposure dose. Because of antioxidants and anti-inflammatory activity of Cranberry, the investigated parameters; lipid peroxidation and GSH contents, SOD, CAT & GSH-Px activities as well as MMPs (2&9) were detected. The results revealed that administration of Cranberry extract to irradiated rats significantly ameliorates the changes induced in antioxidant system and TBARs; lipid peroxidation index. MMP2&9 revealed significant inflammatory responses to gamma-rays increased activity area, while in case of Cranberry treated animals post radiation showed demented MMP2&9 activity areas. TBARS; lipid peroxidation index were significantly decreased when compared with their equivalent values in irradiated rats. In conclusion, the administration of Cranberry extract to irradiated rats might provide substantial protection against oxidative damages due to its free radical scavenging and antioxidant properties. It could be suggested that, Cranberry extract may have a potential benefits in radiotherapy.

Keywords: Cranberry extract, γ-rays, oxidative stress, Mitochondria, Matrix metalloproteinases.

Exposure to ionising radiation initiates a cascade of events that are based not only on direct DNA damage (Moulder, 2002), but also on other effects including oxidative-damage that leads to alteration of tissue physiological functions (Ropenga et al., 2004). Meanwhile, lipid peroxidation of biological membranes can cause alterations in fluidity, reductions in membrane potential, increased permeability to $\text{H}^+$ and other ions and eventual membrane rupture leading to release of cell and organelle contents (Geetha et al., 2004). Oxidative stress initiated and propagated by active oxyradicals and various other free radicals in the presence of catalytic metal ions not only can damage the phospholipid, protein and DNA molecules within the cell but can also modulate cell signaling pathways and gene expression pattern (Chakrabarti et al., 2011). Cranberry (Vaccinium macrocarpon), as native American fruit, has traditionally been used to treat urinary tract infections and blood poisoning (Vattem et al., 2005). Cranberries contain many bioactive compounds such as proanthocyanidins and flavonols that have antioxidant, antimutagenic, antihypercholesterolemic and other health benefits (Neto, 2007). The proanthocyanidins and flavonols are found to be major microbial inhibitors of cranberry other than benzoic acids (Aref et al., 1986). Cranberry's antimicrobial effects offer considerable promise as a natural and effective tool to prevent food borne outbreaks (Wu et al., 2009).

Soliman et al. (2005) reported that irradiation markedly reduced antioxidants-stores and suggested that such modifications could be attributed to radiation-induced vascular damage. Cranberry ranks high among fruit in both antioxidant quality and quantity because of its substantial flavonoid content and a wealth of phenolic acids (Neto, 2007).
The present study was designed to experiment whether cranberry promote systemic-antioxidant effects, endothelial functions and anti-inflammatory properties. The effect of cranberry on various biochemical changes induced by ionising-radiation will be reviewed.

**Material and Methods**

**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 under standard laboratory condition including all hygienic measures with constant illumination and ventilation, temperature and humidity. Animals were maintained on standard rat pellets and water *ad libitum*. All animals received human care in compliance with institutional guidelines.

**Drug administration:**

Cranberry extract from *Vitis vinifera*, USA was obtained from the Healthy Shop. The product is supplied as tablets of 100 mg. Tablets were dissolved in water and animals received by gavages the equivalent of 100 mg/ kg body weight/ day after day for 14 days (Elberry et al., 2010).

**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-rays; delivered as 2Gy every other day up to total dose of 8 Gy, source offers a dose rate of 0.42 Gy/ min at the time of experiment.

**Animal groups:**

The rats were randomly distributed into 4 groups (n=12, 6 rats/each time interval). Group (I): served as controls. Group (II): Animals received Cranberry extract by stomach tube in a dose of 100 mg/kg body weight for 14 successive days. Group (III): subjected to fractionated whole body γ-rays 2 Gy day after day up to total dose of 8 Gy. Group (IV): Animals were received Cranberry extract for 7 days and continue for another 7 days within exposure to γ-rays.

**Samples collection and mitochondrial liver preparation:**

Animals were fasted over night prior to sacrificing. Samples were collected at 1 st and 14 th days post after end of the experiment. Whole blood was withdrawn via heart puncture technique. Blood samples were allowed to clot at room temperature then centrifuged at 5000xg for 15 min. Serum samples were aspirated, divided into two Eppendorff vials, and stored at –20 oC until analysis. Part of liver tissues was dissected, weighed and homogenized in physiological saline (10% w/v). Homogenate was centrifuged at 9000xg for 20 min using cooling centrifuge (Memmert, MLW, Germany). The pellet was discarded and the supernatant was stored at –20 oC until used for the biochemical analysis. Another part of Liver was removed and homogenized in 0.25M 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 10,000xg for 10 min to sediment mitochondria in the centrifuge tubes. The mitochondrial pellets that obtained were washed 3 times with 5 mM potassium phosphate buffer pH 7.4 to remove sucrose and were suspended in the same buffer (Kamat et al., 1997).
Biochemical analysis:

Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substances (TBARs) level in liver tissues and mitochondrial liver 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).

Gelatin Zymography:

The presence and activity of specific MMP species (MMP -2 and 9) were initially detected in the serum using substrate (gelatin) gel electrophoresis (Birkedal-Hansen and Taylor 1982). A buffer of 4% SDS, 0.15 mol/L Tris (pH 6.8), 20% glycerol and 0.5% (w/v) bromophenol blue was added to the serum sample. Serum samples mixed with buffer were directly added to 10% SDS–acylamide gel containing 0.1% (w/v) gelatin (sigma) and separated by running on a mini gel apparatus at15 mA/gel, and then gels were gently rocked in a 2.5% Triton X-100 solution for 30 min at room temperature. Gels were then incubated overnight at 37 °C in substrate buffer containing 50mmol/L Tris–HCl (pH 8), 5 m mol/L CaCl2 and 0.02% NaN3. Gels were subsequently stained for 30 min in 0.5% Coomassie Blue R-250 dissolved in a 1:3:6 solution of acetic acid, isopropyl alcohol and water. The gel was scored for the presence/absence MMP activity by a blinded evaluator and photographed. MMP-2 and MMP-9 could be detected on the SDS gel as transparent bands.

Statistical analysis:

Statistical analysis was performed by using Duncan's multiple range tests using SAS "Statistical Analysis System" Institute, (1988). The results were presented as means± S.E.

RESULTS

The TBARs concentrations in liver tissues of animals exposed to fractionated γ-rays with or without Cranberry extract treatment are presented in Table (1). There were elevations in liver TBARs concentration of irradiated groups as compared to control and Cranberry extract treated groups all over the experimental periods. The prolonged administration of Cranberry extract before and within exposure to fractionated γ-rays 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 γ-rays in comparison with respective control groups all over the experimental periods. Administration of Cranberry 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 mitochondria of animals exposed to fractionated γ - irradiation with or without Cranberry extract treatment are presented in Table (2). There were elevations in liver mitochondrial (TBARs) concentration of irradiated groups as compared to control and Cranberry extract groups all over the experimental periods. The prolonged administration of Cranberry extract throughout exposure to fractionated γ-irradiation induced a decrease in TBARs contents of liver mitochondria compared to irradiated animals (P < 0.05), while a significant decrease of SOD, GSH and GSH-Px activity in liver mitochondria of animals exposed to fractionated γ-radiation in comparison with respective to control groups all over the experimental periods. Administration of Cranberry 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.
Gelatin zymographic analysis of hepatic MMP2&9 in Fig (1&2) for 1 day and 14 days time intervals, respectively represented a dramatic response in group of animals exposed to gamma rays where an enzyme activity area expanded clearly comparing to normal and positive controls (lane 6 &7, respectively). Meanwhile, an obvious improvement and diminish activity area of MMP 2&9 are shown in lanes 8&9 where rats administered with Cranberry extract as a treatment.

Table (1): Effect of Cranberry extract on TBARS, GSH contents and SOD, CAT activities in liver tissues of different animals groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experiment periods</th>
<th>Groups (n=6 for each interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>Cranberry</td>
</tr>
<tr>
<td>TBARS (nmole/g wet tissue)</td>
<td>1 day</td>
<td>267.85± 2.16c</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>260.50±1.35c</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>1 day</td>
<td>2.76±0.05b</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>2.86±0.05a</td>
</tr>
<tr>
<td>GSH (mg/g wet tissue)</td>
<td>1 day</td>
<td>22.43±0.42a</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>23.02±0.33a</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>1 day</td>
<td>4.36±0.10a</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td>4.30±0.08a</td>
</tr>
</tbody>
</table>

Data are presented as mean± S.E. Similar characters denote non-significance difference between groups using Duncan Multiple range Test for comparative Means at (P< 0.05)
Table (2): Effect of Cranberry extract on TBARS, GSH contents and SOD, GSH-Px activities in mitochondrial liver of different animals groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experiment periods</th>
<th>Groups (n=6 for each interval)</th>
<th>control</th>
<th>Cranberry</th>
<th>Irradiated</th>
<th>Cranberry+ γ-rays</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmole/g wet tissue)</td>
<td>1 day</td>
<td></td>
<td>97.97±0.66</td>
<td>97.85±0.78</td>
<td>147.30±1.40</td>
<td>124.42±1.30b</td>
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<td></td>
<td>14 day</td>
<td></td>
<td>93.18±0.70</td>
<td>93.10±0.81</td>
<td>131.37±1.75</td>
<td>112.93±1.24 b</td>
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<tr>
<td>SOD (U/mg protein)</td>
<td>1 day</td>
<td></td>
<td>2.36±0.05a</td>
<td>2.44±0.06a</td>
<td>1.20±0.08c</td>
<td>1.71±0.08b</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td></td>
<td>2.40±0.01a</td>
<td>2.41±0.01a</td>
<td>1.33±0.11c</td>
<td>2.06±0.07 b</td>
</tr>
<tr>
<td>GSH (mg/g wet tissue)</td>
<td>1 day</td>
<td></td>
<td>17.01±0.19</td>
<td>17.33±0.26</td>
<td>13.30±0.11c</td>
<td>15.09±0.06b</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td></td>
<td>19.15±0.20</td>
<td>19.21±0.30</td>
<td>14.70±0.34c</td>
<td>17.18±0.12b</td>
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<tr>
<td>GSH-Px (U/mg protein)</td>
<td>1 day</td>
<td></td>
<td>0.51±0.01a</td>
<td>0.52±0.02a</td>
<td>0.32±0.01c</td>
<td>0.37±0.02b</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
<td></td>
<td>0.53±0.00a</td>
<td>0.53±0.00a</td>
<td>0.38±0.01c</td>
<td>0.46±0.00b</td>
</tr>
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</table>

Data are presented as mean± S.E. Similar characters denote non-significance difference between groups using Duncan Multiple range Test for comparative Means at (P< 0.05)
Fig. (1): Gelatin Zymography for liver tissue MMP-(2&9) activity (72&92 kDa) (1 day) in all the studied groups: (M) Protein marker; (1$^{st}$ standard collagenase; (3&2) Liver tissue samples from normal controls; (5&4)Liver tissue samples from cranberry treated animals; (7&6) Liver tissue samples from gamma rays exposed animals; (9&8) Liver tissue samples from cranberry treated animals pre-exposed to gamma rays.

Fig.(2): Gelatin Zymography for liver tissue MMP-(2&9) activity (72&92 kDa) (14 days) in all the studied groups: (M) Protein marker; (1) Standard collagenase; (3&2)Liver tissue samples from normal controls; (5&4)Liver tissue samples from cranberry treated animals; (7&6) Liver tissue samples from gamma rays exposed animals; (9&8)Liver tissue samples from cranberry treated animals pre-exposed to gamma rays.
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). Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins (Yan et al., 2002), oxidative damage to rat neurons during simulated ischemia (Neto et al., 2005), and oxidative and inflammatory damage to the vascular endothelium (Youdim et al., 2002). The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activities of cranberry extracts, but recent studies suggest that cranberry’s anticancer activity may involve a variety of mechanisms. Mitochondria are the driving force behind life, as mitochondrial oxidative phosphorylation provides the main source of energy in the cell. In addition to energy production, mitochondria play a crucial role in mediating amino acid biosynthesis, fatty acid oxidation, steroid metabolism, intermediate metabolic pathways, calcium homeostasis, and free radical scavenging (Kwong et al., 2006). Mitochondria are a major source of ROS, which are a byproduct of mitochondrial electron transfer activity (Gustafsson et al., 2008).

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.

Cranberry 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.

SOD catalyses the dismutation of the highly reactive O2- to oxygen and to the less reactive species, H2O2 (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 Cranberry extract 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 (Hologram 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 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 (Scibior et al., 2008) 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 Cranberry extract pre-treatment increase GSH, activities in liver and liver mitochondrial tissues; a possible mechanism explains the results that cranberry 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).

Cranberry treated and irradiated group showed an increased of GSH in liver homogenates and liver mitochondria as showed previously (Slemmer et al., 2013)

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 in gamma irradiated animals 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 –treatment with cranberry extract increase CAT activities in liver. The results are in line with that obtained by (Cetin et al., 2008) who suggested that blueberry 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 and its antioxidant activity.

Current study indicated that Cranberry administered animals showed increased activities in liver mitochondria.

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 endogenous antioxidant enzymes (SOD, CAT, and GSHPx), which further increase oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells.

Matrix metalloproteinase-2 &9 (MMP2&9), are the most ubiquitous members of the matrix metalloproteinase family, cleaves collagen type IV of the extracellular matrix maintaining equilibrium between matrix synthesis and degradation, thus providing a critical role in the cell integrity and cell survival(Malemud, 2006).
A flavonoid-rich extract of blueberry was observed to inhibit MMP expression (Matchett et al., 2006), and this activity was attributed in large part to the proanthocyanidins. These compounds strongly inhibited expression of both MMP-2 and MMP-9 at micromolar concentrations (Kondo et al., 2004), a finding that is consistent with the observed ability of Cranberry to inhibit MMP expression.

In conclusion, the present results suggest that Cranberry extract constituents controlled the excess production of free radicals produced by gamma irradiation and have 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 mitochondria from oxidative damage and preserve the integrity of tissue functions.

REFERENCES

C. J., Malemud; Front Biosci; 11, 1696 (2006).
J. Kamat, H. Sarma, T. Devasagayam, K. Nesaretnam, Y. Basiron; Molecular Cellular Biochemistry; 170, 131 (1997).
L. Shijun, Y. Tao, Y. Qin, D. Terry, W. Larry; *Cancer Res.*; 60, 3927 (2000).
V. Wu, X. Qiu, B. Reyes, Y. Lin, Y. Pan; Food Microbiol.; 1, 7 (2009).
X. Li, A. Zhou, X. Li; *Carbohydrate Polymers*, 69, 172 (2007).
Y. Fang, S. Yang, G. Wu; *Nutrition*; 18, 872 (2002).