Studying the effect of γ-irradiated celery leaves on antioxidant status and cardiac enzymes in hypercholesterolemic rats

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ABSTRACT
This work aimed to study the effects of gamma (γ) irradiation on the total phenols and total flavonoid contents of celery leaves and to investigate effect of γ-irradiated celery leaves powder (γ-Irr.CLP) against hypercholesterolemia in rats. Results obtained that γ-irradiation increased the amount of total phenolic and total flavonoid contents of dried celery. The results of biological study showed marked increases in the concentrations of serum lipid contents, activities of liver and cardiac marker enzymes and level of TBARS with a significant decrease in serum high-density lipoprotein concentration and reduction in the activities of anti-oxidant enzymes and glutathione contents of serum and heart tissues of hypercholesterolemic rats compared to control rats. Treatment of hypercholesterolemic rats with raw CLP or γ-Irr.CLP showed significantly less severe damage and remarkable improvement in all of the measured parameters when compared to hypercholesterolemic rats lowered the concentrations of serum lipid contents, liver and cardiac marker enzymes and TBARS level and ameliorate anti-oxidant enzymatic status in serum and cardiac tissue. These results suggest that, treatment with γ-Irr.CLP has a powerful modulating effect on hypercholesterolemia induced oxidative stress and has the potential in reducing cardiovascular complications.

Key words: Anti-oxidant status, Cardiac marker enzymes, Celery, Hypercholesterolemia, Oxidative stress, γ-irradiation

INTRODUCTION
Eating foods such as continuous ingestion of elevated levels of triglycerides, cholesterol and saturated fats intake are believed to be directly related to hypercholesterolemia (Otinola et al., 2010). Hypercholesterolemia (Dyslipidemia) is a common clinical situation that lipoprotein metabolic disorder characterized by elevated serum low density lipoprotein (LDL) and blood cholesterol and considered as one of the significant important risk factors of atherosclerosis that leads to cardiovascular diseases and can cause morbidity and mortality (Akinymi et al., 2016). Hypercholesterolemia state diminishes the activity of antioxidant defense and decreases the activities of superoxide dismutase (SOD) and catalase (CAT) and accelerate oxidative stress and leads to elevating the lipid peroxide content which lead to tissues damage (Anila and Vijayalakshmi, 2003 and Akinymi et al., 2016). Although numerous synthetic lipid-lowering drugs that causing lower high blood cholesterol, they have been reported to have serious toxic side effects, particularly hepatic damage (Suanarunsawat et al., 2011). Moreover, they lack several desirable properties such as efficacy and safety on long-term use; they are costly and associated with side effects, and simplicity of administration. Therefore, attention is being directed to the medicinal plants and herbal origin with hypolipidaemic activity which is safer for health without side effects. Several kinds of medicinal plants contain anti-oxidant and lipid-lowering effect levels and improve lipid profiles (Suanarunsawat et al., 2011). Celery (Apium graveolens L.) is an aromatic vegetable plant used as food and considered as an important medicinal herb (Sivashanmugam et al., 2011). It’s used in pharmaceutical, food; ornamental industries caused considerable commercial value. It found to contain biologically active compounds such as limonene, selinene, furocumarin glycosides, flavonoid and is an excellent source of vitamins A, C, B1, B2, Mg, P, Fe and K cause its most use in medicine and traditional medicine (Kooti et al., 2014a). Previous studies showed that celery has been referred for anti-fungal effects, anti-mutagenic, anti-oxidants, anti-thrombosis, anti-inflammatory effects and anti-hyperlipidemic activities (Kooti et al., 2015). Gamma (γ) irradiation process is an alternative treatment that has been shown to be effective in decreasing growth of microorganisms on ready-to-eat food. This process has been approved by the FDA for use on fresh fruit and vegetables at a maximum level of 1.0 kGy (IFT 1983 and Thayer et al., 2011).
Food irradiation technology has been recognized as an alternative technique to methyl bromide for treating fresh and dried agricultural products to overcome quarantine barriers in world trade, as a mode of decontamination/disinfestation and for improving nutritional attributes and extends shelf-life without changes in nutritional composition or the texture, taste or appearance of food. (Parveen et al., 2015). The purpose of this work was to study the effects of γ-irradiation on the total phenols and total flavonoids contents of celery leaves and to investigate lipid-lowering and antioxidative activities of γ-irradiated celery leaves powder (Irr. CLP) on serum and heart function of rats fed with a high-fat diet.

MATERIALS AND METHODS

Material: Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Celery plant and standard commercial rodent diet were purchased from local herbal market (Cairo, Egypt). After drying, celery leaves were crushed to coarse powder and sieved through No. 20 mesh size.

Irradiation process: Celery leaves powder (CLP) was transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a 60Co source at a dose rate of 2.48 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Determination of Total Phenolic and Flavonoid Content Preparation of plant extract: Twenty five gram of raw and γ-irradiated dried celery leaves were boiled with 250 ml of distilled water for 15 minutes, after cooling and filtering by using funnel and filter paper (Khoun, 2012).

The amount of total phenolic contents in the extracts was determined spectrophotometrically with the Folin-Ciocalteu (FC) reagent using the method of Fukehoto and Mazza (2000) with small modifications (Božin et al., 2008).

Total flavonoid content in the extracts was determined spectrophotometrically according to Jia et al. (1999), using a method based on the formation of a flavonoid-aluminium complex with an absorbance maximum at 430 nm. All measurements were replicated three times.

Animals and biochemical assay: Male albino rats Sprague Dawley (150 ± 20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed ad libitum. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85 – 23, 1996).

Experimental design: The animals were randomly divided into four groups, each consisted of 7 rats. Group C: rats were fed on normal diet for 10 weeks, served as a control, Group HCD: rats were fed on hypercholesterolemic diet (basal diet + 1% cholesterol + 16% fat and 0.2% Cholic acid) (Harnafi, 2009) for 10 weeks.

Group HCD + CLP & HCD + Irr.CLP: rats were fed on HCD supplemented with 10% of raw CLP or γ-irradiated CLP for 10 weeks.

Each rat was weighed at the initial and the end of experiment. At the end of the experiment (10 weeks), rats were fasted for 24 hours and anaesthetized with diethyl ether. Blood sample were collected through heart puncture and allowed to coagulate and centrifuged to obtain serum for biochemical analysis. Also, cardiac tissue was removed for biochemical investigation.

Cardiac tissue (100 mg tissue/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.2; St. Louis, MO, USA); the homogenate was then centrifuged at 1,200 x g for 15 min and the supernatant was used for biochemical analysis.

Biochemical analysis: Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain et al. (1974), Fossati and Prencipe (1982) and Demacker et al. (1980), respectively. Low-density lipoprotein-cholesterol, very low-density lipoprotein-cholesterol and risk ratio were evaluated according to Friedwald’s formula (Friedwald et al., 1972) by the following equations: LDL-C (mg/dl) = TC -(TG/5+HDL-C), vLDL (mg/dl) = TG/5. The levels of markers of myocardial tissue damage, such as lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of King (1965), aspartate transaminase (AST) and alanine transaminase (ALT), were determined according to the Reitman and Frankel (1957). Serum samples and cardiac tissue homogenate were used for determination of thioarbituric acid reactive substances (TBARS) (Yoshioka et al., 1979), glutathione content (GSH) (Beutler et al., 1963), and for the assays of the activity of superoxide dismutase (SOD) (Minami and Yoshikawa, 1979) and catalase (CAT) (Johansson and Borg, 1988).

Statistical analysis: Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan’s multiple range test was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) (SPSS, 1998). Differences between means were considered significant at P < 0.05.

RESULTS AND DISCUSSION

The effects of gamma irradiation on the total phenols and total flavonoids are shown in (Table 1). The data demonstrated that the irradiated celery up to 10 kGy had higher phenolic compounds and flavonoids than non-irradiated control. The results show that the HCD resulted in significant increase in the level of serum TC, TG, LDL-C
Table 1: Total phenolic and total flavonoids contents of raw and γ-irradiated celery.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Celery samples</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content (mg GAE/g d.wt. ± S.D)</td>
<td>46.74 ± 0.45</td>
<td>48.32 ± 0.37</td>
</tr>
<tr>
<td>Total flavonoids (µg RE/g d.wt. ± S.D)</td>
<td>0.76 ± 0.01</td>
<td>0.79 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means of three replicates (± S.D)

Table 2: Effect of administration of raw CLP and γ-Irr.CLP on serum lipid profile in diet induced hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>TC (mg/dl) ± S.E.</th>
<th>TG (mg/dl) ± S.E.</th>
<th>HDL-C (mg/dl) ± S.E.</th>
<th>LDL-C (mg/dl) ± S.E.</th>
<th>v-LDL-C (mg/dl) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>123.27 ± 6.12</td>
<td>116.45 ± 5.48</td>
<td>56.15 ± 4.42</td>
<td>43.83 ± 5.54</td>
<td>23.29 ± 4.62</td>
</tr>
<tr>
<td>HCD</td>
<td>181.63 ± 9.24</td>
<td>231.15 ± 7.86</td>
<td>48.36 ± 6.27</td>
<td>62.16 ± 7.62</td>
<td>33.71 ± 6.08</td>
</tr>
<tr>
<td>HCD+ RCLP</td>
<td>144.23 ± 6.60</td>
<td>168.52 ± 6.24</td>
<td>45.73 ± 4.56</td>
<td>64.96 ± 4.55</td>
<td>31.84 ± 4.52</td>
</tr>
<tr>
<td>HCD+ Irr. CLP</td>
<td>142.53 ± 4.96</td>
<td>159.20 ± 6.77</td>
<td>45.73 ± 4.56</td>
<td>64.96 ± 4.55</td>
<td>31.84 ± 4.52</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7)

Table 3: Effect of administration of raw CLP and γ-Irr.CLP on serum LDH, CPK, ALT and AST activities in diet induced hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HCD</th>
<th>HCD+ RCLP</th>
<th>HCD+ Irr. CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (U/L)</td>
<td>9.02 ± 1.42</td>
<td>16.71 ± 2.35</td>
<td>12.63 ± 1.15</td>
<td>12.11 ± 1.17</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>5.14 ± 0.19</td>
<td>2.35 ± 0.23</td>
<td>3.82 ± 0.26</td>
<td>4.14 ± 0.29</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>1061.73 ± 40.6</td>
<td>49.28 ± 1.16</td>
<td>49.28 ± 1.16</td>
<td>51.37 ± 2.53</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>28.54 ± 1.25</td>
<td>17.95 ± 0.97</td>
<td>22.81 ± 1.18</td>
<td>24.37 ± 1.36</td>
</tr>
<tr>
<td>Cat (U/g protein)</td>
<td>39.28 ± 1.14</td>
<td>23.67 ± 1.07</td>
<td>29.17 ± 1.16</td>
<td>31.82 ± 1.17</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7)

Table 4: Effect of administration of raw CLP and γ-Irr.CLP on serum and cardiac level of TBARS and GSH and activity of SOD and CAT in diet induced hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HCD</th>
<th>HCD+ RCLP</th>
<th>HCD+ Irr. CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (ng/mL)</td>
<td>9.02 ± 1.42</td>
<td>16.71 ± 2.35</td>
<td>12.63 ± 1.15</td>
<td>12.11 ± 1.17</td>
</tr>
<tr>
<td>GSH (mg/g tissue)</td>
<td>5.14 ± 0.19</td>
<td>2.35 ± 0.23</td>
<td>3.82 ± 0.26</td>
<td>4.14 ± 0.29</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>28.54 ± 1.25</td>
<td>17.95 ± 0.97</td>
<td>22.81 ± 1.18</td>
<td>24.37 ± 1.36</td>
</tr>
<tr>
<td>CAT (U/g protein)</td>
<td>39.28 ± 1.14</td>
<td>23.67 ± 1.07</td>
<td>29.17 ± 1.16</td>
<td>31.82 ± 1.17</td>
</tr>
<tr>
<td>Serum antioxidants and lipid peroxide</td>
<td>11.36 ± 1.13</td>
<td>17.65 ± 1.19</td>
<td>14.35 ± 1.14 b</td>
<td>13.18 ± 1.12</td>
</tr>
<tr>
<td>TBARS (µmol/mL)</td>
<td>10.73 ± 0.57</td>
<td>13.25 ± 0.49</td>
<td>17.19 ± 0.61 b</td>
<td>18.92 ± 0.49 b</td>
</tr>
<tr>
<td>GSH (µmol)</td>
<td>30.46 ± 0.49</td>
<td>27.24 ± 0.51</td>
<td>26.63 ± 0.52 b</td>
<td>28.12 ± 0.57 b</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7).
which might induce chemical reaction in the components of celery by degradation or decomposing the higher molecules weight into simple molecules readily soluble in methanol and then resulting in the production of more methanol soluble substances. These data are in agreement with the study done by Huang and Mau (2006). Also, Villavicencio et al. (2000) presented that γ-irradiation could be results in increasing in the level of total phenolic contents compared with non-irradiated samples and that might be due to the break this complex of some insoluble phenolic compounds which were contributed to increase the total phenolic content.

The results of biological study showed marked increases in the concentrations of serum lipid contents, activities of liver and cardiac marker enzymes and level of TBARS with a significant decrease in serum high-density lipoprotein concentration and reduction in the activities of antioxidant enzymes and glutathione contents of serum and heart tissues of hypercholesterolemic rats compared to control rats. Kooti et al. (2014a) reported that due to the high fat diet, the amount of cholesterol, triglycerides and chylomycin elevated due to the high fat diet then the level of LDL increased. Elevated in LDL-C level in blood has been pointed out as one of the major risk factors for the development of atherosclerosis and related cardiovascular diseases (Abd El-Mageed, 2011). High cholesterol diet markedly suppressed hepatic and cardiac functions as expressed by an augmentation of serum levels of AST, ALT, LDH and CK-MB activities (Suanarunsawat et al., 2011). Cellular damage, loss of functional integrity, and/ or permeability of cell membrane resulted in elevation in the activities of these enzymes in serum (Dikshit et al., 1995). The effect of HCD on lipid peroxidation (TBARS) in this work is consistent with several clinical and experimental studies which have shown that hypercholesterolemia leads to increased lipid peroxidation (Dutta and Bishayi, 2009). Oxidative stress has been considered as an important pathogenic factor in the development of hypertension and also most of the complications related to hypertension are associated with oxidative stress, induced by the production of free radicals (Soanker, 2012). The reduction in activities of serum and heart antioxidant enzymes (SOD and CAT) could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the HCD (Ma et al., 2011) or insufficient availability of GSH. This observation is further substantiated by the elevated TBARS levels.

Treatment of hypercholesterolemic rats with raw CLP or γ-Irr.CLP showed significantly less severe damage and remarkable decrease in the concentrations of serum lipid contents, significant reduction in the activity of LDH, CPK, AST and ALT with significant increase in HDL-C level, GSH concentration and the activity of SOD and CAT with decrease in TBARS concentration of the serum and cardiac tissue compared to HCD-rats. Kooti et al. (2014b) found that celery could be effective in the treatment of hyperlipidemia due to the its anti-oxidant properties leads to appropriate changes in serum lipid profiles and reduces them. Tsi et al. (2000) studied the effect of anti-hyperlipidemia of the hydroalcoholic celery in the rat and observed a significant reduction in the concentration of serum TC, TG levels and hepatic lipase triacyl glycerol in the treatment group. The lipid lowering action of celery may be mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acids excretion, and enhanced plasma lecitin: cholesterol acyltransferase activity, and reduction of lipid absorption in the intestine, thereby reducing the bad cholesterol (Mansi et al., 2009). Alhat et al. (2007) reported that *Apium graveolens* traditionally used for heart palpitations and liver disorders. The reduction in the activity of AST and ALT by celery in this study might due to the biochemical compounds of celery as (D-carvon, D-limonen and myrcen), which have biological effects on aminotransferase enzymes and important role for elevation the antioxidant enzymes (Sorbitol dehydrogenase and Glutamic dehydrogenase) (Taher et al., 2007). Aravind et al. (2013) suggested that the volatile compounds of the celery extract may be beneficial in the treatment of myocardial infarction and prevention of the increase of the marker enzymes released due to the necrosis of cardiac muscles. The antioxidant activity of celery may be due to the presence of flavonoids (Popovic et al., 2006), β-carotene, lutein and the flavones, luteolin and apigenin (Lugasi et al., 2003). Manju et al. (2005) observed enhancement of anti-oxidant status (glutathione GSH, pyrogallol peroxidase PPx, glutathione-S-transferase GST, glutathione reductase GR, SOD, CAT, Vitamin C, Vitamin A and β-carotene) in rats with 1,2-dimethylhydrazine induced colon cancer upon intragastric administration of 0.2 mg/kg luteolin(which is a flavone contained in celery). Moreover, Jain et al. (2009) reported that co-administration of methanolic extract of celery seeds along with Di-(2-ethylhexyl) phthalate (DEHP) induced hepatotoxicity in rats, significantly prevented the rise in TBARS level with a concomitant elevation in the concentration of hepatic glutathione and ascorbic acid suggesting alleviation of oxidative stress and restoration of antioxidant defense system resulting in membrane stabilization.

From the results of this study, it obtained that γ-ray -radiation processing resulted in increasing of the total phenolic content and total flavonoid which confirm the effectiveness of γ-radiation technology to enhance the antioxidant properties of celery. Also, the present study demonstrated that γ-Irr.CLP administration produced an effective treatment against hypercholesterolemia and was able to ameliorate serum biochemical parameters, cardiac marker enzymes and lipid contents and significantly improved the antioxidant defense system with significant
reduction of oxidative stress caused by hypercholesterolemia in cardiac tissue of rats as well as improved the antioxidant defense system. It is recommended that, administration of diet rich in the natural antioxidant is very important for protection of different body tissue and may be beneficial for patients who suffer from hyperlipidemia, hypercholesterolemia and/or arteriosclerosis.

REFERENCES


