Efficiency of Broccoli in Attenuating of Some Biochemical Disorders in Rats exposed to γ-irradiation

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ABSTRACT

Administration of dietary antioxidants has been suggested to protect against ionizing radiation induced injury. Broccoli is a floral vegetable with an important nutritional value due to its content of antioxidants and anti-carcinogenic compounds. Thus, the aim of this study was oriented to study the ameliorative effect of broccoli administration against γ-radiation induced oxidative stress. Exposure of rats to γ-radiation (6 Gy) induce a significant increase in the level of malondialdehyde (MDA) and hepatic xanthine oxidase (XO) activity, the concentration of some lipid contents, the activity of some liver enzymes and glucose concentration. Whereas, the value of hepatic glutathion content and the activity of hepatic xanthine dehydrogenase (XDH), superoxides dismutase (SOD) and catalase (CAT) activity, high-density lipoprotein-cholesterol (HDL-C) concentration and the level of insulin and testosterone were significantly decreased. On the other hand, dietary supplementation with broccoli to γ-irradiated rats resulted in an obvious amelioration of the hazardous effects induced by γ-irradiation. In conclusion, the results suggested that broccoli has remarkable radioprotective effects against radiation induced oxidative damage and that might be attributed to its phytochemical contents.

INTRODUCTION

Exposure to ionizing radiation represents a genuine, increasing threat to mankind and our environment. The major destructive effect of ionizing radiation in biological systems is based on radiolysis of water and generation of reactive oxygen species (ROS) in cells (1&2). Among them, particularly, the highly damaging hydroxyl radical (•OH) can cause injury by reacting with biomolecules and also can cause DNA damage and introduce mutations (2&3).

Therefore, control of radiation hazards is considered as one of the most important challenges in order to protect our lives from radiation damage (4). Dietary antioxidants including polyphenolic compounds, vitamins E, vitamin C and carotenoids are believed to be the effective nutrients in the protection against free radicals (5) and prevention of the oxidative stress related diseases (6).

Broccoli (Brassica oleracea L. var. italica) is distinguished for the high number of health-promoting compounds such as carotenoids, glucosinolates, chlorophylls, different vitamins and phenolic compounds that can cause interference with oxidation of lipids and other molecules by the rapid donation of hydrogen atoms to free radicals (7&8). Another major health-promoting compound present in broccoli is vitamin C (9&10) which includes ascorbic acid and its oxidized product dehydroascorbic acid, participates in redox reactions in intra- and extracellular spaces of biological mechanisms. Also, vitamin C protects against cell death, directly scavenge superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radicals, and acts as a lipid peroxidation chain-breaking agent (11).
The aim of this study was to investigate the efficiency of broccoli in attenuating of some biochemical disorders in rats exposed to γ-irradiation.

MATERIAL AND METHODS

Material:
Fresh broccoli was purchased from the local market, Cairo, Egypt.

Radiation Facility:
Whole body gamma irradiation of rats at a dose level of 6 Gy was performed using a Canadian gamma cell-40, (137)Cs housed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate was 0.43 Gy/min at the time of the experimentation.

Experimental Animals:
Adult male albino rats, reared in the NCRRT, Egypt, were used in the present work. Matched weight animals (120-140g) were selected and housed in plastic cages under controlled condition and fed on well balanced diet (60% corn, 20% soy-been, 10% growth additives, 5% wheat bran and fibers 2.75% molas, 1.5% powdered bone, 0.5% table salt and 0.25% vitamins).

Experimental Design:
Animals (28 rats) were randomly divided into 4 groups each of seven rats as follows:

Group 1: fed on balanced diet for 8weeks, served as control group.
Group 2: rats fed on balanced diet with 15% fresh broccoli for 8weeks.
Group 3: rats were exposed to whole-gamma-irradiation (6Gy) at the beginning of the experiment and fed on balanced diet for 8weeks (irradiated group).
Group 4: rats were exposed to whole-gamma-irradiation (6Gy) at the beginning of the experiment and fed on balanced diet supplemented with 15% broccoli for 8weeks.

At the end of the experiment, animals were sacrificed 24 hrs after the last dose of treatment. Blood samples were collected through heart puncture after light anesthesia. Blood samples were allowed to coagulate and centrifuged to obtain serum for biochemical analysis. The liver was removed immediately by dissection, washed in ice-cold isotonic saline and stored. A 10% (w/v), liver homogenates were prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5 using Branson sonifier (250, VWR Scientific, USA).

Biochemical Analysis:
The lipid peroxide content was determined colorimetrically as malondialdehyde (MDA) according to Yoshioka et al. (12). Hepatic xanthine oxidase (XO) and xanthine dehydrogenase (XDH) were determined according to Kaminski and Jeweszka (13). Whereas, the value of hepatic glutathione content (GSH) and the activity of superoxides dismutase (SOD) and catalase (CAT) were measured by the method of Gross et al. (14), Minami and Yoshikawa (15) and Aebi (16), respectively. In addition, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined in the serum according to procedure described by Allain et al. (17), Fossati and Prencipe (18) and Demacker et al. (19), respectively, while low-density lipoprotein cholesterol and very Low-density lipoprotein-cholesterol were evaluated according to Friedwald et al. (20) and Norbert (21) formulas, respectively by the following equations: LDL-C (mg/dl) = TC - (TG/5+HDL-C), vLDL (mg/dl) = TG/5. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (22), serum gamma glutamyl transferase (GGT) was assessed according to Rosalk (23), and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (24). Serum glucose was evaluated by the method of Trinder (25). Finally, the serum testosterone concentration was measured by the enzyme linked immunosorbent assay.
(ELISA) according to the method of Engrall and Perlman (26) and also insulin hormone level was determined by radioimmunoassay kit supplied by Diasari, Italy.

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. Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) (27). Differences between means were considered significant at P < 0.05.

RESULTS

The data presented in Table (1) revealed that γ-irradiated rats had a significant decrease in the value of hepatic GSH content and the activity of XDH, SOD and CAT associated with a significant increase in MDA level and XO activity of rats exposed to γ-radiation as compared to the corresponding values of control and other groups. Whereas, γ-irradiated rats fed on broccoli had a lower concentration of MDA and XO activity and higher level of GSH as well as the activity of XDH, SOD and CAT was increased in this group comparing to those of the γ-irradiated group.

Table (1): Effect of broccoli supplementation to γ-irradiated rats on the level of MDA, xanthine oxidoreductase system (XO and XDH), GSH, SOD and CAT.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>Broccoli</th>
<th>Irradiated</th>
<th>Irr.+Broccoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(nmol/ml)</td>
<td>200.1±5.51</td>
<td>186.67±4.69</td>
<td>391.71±6.84</td>
<td>279.33±5.81</td>
</tr>
<tr>
<td>XO(mU/mgprotein)</td>
<td>2.52±0.08</td>
<td>2.47±0.07</td>
<td>3.76±0.09</td>
<td>2.78±0.06</td>
</tr>
<tr>
<td>XDH(mU/mgprotein)</td>
<td>3.29±0.12</td>
<td>3.35±0.15</td>
<td>1.62±0.13</td>
<td>2.87±0.12</td>
</tr>
<tr>
<td>GSH(mg/g tissue)</td>
<td>27.94±0.95</td>
<td>28.04±0.76</td>
<td>15.72±0.72</td>
<td>23.90±0.81</td>
</tr>
<tr>
<td>SOD(U/mg protein)</td>
<td>45.92±1.13</td>
<td>46.26±0.97</td>
<td>30.42±0.87</td>
<td>43.58±1.06</td>
</tr>
<tr>
<td>CAT(U/g protein)</td>
<td>3.30±0.07</td>
<td>3.33±0.05</td>
<td>1.84±0.04</td>
<td>2.75±0.05</td>
</tr>
</tbody>
</table>

Table (2): Effect of broccoli supplementation to γ-irradiated rats on lipid profile.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Broccoli</th>
<th>Irradiated</th>
<th>Irr.+Broccoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>154.12±4.54</td>
<td>150.45±5.18</td>
<td>211.71±6.57</td>
<td>182.33±4.25</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>117.16±3.63</td>
<td>114.35±3.51</td>
<td>183.04±2.72</td>
<td>149.56±3.26</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>47.62±1.87</td>
<td>47.77±1.81</td>
<td>37.97±1.96</td>
<td>41.57±1.68</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>83.07±2.17</td>
<td>79.81±2.08</td>
<td>137.13±2.83</td>
<td>110.85±2.51</td>
</tr>
<tr>
<td>vLDL-C (mg/dl)</td>
<td>23.43±1.24</td>
<td>22.87±1.28</td>
<td>36.61±1.86</td>
<td>29.91±1.32</td>
</tr>
</tbody>
</table>

Legend as table 1

The concentration of TC, TG, LDL-C and vLDL-C in the serum of γ-irradiated rats was highly increased with a significant decrease in HDL-C concentration as compared with control and other groups, while adding broccoli to the diet of rats post γ-irradiation minimized the hyperlipidemic effects of γ-irradiation by reducing the concentration of TC, TG, LDL-C and vLDL-C and increased the level of HDL-C comparing with irradiated group only (Table 2).

The activity of some liver enzymes (AST, ALT, ALP and GGT) in the serum of γ-irradiated group showed a remarkable increase as compared to control rats. On the other hand, the activity of these enzymes was elevated in γ-irradiated group dietary supplemented with fresh broccoli (Table 3).

The obtained results in this work indicated that exposure of rats to γ-radiation resulted in an obvious rising in the glucose concentration (Table 3) associated with reduction in the level of insulin
In the same time, it was noticed that broccoli consumption post γ- irradiation significantly reduced the level of glucose parallel with enhancement of insulin level.

Table (3): Effect of broccoli supplementation to γ-irradiated rats on the activity of some liver enzymes and glucose concentration.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>Broccoli</th>
<th>Irradiated</th>
<th>Irr.+Broccoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/ml)</td>
<td>28.83±0.76a</td>
<td>28.62±0.64a</td>
<td>51.75±0.91c</td>
<td>36.62±0.68b</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>17.95±0.62a</td>
<td>17.68±0.52a</td>
<td>39.82±0.92c</td>
<td>28.15±0.87b</td>
</tr>
<tr>
<td>ALP(U/100ml)</td>
<td>9.26±0.42a</td>
<td>9.11±0.50a</td>
<td>16.02±0.67c</td>
<td>11.78±0.56b</td>
</tr>
<tr>
<td>γGT (U/ml)</td>
<td>3.38±0.32a</td>
<td>3.29±0.39a</td>
<td>6.61±0.49c</td>
<td>4.88±0.57b</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>118.41±3.88a</td>
<td>116.83±3.71a</td>
<td>181.56±5.54c</td>
<td>139±4.28b</td>
</tr>
</tbody>
</table>

Also, the reducing effect of γ-irradiation on the level of testosterone is illustrated in Figure 2. While, an obvious enhancement in the level of this hormone was observed in γ-irradiated group received fresh broccoli.

DISCUSSION

Ionizing radiation is known to induce oxidative stress through generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cells, which is suggested to culminate in cell death (28). Broccoli is a cruciferous vegetable that contains high concentrations of antioxidant vitamins, glucosinolates and polyphenols that may act as antioxidants and protect against oxidative stress (29&30).

In the present work, the results revealed that whole body gamma irradiation of rats resulted in an alteration of XOR system and conversion of XDH into XO activity as well as a significant increase in the level of MDA. The results are compatible with previous findings that ionizing radiation induces the conversion of XDH into XO (31&32). Also, Saada et al (33) reported that the significant increase in XO activity might be attributed to radiation-induced hypoxia where insufficient oxygen availability elevates calcium concentration, which activates protease capability of converting the dehydrogenase to...
oxidase form. The increased MDA level in irradiated rats could be attributed to the peroxidation of membranes lipid resulted in cellular structure modifications and oxygen radicals- mediated tissue damage (34).

Moreover, exposure of rats to γ-rays reduces GSH content as well as the activity of SOD and CAT. The decrease in reduced glutathione by irradiation could be due to it enhanced utilization in large amount to combat the radiation-induced free radical damage, as glutathione is a major non-enzymatic antioxidant (18&35). The observed decrease in SOD activity suggests inactivation of the enzyme possibly due to increased superoxide radical production or an inhibition by the H2O2 as a result of corresponding decrease in the activity of CAT which selectively degrades H2O2. Furthermore, the decrease in CAT activity might be interpreted by the prospect of oxidative modifications of various protein types which leads to functional alteration which can have substantial physiological impact, as oxidative damage to enzymes associated with a modification of their activity (36).

On the other hand, dietary supplementation with broccoli to γ-irradiated rats caused a significant reduction in the level of MDA content and XO activity with concomitant significant increment in the activity of XDH, SOD and CAT, and in the content of GSH. Fernández-León et al. (8) reported that broccoli is one of Brassicaceae family that possesses different type of phenolic and flavonoids compounds that can decrease the risk of development of different type of diseases. These dietary antioxidants work synergistically or independently to scavenge the free radicals and terminate the propagation of the free radicals reactions in addition to limiting the formation of new free radicals. Also, they can protect against oxidative stress induced by gamma radiation (37).

This study demonstrated that whole body gamma irradiation of rats was accompanied by elevated activity of ALT, AST, GGT and ALP. These results are in accordance with those found by (38 &39). It is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation. Also, the increase in aminotransferase activities by radiation may be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in liver and blood serum (40).

However, consumption of broccoli by γ-irradiated rats resulted in an obvious reduction in the activity of ALT, AST, GGT and ALP. A possible mechanism of reduced activities of the tested enzymes and hepatoprotective effect of broccoli may be related to the antioxidant effect of their phenolic and flavonoids compounds. Previous study reported that polyphenols can inhibit nitrosation and flavonoids have hepatoprotective activities (41).

In this work, the levels of TC, TG, LDL-C and vLDL-C in serum were significantly higher in irradiated groups after radiation exposure than those of the control group. Whereas, a remarkable decreases was observed in the concentration of HDL-C in the serum of γ-irradiated rats. These data are in accordance with previous results of Ragab and Ashry (42) and Abou-Safi et al (43) who observed that the elevation in serum lipid fractions might result from ionizing radiation ability to accelerate other pathways of cholesterol formation like increasing its rate of biosynthesis in the liver and other tissues, or destruction of cell membrane by radiation and also to disturbance of LDL cholesterol receptors, leading to hypercholesterolemia. Also, it is suggested that oxidative stress might be an important determinant of altered lipid metabolism due to radiation exposure (39). The recorded elevated level of triglycerides correlates previous finding of Osman (44) and Abou-Safi et al. (45), who observed that after irradiation insulin level increased and synthesis of triglycerides was increased in both adipose tissues and liver which was accompanied by an acceleration of fatty acids mobilization from fat dopots to blood.
On the other hand, γ-irradiated rats fed diet containing broccoli had lower level of serum TC, TG, LDL-C and vLDL-C and higher level of HDL-C than those of γ-irradiated group. A previous study demonstrated that consumption of broccoli improved cholesterol metabolism in rats \(^\text{(46)}\). The hypcholesterolemic effect associated to broccoli consumption could be explained to its high content of phenolic and flavonoids \(^\text{(47 & 48)}\). Hermansen et al. \(^\text{(49)}\) stated that flavonoid intake reduced LDL-C: HDL-C ratio only by 27%. Also, Weggemans and Trautwein \(^\text{(50)}\) reported that flavonoids intake decreased LDL-C and increased HDL-C that may enhance removal of cholesterol from peripheral tissue to liver for catabolism and excretion.

The results of γ-irradiated rats indicated that this group exhibited a higher glucose concentration and lower insulin level in comparison with the control rats. The increased glucose level might be related to endocrine abnormalities induced by irradiation that promote the secretion of biologically active peptide which increase gluconeogenesis in liver \(^\text{(51)}\). In addition, the lowering effect of γ-irradiation exposure on insulin level was observed due to production of free radicals that induced oxidative stress resulted in reduction in insulin secretion and DNA damage \(^\text{(52)}\). Whereas, consumption of broccoli by γ-irradiated rats resulted in decreasing the glucose level and increasing the insulin concentration. The hypoglycemic effects of broccoli may be due to its inducing nature on pancreatic β-cells for insulin secretion, or the phenolic compounds such as flavanols, flavones and anthocyanins present in this crop may themselves be acting as insulin like molecules or insulin secretagogues \(^\text{(53)}\).

Moreover, this study reveals that gamma irradiation of rats induced a significant decrease in testosterone concentration. Michael and Amer \(^\text{(54)}\) recorded that decreased testosterone level after whole-body irradiation dose of 4 and 5 Gy was due to alterations in DNA-single strand break, cell apoptosis and oxidative stress. Also, Popoff and Kapich \(^\text{(55)}\) found a positive correlation between a decline in testosterone affinity and exposure to gamma irradiation.

According to the above mentioned results of this study, a significant increase in the level of testosterone was observed in the group of γ-irradiated rats dietary supplemented with broccoli in comparison with that of γ-irradiated group. Besides being loaded with vitamins and minerals, broccoli may have a positive impact on testosterone. Broccoli contains the compound indole-3-carbinol (I3C) \(^\text{(56)}\), which provides powerful antioxidant protection, helping to prevent cellular damage caused by free radicals. In addition I3C can help to maintain healthy hormonal balance for both men and women and therefore may support the health of the breast, prostate, and other reproductive organs. Furthermore, I3C enhances testosterone's effects by reducing the strength of estrogen.

**CONCLUSION**

In conclusion, broccoli and its antioxidant contents might has the capability to protect against gamma irradiation induced oxidative stress, stimulate antioxidant enzymes, enhance liver function and exert hypolipidemic impact in γ-irradiated rats. Thus, dietary supplementation of broccoli may has a valuable effect during the application of radiation technology in medicine and industry.

**REFERENCES**