Utilization of Basil Extract as a Radioprotector in Male Rats

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ABSTRACT

Man is exposed to natural radiations from cosmic or terrestrial origins. Furthermore, it is well known that the gamma irradiation-induced biochemical alteration depends mostly on oxidative stress. Basil or sweet basil (Ocimum basilicum) is known to have numerous pharmacological activities. Therefore, the present study was carried out to investigate the radioprotective activity of basil in albino rats. The effect of basil aqueous extract (BAE) was evaluated on hepatic marker enzymes, sex hormones, lipid profile and antioxidant status. The results showed that γ-rays caused a significant increase in serum level of alanine and aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase (ALT, AST, ALP & ßGT), cholesterol (TC), triglyceride (TG), low and very low density lipoprotein cholesterol (LDL-C & VLDL-C) and thiobarbituric acid reactive substances (TBARS). A significant decrease in high density lipoprotein cholesterol (HDL-C), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in serum was observed, compared with control group. Moreover, γ-rays induced a significant drop in the serum sex hormones levels testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormone (LH). The BAE administered orally to rats has significantly modulated all the radiation-induced biochemical alterations. These findings showed that basil would exert a radioprotective properties.

Key words: Gamma Irradiation / Basil / Liver Function / Lipid Profiles / Sex Hormones.

INTRODUCTION

Radiotherapy is one of the most common therapies for treating human cancers. Several studies have indicated that irradiation induces reactive oxygen species (ROS), which play an important role in damage of the cell. Scientific and technological advancements have further increased the radiation burden in humans, because exposure to low levels of radiation has become common during medical diagnostic procedures, space or air travel, cosmic radiation and through the use of certain electronic gadgets. Other sources of radiation exposure include radon in houses, contamination from weapons testing sites, nuclear accidents and radiotherapy (1). Ionizing radiation may cause cancer, death, and loss of neural function in humans and animals. It also induces damage to skin and fertility impairment, mutation, chromosomal aberrations and apoptosis in cells (2, 3).

Radiation is an important source in the generation of oxygen-derived free radicals and excited states. In actively metabolizing cells, there is considerable water apart from the target macromolecules of DNA, proteins, lipids and so on. The exposure of biological systems to radiation results in a radiolytic cleavage of water, giving rise to hydroxyl radicals. Moreover, ionizing radiation can break chemical bonds and cause ionization of biologically important macromolecules such as nucleic acids, membrane lipids and proteins (4).
Plants and natural products are extensively used in several traditional systems of medicine, so screening these products for radio-protective compounds has several advantages, because they are usually considered non-toxic and are widely accepted by humans. Many natural antioxidants, whether consumed before or after radiation exposure, can confer some level of radioprotection. In addition to beneficial effects accrued from established antioxidants, such as vitamin C and E, and their derivatives, vitamin A, beta carotene, curcumin, Allium cepa, quercetin, caffeine, chlorogenic acid, ellagic acid and bixin. Protection is also conferred by several novel molecules, including flavonoids, eppigallocatechin and other polyphenols (4, 5, 6 &7).

Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. Ocimum sanctum L. (Tulsi), Ocimum basilicum (Ban Tulsi), Ocimum canum (Dulal Tulsi), and Ocimum gratissium (Ram Tulsi) are examples of known important species of genus Ocimum which grow in different parts of the world (8). Basil or sweet basil (Ocimum basilicum) is commonly known as Tulsi in Hindi, Holy basil in English and Rehan in Egypt. It is known to have numerous pharmacological activities, so it is widely used in folk medicine to treat a wide range of diseases. For example, the aerial part of O. basilicum is traditionally used as an antispasmodic, aromatic, digestive, carminative, stomachic and tonic agent. O. basilicum has also been used externally for the topical treatment of acne, insect stings, snake bites, and skin infections (9). Many studies have established that basil leaves extracts have potent antioxidant, anti-aging, anticancer, antiviral and antimicrobial properties (10, 11, 12 & 13).

The ethanolic extract of Ocimum sanctum leaves was found to prevent noise induced oxidative stress in discrete regions of the brain (14). Rupert (15) reported that basil or basil oil have agents for prevention and treatment of cardiovascular disease. Recent study by Khaki et al., (16) showed that basil could increase sperms health parameters and protect exposed animals by electromagnetic field.

The present investigation was undertaken to determine the protective effect of concurrent use of basil leaves extract on γ-irradiation induced oxidative stress and some biochemical alterations in rats.

**MATERIALS AND METHODS**

**Material:**
Dry plant leaves of basil (*Ocimum basilicum* L.), as well as standard commercial rodent diet were purchased from a local market in Cairo, Egypt.

Preparation of basil aqueous extract:

Basil leaves were dried (40–60 ºC) and the basil aqua extract was prepared by boiling dried plant leaves with distilled water for 15 min. The extract was then filtered through a clean cotton cloth. The aqua extract was given to rats intra-gastric (250 mg/kg B.wt) (17).

**Radiation treatment:**
Whole body gamma irradiation of rats at the dose level of 6.5 Gy was performed using a Canadian gamma cell-40, (137Cs) housed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The irradiation dose rate calculated by Dosimetry Department in our NCRRT was 0.44 Gy/min at the time of the experimentation.

**Experimental animals:**
Adult male albino rats, reared in NCRRT animal house, were used in the present experiment. Matched weight animals (150 ± 10g) were selected and housed in plastic cages under controlled condition and fed on standard commercial rodent diet.
Experimental protocol:
The rats were randomly divided into 4 groups each of eight animals as follows:

**Group I:** Rats didn’t receive any treatment, served as control.

**Group II:** Rats whole body gamma irradiated with 6.5 Gy administered as one shot dose

**Group III:** Rats were orally supplemented with basil extract at dose 250 mg/kg B.wt daily, during 6 weeks.

**Group IV:** Rats were orally supplemented with basil extract at dose 250 mg/kg B.wt during three weeks before and three weeks after irradiation.

Rats were sacrificed under light chloroform anesthesia at the end of experimental period. Blood samples were collected for biochemical estimation.

Biochemical analysis:

The determination of serum AST and ALT activities was done using the method of Reitman and Frankel[18]. ALP activity was estimated according to Kind and King[19]. γ- GT was determined using the method of Szasz [20]. In addition, concentration of TC, TG and HDL-C was determined according to procedure described by Allain et al. [21], Fossati and Prencipe, [22] and Demacker et al. [23], respectively while LDL-C and VLDL-C were evaluated according to Friedwald et al. [24] and Norbert [25] formulas, respectively, by the following equations: LDL-C (mg/dl) = TC - (TG/5+HDL-C), VLDL (mg/dl) = TG/5. The level of LH and FSH was determined using radioimmunoassay (RIA) methods according to the procedure instructions of the corresponding kits. Serum T level was estimated using a test reagent kit based on a solid phase enzyme linked immunosorbent assay (Sanchez et al..). The extent of lipid peroxidation was assayed by the measurement of TBARS according to Yoshioka et al. [27]. SOD and CAT activities were determined according to Minami and Yoshikawa et al. [28], and Aebi [29], respectively. The content of GSH was determined according to Beutler et al. [30].

Statistical analysis:

Analysis of variance (ANOVA) was conducted for all data using the general linear model (GLM) (SAS Institute[31]). Duncan's multiple-range test was used for comparison between treatments [32]. Data were presented as means ± standard error. A value of P<0.05 was taken as criterion of significance.

RESULTS

The results of the present study showed that the rats supplemented with BAE under normal conditions; for a period of six weeks, did not show any significant changes in all measured parameters except a significant increase occurred in serum T level (Tables 1-4).

Significant (P<0.05) rise was observed in the activity of diagnostic marker enzymes ALT, AST, ALP and γ-GT in serum of irradiated rats versus control animals (Table1). The orally supplemented rats with BAE three weeks before and after irradiation significantly reduced the release of these hepatic marker enzymes (Table 1).

The level of TC, TG, LDL-C and VLDL-C in serum was significantly higher in irradiated group than that of the control group. Treatment of rats with BAE before and after irradiation significantly ameliorated the elevation in the levels of lipid profile (Table 2). On the other hand, and in comparison with normal conditions, radiation exposure of rats caused a significant decrease in serum HDL-C level This effect was significantly prevented by treatment with BAE (Table 2).
Table (1) Effect of BAE administration on the activity of serum hepatic marker enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/ml)</th>
<th>AST (U/ml)</th>
<th>ALP (U/l)</th>
<th>5'-GT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.38±3.14</td>
<td>74.53±4.64</td>
<td>82.28±3.27</td>
<td>1.87±0.11</td>
</tr>
<tr>
<td>BAE</td>
<td>32.87±4.07</td>
<td>71.82±5.37</td>
<td>81.42±2.07</td>
<td>1.98±0.17</td>
</tr>
<tr>
<td>Irrad.</td>
<td>44.25±3.46</td>
<td>87.34±6.24</td>
<td>139.65±4.06</td>
<td>3.47±0.29</td>
</tr>
<tr>
<td>BAE + Irrad.</td>
<td>35.16±2.63</td>
<td>82.26±5.43</td>
<td>101.12±3.58</td>
<td>2.15±0.20</td>
</tr>
</tbody>
</table>

Data are expressed as means± S.E. of eight rats per group. Values with different superscript in the same columns are significantly different at P = 0.05.

Table (2) Effect of BAE administration on serum lipid profile.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.73±6.54</td>
<td>87.77±3.87</td>
<td>62.40±4.28</td>
<td>38.86±3.29</td>
<td>21.27±1.82</td>
</tr>
<tr>
<td>BAE</td>
<td>110.22±5.22</td>
<td>75.47±3.92</td>
<td>63.77±4.24</td>
<td>38.03±1.25</td>
<td>18.89±2.09</td>
</tr>
<tr>
<td>Irrad.</td>
<td>132.7±6.5</td>
<td>117.25±6.5</td>
<td>42.10±4.89</td>
<td>70.60±5.05</td>
<td>26.97±2.20</td>
</tr>
<tr>
<td>BAE + Irrad.</td>
<td>119.3±5.38</td>
<td>96.27±4.21</td>
<td>52.87±4.34</td>
<td>52.57±3.09</td>
<td>22.77±1.74</td>
</tr>
</tbody>
</table>

Legend as table 1

The data in Table (3) exhibited a significant decline (p < 0.05) in serum T, LH and FSH concentration after exposure of rats to γ-radiation. This reduction was improved in irradiated rats treated with BAE (Table 3).

Table (3): Effect of BAE administration on serum level of T, LH and FSH .

<table>
<thead>
<tr>
<th>Groups</th>
<th>T (n mol/l)</th>
<th>LH (U/l)</th>
<th>FSH (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.76±0.13</td>
<td>0.91±0.14</td>
<td>0.69±0.05</td>
</tr>
<tr>
<td>BAE</td>
<td>4.51±0.15</td>
<td>0.84±0.023</td>
<td>0.79±0.05</td>
</tr>
<tr>
<td>Irrad.</td>
<td>1.23±0.14</td>
<td>0.52±0.018</td>
<td>0.61±0.06</td>
</tr>
<tr>
<td>BAE + Irrad.</td>
<td>2.44±0.15</td>
<td>0.68±0.03</td>
<td>0.67±0.06</td>
</tr>
</tbody>
</table>

Legend as table 1

Exposure of rats to γ -radiation resulted in a significant enhancement (P=0.05) in lipid peroxidation, as measured by the formation of TBARS in the serum (Table 4). Daily supplementation of BAE before and after irradiation significantly attenuated the radiation-induced elevation in serum TBARS level (Table 4).
The findings of the current study revealed that exposure of rats to gamma radiation (6.0 Gy) led to a significant depression (P< 0.05) in serum antioxidants activity SOD, CAT and GSH content, relative to control group as illustrated in Table (4). Daily treatment of irradiated rats with BAE significantly reduces radiation-induced alternation in serum antioxidants (Table 4).

Table (4) Effect of BAE administration on serum level of TBARS, GSH, SOD and CAT.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (n mol/ml)</th>
<th>GSH (mg/dl)</th>
<th>SOD (U/ml)</th>
<th>CAT (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.20±5.47a</td>
<td>62.86±4.34a</td>
<td>7.91±1.46a</td>
<td>28.73±2.17a</td>
</tr>
<tr>
<td>BAE</td>
<td>67.80±7.06a</td>
<td>63.61±5.25a</td>
<td>9.42±1.83a</td>
<td>28.19±3.74a</td>
</tr>
<tr>
<td>Irrad.</td>
<td>119.84±8.73c</td>
<td>49.98±4.52b</td>
<td>4.32±0.86b</td>
<td>21.82±1.63c</td>
</tr>
<tr>
<td>BAE+ Irrad.</td>
<td>82.62±6.65b</td>
<td>55.27±5.73ab</td>
<td>7.23±0.74a</td>
<td>26.54±2.82a</td>
</tr>
</tbody>
</table>

Legend as table 1

**DISCUSSION**

It is known that ionizing radiations cause generation of reactive oxygen species (ROS) leading to oxidative stress and hence an imbalance in the pro-oxidant, antioxidant status in the cells (33). It could be noticed that in rats treated with gamma rays the serum levels of AST, ALT, ALP and ?-GT were increased compared to those of the control rats (Table 1). These findings are in harmony with those of El-Gawish et al. (34). This rise in the AST and ALT levels could be attributed to the drastic dysfunction of the hepatic cells as a consequence of radiation interaction with membranes of cells and also related to extensive breakdown of liver parenchyma (35). ALP is an ecto-enzyme of hepatocyte plasma membrane; an increase in serum ALP reflects the pathological alteration in biliary flow and damage to the liver cell membrane (36). High concentration of serum transaminases is considered to be an index of hepatic injury where elevation of ALT is regarded as a more sensitive indicator and is usually accompanied by a rise in AST (37). ?-GT is a microsomal enzyme, which is widely distributed in tissues including liver. Serum ?-GT is most useful in diagnosis of liver diseases (38). Changes in ?-GT is parallel to those of aminotransferases.

The present findings demonstrated that *O. basilicum* alleviates the increase in liver function enzymes activity induced by exposure of rats to ?-rays. This indicates the effectiveness of *O. basilicum* in the prevention of radiation induced hepatotoxicity. The hepatoprotective effects of *O. basilicum* have been shown in studies on experimental liver damage. Yamamoto et al., (39) proved that *ocimum* suppressed hepatic fibrosis and protected liver against parenchymal damage induced by CCL4. It has been shown that *O. sanctum* leaf extracts can protect the liver from heavy metals (40) and prevent isoproterenol induced myocardial necrosis in rats (41). Adhvaryu et al., (42) reported that *O. sanctum* have hepatoprotective and immunomodulatory effects on liver injury and immunosuppression induced by Isoniazid, Rifampicin and Pyrazinamide in guinea pig. Furthermore, *Ocimum gratissimum* extracts showed a hepatoprotective effect by reducing damage due to radiation exposure, environmental pollution or toxicants (43).

As compared with control rats group, the recorded events in this study pointed to a significant rise in the serum level of TC, TG and LDL-C accompanied by a decline in the HDL-C value after irradiation treatment. El-Khafif et al., (44) and Mansour, (45) claimed that whole body gamma irradiation of rats produced high level of serum cholesterol fractions due to its release from tissues, destruction of cell membranes and increase rate of cholesterol biosynthesis in the liver and other tissues. In addition, irradiation could modify low- and high density lipoproteins metabolism indirectly through the action
of various inflammatory products. Gamma-irradiation might decreased the lipoprotein lipase activity in adipose tissue, leading to reduction in the uptake of triacylglycerols.

It has been shown that 2% of dried O. sanctum leaf powder supplemented in the diet can lower serum lipid profile and partially protect the liver in diabetic rats \(^{(46)}\). Also, consumption of basil or basil oil has been associated with a reduction in total cholesterol, low-density lipoprotein and triglyceride in hypercholesterolemic rats \(^{(47)}\). The results of the present study are consistent with these observations where O. basilicum treatment attenuated the radiation induced alterations in serum lipid profile. This may be due to the anti-hyperlipidemic action of the components of O. basilicum leaves. Suanarunsawat et al.,\(^{(48)}\) mentioned that the anti-hyperlipidemic activity of O. basilicum may be due to the suppression of liver lipid synthesis.

The present results indicated that whole body gamma irradiation of rats caused a dramatic decrease in serum T, FSH and LH level. These findings are in accordance with those of Jegou et al\(^{(49)}\), who reported that radiation has particularly severe adverse effect on gonads and therefore on fertility in both animals and man. Also, Dygalo et al \(^{(50)}\) observed that exposure of rats to gamma radiation at 7Gy (single dose) significantly diminished the serum levels of testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH). Consumption of basil extract was able to improve the levels of sex hormones (T, LT and FSH) in the serum of irradiated rats. In accordance, Khaki et al.,\(^{(16)}\) have shown that nourished animals with basil extract showed elevated level of testosterone and increased sexual desire as more sexual intercourse episodes.

Exposure of rats to ?-radiation resulted in a significant increase in lipid peroxidation, associated to a significant decrease in the antioxidants, indicating oxidative stress (Table4). Similar observations were reported on radiation-induced oxidative damage in several organs \(^{(51, 52)}\). Ionizing radiation generates ROS as a result of water radio lysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes \(^{(53)}\). Products of lipid peroxidation such as malondialdehyde (MDA) and 4NHE (4-hydroxynonenal) have the ability to interact with and alter macromolecules, possibly resulting in diseases \(^{(54)}\).

Superoxide dismutase, catalase and glutathione peroxidase constitute the major enzymatic antioxidant defenses which convert active oxygen molecules into nontoxic compounds. In the present study the decline in the level of these enzymes could be explained by the assumption that excess superoxide radicals may inactivate H_2O_2 scavengers, thus resulting in inactivation of superoxide dismutase \(^{(55)}\). Excessive oxidative stress caused by ?-irradiation might be responsible for the depletion of GSH. Oxidative stress induced by ?-irradiation of animals resulted in an increased utilization of GSH and subsequently a decreased level of serum GSH. Depletion of GSH is known to cause an inhibition of the glutathione peroxidase activity and has been shown to increase lipid peroxidation \(^{(56)}\). A similar correlation between the depletion of GSH and increase of lipid peroxidation was observed in the present investigation.

Oral administration of basil was able to improve the level of endogenous antioxidants (SOD, CAT and GSH) and to decrease the level of TBARS in the serum of irradiated rats. The recorded data are on the same line with those observed previously by Meera et al.,\(^{(57)}\) who sustained that the ethanolic extract of O. basilicum leaves showed significant anti-lipid peroxidation effects in vitro in a liver damage induced by H_2O_2 and CCl_4 in goat. Moreover, Khaki et al.,\(^{(16)}\) stated that the hydroalcoholic extract of O. basilicum increased the antioxidant capacity and had potential beneficial effects by neutralizing free radicals in the electromagnetic field exposed group, and led to a decreased level of MDA.
The present study showed that *O. basilicum* extract has a protective effect on the exposed animals to gamma radiation induced oxidative stress. Protective property of basil against γ-rays may be referred to its antioxidative potency and free radical scavenging activity. Some previous studies have investigated basil antioxidative property in vital organs\(^{58,59}\). As well as, Dorman and Hiltunen\(^{60}\) documented the antioxidant and radical scavenging activity of *O. basilicum*. Chinnasamy et al.,\(^{61}\) reported that the protective action of *ocimum* was attributed to its antioxidant action. They added that this protection may be due to anti-inflammatory property of *ocimum* which reduces formation, release, and activity of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes.

The antioxidative effect of basil is mainly due to its content of phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes. Niwano et al.,\(^{62}\) stated that the herbal antioxidant effects from *O. basilicum* ingestion were due to the presence of flavonoids, phenylpropanoids, and rosmarinic acid in the aerial parts of the plant. The antioxidative activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides\(^{63}\). The antioxidants interrupt the free-radical chain of oxidation by donating hydrogen from phenol’s hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids. Furthermore, some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties\(^ {64}\).

**CONCLUSION**

Treatment of rats with basil aqueous extract (BAE) three weeks before and after whole body γ-irradiation seems to exert protective effects that might be attributed to its antioxidants and free radicals scavenging properties.

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