POSSIBLE ANTI-HEMOLYTIC AND ANTIOXIDANT ROLE OF ETHANOLIC EXTRACT OF CORIANDER ON IRRADIATED RATS

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Abstract: Foods wealthy in antioxidants play an essential role in the prevention of diseases. Coriander (Coriandrum sativum L.), a conventional annual herb selected for assessing antihemolytic and antioxidant properties. To study the effectiveness of Coriandrum sativum as an antihemolytic and antioxidant agent, the oxidative stress was induced in rats by gamma radiation exposure with 4 Gy shot dose after 14 days oral administration of coriander extract (600 mg/kg b.wt). Exposing rats with 4 Gy gamma rays was significantly decreased RBCs, WBCs and Platelets counts. But the percentage of segmented cells as well as the activity of serum aspartate transaminase (AST) and endotheline 1 (ET1) level was significantly increased after radiation exposure. Interestingly, among the tested parameter, the ethyl extract of coriander showed marked improvement in both haematological and biochemical changes. In conclusion, addition of coriander to food will increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes.

Keywords: Antioxidants, Antihemolytic, Flavonoids, Coriandrum sativum L., Antioxidant, Gamma radiation.

Introduction

Plants ingredient is an important source of natural products which differ broadly in their structures, biological properties and mechanism of action. Different phytochemical components especially polyphenols, flavonoids, phenolic acids etc. are responsible for the free radical scavenging and antioxidant activity of the plants. Polyphenols possess many biological effects, mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelation of transition metals (Nickavar et al., 2006). Antioxidants reduce oxidative injury to cells and bio-molecules caused by reactive oxygen/nitrogen species (ROS/RNS) (Fiorentino et al., 2006). The well known protective effects of antioxidants against oxidative stress-induced diseases have received increasing attention in recent times, especially within biological, medical and nutritional areas. Among the dietary antioxidants, phenolic compounds, secondary metabolites from plants, are the most plentiful natural antioxidants (Fiorentino et al., 2006). Phenolics act as antioxidants in a number of ways such as reducing agents, hydrogen donors, free radical scavengers, and singlet oxygen reducer, therefore, act as cell saviours (Fattouch et al., 2007).

Coriander (Coriander sativum L.) is rich in beneficial phytounutrients and has a health-supporting reputation (Rajeshwari & Andallu, 2011). It was shown that coriander extracts have phenolic compounds and flavonoides, suggesting that these compounds contribute to the
antioxidative activity (Wangensteen et al., 2004). Phenolic substances such as flavonoids, cumarins, cinnamic acid and caffeic acids are believed to have antioxidant properties, which may play an important role in protecting cells and any organ from oxidative degeneration (Wiseman et al., 2000). It has been reported to exhibit several pharmacological effects such as antioxidant, anti-diabetic, anti-mutagenic, sedative-hypnotic, anticonvulsant, diuretic activities as well as has a protective role against lead toxicity, anti-feeding activity, anticancer activity, hepatoprotective activity, anti-protozoal activity, anti-ulcer activity, post-coital anti-fertility activity, heavy metal detoxification (Momin et al., 2012). In addition, it possesses hypoglycemic, hypolipidemic, antibacterial, antihemolytic, antimutagenic activity, insecticidal and aflatoxin controlling effects (Rajeshwari et al., 2012).

Ionizing radiation is a form of radiation with sufficient energy to remove electrons from their atomic or molecular orbital shells in the tissues they penetrate (Djeridane et al., 2007). These ionizations, received in sufficient quantities over a period of time, can result in tissue damage and disturbance of cellular function at the molecular level. Ionizing radiation may break the cell’s DNA (which the cell relies on to manufacture proteins and enzymes, perform routine cell functions, and maintain cell integrity and homeostasis) to the point that normal cell functions are markedly decreased or stopped, resulting in cell injury and death. Once damaged, the cell can either repair the damage or die. Repair or mis-repair may or may not result in cell lethality. When precursor cells in the hematopoietic system (which multiply quite frequently to replenish aging leukocytes) are damaged or die, leukopenia may occur in the peripheral blood, leaving the body susceptible to infections and disease.

Erythrocytes, which are the rich cells in human body, possessing desirable physiological and morphological characteristics, are broken extensively in drug delivery (Hamidi and Tajerzadeh, 2003). Oxidative damage to the erythrocyte membrane (lipid/protein) may be implicated in haemolysis associated with radiation, and reduction in some haematological parameters (Ko et al., 1997).

In the light of abovementioned properties of coriander, this study was carried out to evaluate the possible antihemolytic and antioxidant properties of coriander extract against radiation exposure induced oxidative stress in rats.

**MATERIALS AND METHODS**

**Plant collection**
*Coriandrum sativum* leaves has been kindly supplied from Medicinal and Aromatic Plants Department, National Research Centre, Cairo, Egypt

**Extract preparation**
The dried and powdered leaves (200 g) were extracted successively with 70% ethanol in a soxhlet extractor for 48 hours at 60 °C. After extraction, the solvent was evaporated to dryness at 50-55 °C using a rotary evaporator. Finally, the lyophilization of the dried extract was done to yield the Coriander (Kil et al., 2009). The extract was stored at 4°C till the analysis of different parameters.
Chemicals:
The chemicals used in this experiment were obtained from Sigma Chemical (USA). Kits used in this experiments were purchased from Bio-Diagnostics (UK).

Radiation process
A shot dose whole body irradiation (4 Gy) was performed with rats, using gamma rays by Cesium 137 irradiation unit, National Center for Radiation Research and Technology (NCRRT), with the dose rate 0.7488 rad/sec. The gamma cesium cell was calibrated by alanine dosimetry relative to a primary standard. Correction were made daily for humidity, temperature, and barometric pressure.

Animals and treatment:
Female albino rats, each weighing 120-150 gm, were purchased from the animal breeding unit of the Cancer Institute, Giza, Egypt. Rats were housed under appropriate conditions of controlled humidity, temperature and light. The animals were allowed free access to water and were fed a standard pellet rat diet. The study was conducted accordance with the guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical Committee at the Faculty of Pharmacy, Cairo University. Whole body irradiation of animals was carried out at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt using the gamma Cell-40 biological irradiator furnished with a Caesium source (Atomic Energy of Canada LTD). Rats were segregated into groups of six animals each. Each group was subjected to one of the following treatments described in the next sections.

Control group. Irradiated group: The animals in IRR group were exposed to whole body gamma irradiation with a shot dose of 4 Gy. Coriander administered group: Animals in Coriander group were administered orally with a cumulative dose of coriander (600 mg/Kg b.wt. for 14 days) diluted in 0.4 ml distilled water. Curative group: Animals in Curative group were administered orally with a cumulative dose of (600 mg/Kg b.wt for 14 days) diluted in 0.4 ml distilled water then after 30 min. of the last dose of coriander treatment the animals were exposed to 4 Gy whole body γ- irradiation as a shot dose. Animal groups were sacrificed after 48 hours of radiation exposure, blood and heart organ were collected for biochemical analysis.

Biochemical Analysis:
The blood samples were collected directly from the animals by heart puncturing and divided into two portions, one of them collected on EDTA for haematological parameters and the other portion was centrifuged using universal 16R/ Germany centrifuge at 3000 rpm for 15 min; clear serum was collected and stored in a refrigerator. The levels of serum endothelin 1 as well as serum AST activity were estimated. Heart were excised from the rat, homogenization was carried out using a homogenizer (universal laboratory AID type MPW-309, Poland). Estimation of the haematological changes (platelets counts, RBCs counts, Hb concentration, PCV%, WBCs counts and its deferential percentages) was done according to Vankampen and Ziglstra (1961). The quantitative determination of AST activity was done using kit according the method of Reitman and Frankel (1957). Endothelin 1 measurements were accomplished by the method Kappers et al., (2011). The homogenate of heart was used to analyze nitric oxide and GSH levels. Nitric oxide level in heart was estimated according to
the method of Geng et al. (1994). GSH content was determined using the method of Beutler et al. (1963).

Statistical analysis:
The data were expressed as mean ± standard error (SD) and were analyzed by one way analysis of variances (ANOVA) followed by Tukey's multiple comparison test. Statistical analysis was performed by using Graph-Pad software, San Diego, CA, USA. Differences were considered statistically significant when P<0.05.

RESULTS

Does radiation exposure cause a disturbance in haematological parameters?
Radiation exposure to rats significantly decreased the level of most haematological parameters; RBCs, PCV%, Platelets counts, as well as leucocytes and leucocytes differential counts; as compared with control group except Hb concentration, esinophil counts and neutrophils counts (Table 1, 2). The most pronounced decrease was for leucocytes counts that recorded -292% comparing to normal control group. On the other hand, Hb concentration and esinophil counts of IRR group showed no significant change, while the neutrophil counts showed a highly significant increase due to the radiation exposure that recorded 291% as compared with the normal control level (Table 2).

Table 1. Effects of coriander extract administration and/or radiation exposure on RBCs (M/Cmm), Hb (g/ dl), PCV% as well as platelets counts/ Cmm after 48hours of radiation exposure (n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>Coriander Group</th>
<th>IRR Group</th>
<th>Curative Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs M/ Cmm</td>
<td>6.60±0.84</td>
<td>6.00±0.86</td>
<td>4.70± 0.89*</td>
<td>5.73± 0.90</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.92± 1.64</td>
<td>12.82± 0.891</td>
<td>12. 88± 1.15</td>
<td>12.18± 1.85</td>
</tr>
<tr>
<td>PCV%</td>
<td>38.05± 5.32</td>
<td>35.90± 2. 09</td>
<td>29.41± 3.98*</td>
<td>31.35± 5.35</td>
</tr>
<tr>
<td>Platelets counts/ Cmm</td>
<td>586400± 143400</td>
<td>621333± 124779 (#)</td>
<td>336000± 58570*</td>
<td>487000± 89297</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *Significantly different from the control group (P<0.05). (#) Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 4 Gy as a shot dose.

Serum endothelin-1 level and AST activity were markedly increased in IRR groups comparing to the control group. The levels of this increase were 55% and 53% regarded to the normal control levels (Table 3).
Table 2. Effects of coriander extract administration and/or radiation exposure on total leucocytes/ Cmm and its differential % after 48 hours of radiation exposure (n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leucocytes/ cmm</th>
<th>Differential Leucocytic Count (%)</th>
<th>Granulocyte</th>
<th>Agranulocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Segmented (Neutrophil)</td>
<td>Eosinophil</td>
<td>Lymphocyte</td>
</tr>
<tr>
<td>Control Group</td>
<td>7133± 508.6</td>
<td>13.67± 1.51</td>
<td>1.333± 0.52</td>
<td>80.67± 8.24</td>
</tr>
<tr>
<td>Coriander Group</td>
<td>6267± 697.6(#)</td>
<td>10.33± 2.32(#)</td>
<td>1.00± 0.89</td>
<td>84.50± 2.74(#)</td>
</tr>
<tr>
<td>IRR Group</td>
<td>1689± 397.5*</td>
<td>53.50± 12.04*</td>
<td>0.833± 0.41</td>
<td>37.58± 8.065*</td>
</tr>
<tr>
<td>Curative Group</td>
<td>1817± 160.2*</td>
<td>41.67± 9.4*</td>
<td>1.333± 0.516</td>
<td>42.00± 10.04*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. * Significantly different from the control group (P<0.05). #Significantly different from IRR group (P<0.05). IRR: Radiation exposure with the dose of 4 Gy as a shot dose.

Radiation exposure affects the nitrosative stress and antioxidant biomarkers in heart tissues:
Damages in heart tissue can be estimated by measuring the nitrosative stress and antioxidant biomarkers such as NO concentration and GSH levels respectively. NO levels showed a significant increase in IRR group due to the radiation exposure of 4 Gy gamma rays as a shot dose and the percentage of this increase was 46% accompanied with a significant decrease in GSH level recorded -22% as compared with normal control values (Table 4).

Table 3. Effects of coriander extract administration and/or radiation exposure on serum endothelin 1 contents as well as AST (U/ml) activities after 48 hours of radiation exposure (n=6)

<table>
<thead>
<tr>
<th>Treatment (X ± SD)</th>
<th>Control Group</th>
<th>Coriander Group</th>
<th>IRR Group</th>
<th>Curative Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin 1</td>
<td>5.937± 1.031</td>
<td>5.950± 0.224(#)</td>
<td>9.207± 1.22*</td>
<td>6.233± 0.408(#)</td>
</tr>
<tr>
<td>AST activity (U/ml)</td>
<td>30.03± 0.889</td>
<td>29.25± 3.708(#)</td>
<td>45.99± 8.035*</td>
<td>31.34± 1.082(#)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). *Significantly different from the control group (P<0.05). #Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 4 Gy as a shot dose.

The ameliorative role of coriander extract against radiation exposure:
The ameliorative role of coriander extract against radiation exposure is based on the improvement of the parameters measured. Curative groups showed a resistance to radiation detected by increasing the RBCs, PCV%, platelets, leucocytes counts as well as agranulocytes differential counts when compared with IRR group. In addition a highly significant decrease in segmented cells was recorded (-22%) when compared with irradiated...
groups, however the decrease in segmented cell was still significantly increased comparing to the control group (Table 1&2).

Coriander pre- treatment caused a significant restoration and the activity of AST as well as the endothelin 1 level was reached to the normal control level (Table 3).

Table 4. Effects of coriander extract administration and/ or radiation exposure on GSH (mg/ g tissue) and NO (µmol/ g tissue) contents of heart tissue after 48 hours of radiation exposure (n=6)

<table>
<thead>
<tr>
<th>Treatment (X ± SD)</th>
<th>Control Group</th>
<th>Coriander Group</th>
<th>IRR Group</th>
<th>Curative Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/tissue)</td>
<td>16.86±0.4230</td>
<td>16.40±0.7191¹(#)</td>
<td>13.09±0.9532*</td>
<td>14.71±1.392*¹(#)</td>
</tr>
<tr>
<td>NO (µmol/g tissue)</td>
<td>6.185±0.9807</td>
<td>7.004±0.5011¹(#)</td>
<td>9.034±1.517*</td>
<td>6.170±0.8788¹(#)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *Significantly different from the control group (P<0.05). (¹) Significantly different from IRR group (P<0.05). IRR: Radiation exposure with the dose of 4 Gy as a shot dose. According to the data presented in table (4) GSH content showed a slight amelioration and the change was significant as compared to both IRR group and the control one. However, the NO level in the curative group showed a full restoration and the level reached the normal control level.

**DISCUSSION**

Radiation has potentially detrimental effects on living tissue and can destroy the living cells or make them functionally abnormal by free radical mechanism (Meo et al., 2006). Neutrophilia prevents the total white cell count from being a guide to the degree of radiation damage during the first few hours after radiation exposure. Ideally the hematological investigation of radiation casualties should include full blood counts, together with platelet counts, reticulocyte counts, and marrow examinations, and the degree of damage should be focused by the changes occurring in all the cell types (Hulse, 1960). The present data showed that total accounts of WBCs and lymphocyte, esinophil and monocytes percent were decreased on the second day after radiation exposure with the dose of 4 Gy, accompanied with a highly significant increase in segmented neutrophils. The decrease in the total white cell count as an indicator of radiation damage is due to the increase in the neutrophil count which takes place shortly after irradiation (Hulse, 1960). On the other hand, erythrocytes are considered as a major target for the free radicals owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active hemoglobin molecules, which are potent promoters of activated oxygen species (Ebrahimzadeh et al., 2009). The extent of hemolysis was found to be much greater, when the animals exposed to gamma rays with respect to the destruction of cell membrane and subsequent liberation of hemoglobin from the cells. (Kupier-Goodman and Scott., 1989). Nevertheless, the antihemolytic activity is the expression of mutual action of the various antioxidant mechanisms which function in nature. In addition the extract of coriander leaves display potent antihemolytic activity due to the presence of flavonols and glycosides (Rajeshwari et al., 2012), which can guard RBCs from free radical mediated oxidative hemolysis (Dai et al., 2006). Also, binding of flavonoids to the red blood cell
membranes significantly inhibits lipid peroxidation and at the same time, enhances their integrity against lysis (Chaudhuri et al., 2007). In this study, the antihemolytic activity of the ethanolic extract of coriander indicates presence of radical scavenging phytochemicals compounds, especially, flavonols, flavonoids and tannins in coriander that can be supported by Rajeshwari et al., (2012) as well as the composition of coriander as reported by Duke (1992). In addition to the polyphenolic compounds ethanolic extract soluble compounds like coriandrin, linalool also prevented lysis of RBCs as they possessed scavenging activity. Also, coriander contains a lot of bioactive compounds possessing strong anti-radical activities and thereby showed inhibitory effects on hemolysis (Rajeshwari et al., 2012).

On the other hand, endothelin-1 may contribute to the development of endothelial dysfunction, and consequently insulin resistance, by increasing the production of ROS. Reactive oxygen species can react with NO, forming peroxynitrite, and thus decrease the bioavailability of NO resulting in endothelial dysfunction. In addition, increased plasma levels of endothelin-1 have been demonstrated in states of myocardial ischemia and heart failure in human (Goddard et al., 2004) and in the response of endothelial cells to ionizing radiation (Lanza et al., 2007), also it could be used as a biomarker for irradiation of endothelial tissues. Radiation-induced endothelial dysfunction is associated with nitric oxide impairment (Soloviev et al., 2003) and up-regulation of endothelin-1 (Boerma et al., 2008). In the present study ionizing radiation caused a pronounced increase in both Endothelin-1 and AST activity, which is in agreement with the previous explanation.

The removal of free radicals is achieved through enzymatic and non-enzymatic reactions. NO is rapidly oxidized by oxy-hemoglobin to form NO$_3^-$ (nitrate), the major end stable oxidation product of NO in the body (Wu et al., 1999). NO also reacts with GSH to form nitrosothiol or with heme to yield heme-NO. Physiologically, nitrosothiol can serve as a vehicle to transport NO in plasma, thereby increasing the biological half-life of physiologic concentrations of NO (Rassaf et al., 2002). In addition, tyrosine residues of proteins can be nitrosylated by NO or its derivative peroxynitrite. Moreover, GSH can scavenge ONOO$^-$ with the formation of oxidized glutathione (GS-SG), which is converted back to GSH, by the NADPH-dependent glutathione reductase (Sies, 1999). In this study, significant increase in the levels of NO concentration and significant decrease in GSH content were observed in $\gamma$-irradiated-rats. According to Mansour (2013), the decrease in antioxidant enzymes might be due to radiation-induced production of free radicals, which in turn can impair the antioxidant defense mechanism, leading to an increase in membrane lipid peroxidation. The decreased level of GSH in $\gamma$-irradiated rats may be due to their utilization by the enhanced production of reactive oxygen species (Kamat et al., 2000). In this study, administration of ethanolic coriander extract induced an increase in GSH content and significantly decreased the level of NO in rat cardiac tissues. This might be due to the presence of compounds with antioxidant activity in coriander leaves (Rajeshwari & Andallu, 2011).

**CONCLUSION**

Based on our findings we conclude that administration of coriander extract showed a significant antihaemolytic and antioxidant properties against radiation damage, which open up new possibilities for using coriander as a way for radiation injury treatment.
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