The Role of Warfarin Alone or in Combination with Certain Natural Products Against Blood Disorders Induced by Gamma Radiation in Experimental Animals

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ABSTRACT

The effect of warfarin against blood disorder induced by radiation and possible effect of certain natural products in modifying anticoagulant effect of warfarin in irradiated rats was investigated. Prothrombin time (PT), international normalized ratio (INR), partial thromboplastin time (PTT), thrombin time (TT), fibrinogen level and platelet count were evaluated. Furthermore, plasma level of malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) were determined. The obtained data showed that irradiation elevated PT, INR, PTT, TT and fibrinogen level. Moreover, irradiation increased MDA and NO levels while, reduced GSH level. It was found that administration of garlic, fenugreek or green tea with warfarin for one week before irradiation resulted in elevation in PT and INR. Results suggest that patients on warfarin and exposed to radiation are specifically advised to avoid taking herbal medicines or to have their PT and INR measured within two weeks of starting the drug, to be on a safer side.

Keywords: Herb-Drug Interaction; Warfarin; Garlic; Green Tea; Fenugreek; Radiation Blood Disorder.

INTRODUCTION

Whole body exposure to elevated dose of ionizing radiation causes injury to hematopoietic system that will be reflected in the peripheral blood cells and platelets. These reduced circulating blood cells can cause hemorrhage, anemia and death (1). Bleeding is an early presenting complaint or it may occur during the delayed period (months or years) after radiation therapy (2). Coagulation is highly conserved throughout biology; in all mammals, and it involves both a cellular (platelet) and a protein (coagulation factor) components (3). It begins almost instantly after an injury to the blood vessel and has damaged the endothelium lining the vessel. Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called primary hemostasis, while, secondary hemostasis occurs simultaneously as proteins in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (4).

Many compounds with various pharmacological properties have screened to determine if their administration before or during irradiation diminishes radiation-induced damage to the hematopoietic system. Warfarin is an anticoagulant normally used in the prevention of thrombosis, formation of blood clots in the blood vessels and their migration elsewhere in the body; it is the most widely prescribed oral anticoagulant drug in North America (5).

Warfarin inhibits the vitamin K-dependent synthesis of biologically active forms of the calcium-dependent clotting factors II, VII, IX and X, as well as the regulatory factors protein C, protein S and protein Z (6). Despite its effectiveness, treatment with warfarin has several shortcomings. Many
commonly used medications interact with warfarin, as do some foods (particularly leaf vegetable foods or "greens," since these typically contain large amounts of vitamin K) and its activity has to be monitored by blood testing for the international normalized ratio (INR) to ensure that an adequate yet safe dose is taken. A high INR predisposes to a high risk of bleeding, while an INR below the therapeutic target indicates that the dose of warfarin is insufficient to protect against thromboembolic events (6). Theoretically, increased anticoagulant effects could be expected when combined with coumarin-containing herbal medicines as fenugreek or with antiplatelet herba as garlic (7).

Garlic (Allium sativum), is a species in the onion genus, Allium. It contains at least 33 sulfur compounds such as aliiin, allicin, ajoene, allylpropl, diallyl, trisulfide, sallylcysteine, vinylthiones, S-allylmercaptocystein and allixin. These sulfur compounds are responsible for most of the health benefits of garlic (8). It was demonstrated that garlic can reduce platelet aggregation so it may interact with antiplatelet and anticoagulant drugs (9).

Fenugreek seeds (Trigonella foenumgraecum) contain alkaloids, including trigonelline, gentianine and carpaine compounds (10). They also contain coumarins and other constituents that might affect platelet aggregation so it may interact with antiplatelet and anticoagulant drugs (11).

Green tea is made from the leaves of Camellia sinensis that have undergone minimal oxidation during processing. It originated in China, but it has become associated with many cultures throughout Asia. Green tea has recently become more widespread in the West, where black tea has been the traditionally consumed tea. Green tea has become the raw material for extracts which are used in various beverages, health foods, dietary supplements, and cosmetic items. Many varieties of green tea have been created in countries where it is grown. These varieties can differ substantially due to variable growing conditions, horticulture, production processing and harvesting time (12, 13). Multiple substances in green tea may contribute to its health benefits such as caffeine, theanine, theaflavins, theobromine, theophylline and phenolic acids. In vitro, animal and human studies suggest that the polyphenols in green tea are potent antioxidants with antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic properties (14). Green tea extract catechins (GTC) have antiplatelet effect so they may interact with antiplatelet and anticoagulant drugs (additive effect) (15). On the other hand, green tea leaves contain moderate quantities of vitamin K; which may lead to antagonizing effect to coumadins (16).

The present investigation was undertaken to evaluate the effect of warfarin against blood disorder induced by gamma irradiation in rats and the possible effect of certain natural agents such as garlic powder, fenugreek dried seeds and dried extract of green tea in modifying the anticoagulant effect of warfarin in irradiated rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats, purchased from the Nile Company for Drugs (Cairo Egypt) weighing 150-200 g were used in this study. They were housed in wire - mesh cages and kept under appropriate conditions of, temperature, humidity and light (temperature 25±2 °C, relative humidity 60-70 % and 12h cycle light). The animals were allowed free access to food consisting of standard pellets obtained from El-Nasr Chemical Company, (Cairo Egypt) and water ad libitum. The rats were acclimatized in the animal facilities of the National Center for Radiation Research and Technology for at least one week before starting the experiments. The study was carried out according to the international guidelines and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

Drugs

Warfarin (GSK, Egypt), garlic powder (Atos Pharma, Egypt), dried extract of green tea (MEPACO, Egypt) were freshly dissolved in distilled water immediately before administration.
Fenugreek dried ground seeds (MEPACO, Egypt) was freshly dispersed in distilled water, centrifuged at 1000 rpm for ten minutes and the supernatant was administered.

**Experimental Design**

Three sets of experiments were carried out in the current investigation; the first one was designed to select the suitable dose of radiation that induces blood disorder in rats. Four groups of animals \((n = 6)\) were used in this set of experiment. Group (1) received saline and served as normal group. Groups (2-4) received saline and exposed to \(\gamma\) radiation at doses of 2, 4 or 6 Gy, respectively.

The second set of experiment was carried out to select the proper dose of warfarin that provides significant blood disorder. Three groups of animals \((n = 6)\) were used in this set of experiment. Group (1) received saline and served as normal group, while groups (2,3) received a day dose of warfarin \((0.27 \text{ mg/kg, p.o})\) and \((0.45 \text{ mg/kg, p.o})\), respectively for a week followed by exposure to \(\gamma\) radiation \((4 \text{ G})\).

The third set of experiment was performed to study the effect of warfarin alone or combined with garlic powder, fenugreek dried ground seeds or dried extract of green tea against blood disorder induced by \(\gamma\) irradiation in rats. Ten groups of animals \((n = 6)\) were used in this set of experiment. Group (1) received saline and served as normal group. Group (2) was exposed to a single dose of \(\gamma\) radiation \((4 \text{ G})\) and served as control group. Group (3) received warfarin \((0.45 \text{ mg/kg, p.o})\), daily for a week. Group (4) received warfarin \((0.45 \text{ mg/kg, p.o})\), daily for a week followed by exposure to \(\gamma\) radiation \((4 \text{ G})\). Groups (5-7) were pretreated with garlic powder \((80 \text{ mg/kg, p.o})\), fenugreek dried ground seeds \((0.8 \text{ g/kg, p.o})\) or dried extract of green tea \((45 \text{ mg/kg, p.o})\) daily for one week then exposed to \(\gamma\) radiation \((4 \text{ G})\). Groups (8-10) received the same doses of drugs combined with warfarin \((0.45 \text{ mg/kg, p.o})\) daily for one week followed by exposure to \(\gamma\) radiation \((4 \text{ G})\).

**Irradiation of Animals**

Rats were exposed to whole body \(\gamma\) radiation at a single dose level of 4 Gy delivered at a dose rate of 0.47 Gy/min. The radiation source is \(^{137}\text{Cs}\) using a Canadian gamma cell 40 biological irradiator. Irradiation was performed at the National Center for Radiation Research and Technology, Cairo, Egypt.

**Measured Parameters**

Rats were anaesthetized with urethane \((1.2 \text{ g/kg, i.p})\), blood samples were withdrawn from the retro-orbital venous plexus using non heparinized capillary tubes one day after irradiation. Each sample was collected in three tubes, the first tube contained sodium citrate, while the other tubes contained EDTA. The sodium citrated tube was centrifuged at 2500 rpm for 15 min. The separated plasma was used for the estimation of prothrombin time, partial thromboplastin time, thrombin time and fibrinogen using test reagent kits according to the methods reported in previous publications \(^{17,18,19,20}\), respectively.

One of the EDTA containing tubes was used for estimation of platelet count using automated analyzer. The other EDTA containing tube was divided into two aliquots; the first aliquot was used for determination of lipid peroxides and nitric oxide levels according to the methods referred to elsewhere \(^{21,22}\), while the second aliquot was deprotenized with meta-phosphoric acid and the clear supernatant was used for the estimation of glutathione level according to the method of E. Beutler et al \(^{23}\).

Various tests have been found to be useful for measuring the anticoagulant effect of warfarin and other natural products in rats such as prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), fibrinogen and platelet count, but with varying sensitivities. PT is a measure of the activity of the vitamin K dependent factors II, VII, IX and X and also of factor V, which is not vitamin K dependent. Activated partial thromboplastin time is prolonged when any of the factors VIII, IX and X in the intrinsic system are inhibited by warfarin \(^{24}\).
Statistical Analysis

All the values were expressed as means ± S.E. Comparisons between means were carried out using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test using Prism program, version 3 (25).

RESULTS

The first set was designed to select the suitable dose of radiation that induces blood disorder in rats. The data of the present study demonstrated that one day following the exposure to γ radiation at a dose level of 4 Gy induced significant increase in PT and INR by 40 and 55 %, respectively. Moreover, it increased PTT, TT and plasma fibrinogen level by 34, 26 and 69 % respectively as compared to normal values. Irradiation at a dose level of 4 Gy increased state of oxidative stress demonstrated as significant elevation in plasma MDA and NO levels by 18 and 193 % respectively (Table 1, Figure 1), that was accompanied by reduction in blood GSH level by 22 % as compared with that of normal animals (Table 2, Figure 2). Accordingly, A 4 Gy dose was selected to induce experimental blood disorder in the subsequent experiment.

Table (1): Effect of a single exposure to γ radiation (2, 4 and 6 Gy) on blood disorder parameters in rats 1 day following the exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PT (Sec.)</th>
<th>INR</th>
<th>PTT (Sec.)</th>
<th>TT (Sec.)</th>
<th>Fibrinogen (g/l)</th>
<th>Platelet count ( Unit/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22.87 ± 0.31</td>
<td>1.86 ± 0.04</td>
<td>27.53 ± 1.36</td>
<td>35.13 ± 0.30</td>
<td>20.17 ± 2.49</td>
<td>562333 ± 15376</td>
</tr>
<tr>
<td>Irradiation (2 Gy)</td>
<td>23.87 ± 0.32</td>
<td>1.96 ± 0.04</td>
<td>28.17 ± 1.61</td>
<td>32.83 ± 1.22</td>
<td>17.55 ± 1.10</td>
<td>645000 ± 32138</td>
</tr>
<tr>
<td>Irradiation (4 Gy)</td>
<td>32.13 ± 0.34</td>
<td>2.88 ± 0.04</td>
<td>36.78 ± 1.44</td>
<td>44.20 ± 1.45</td>
<td>33.99 ± 0.20</td>
<td>575667 ± 26861</td>
</tr>
<tr>
<td>Irradiation (6 Gy)</td>
<td>23.70 ± 0.46</td>
<td>1.94 ± 0.05</td>
<td>22.63 ± 0.13</td>
<td>35.03 ± 1.14</td>
<td>22.73 ± 1.25</td>
<td>556333 ± 28204</td>
</tr>
</tbody>
</table>

Table (2): Effect of a single exposure to γ radiation (2, 4 and 6 Gy) on oxidative stress biomarkers in rats 1 day following the exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (nmol/l)</th>
<th>GSH (mg%)</th>
<th>NO (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.34 ± 0.07</td>
<td>25.99 ± 0.99</td>
<td>2.19 ± 0.05</td>
</tr>
<tr>
<td>Irradiation (2 Gy)</td>
<td>11.64 ± 0.11</td>
<td>23.79 ± 1.26</td>
<td>2.28 ± 0.05</td>
</tr>
<tr>
<td>Irradiation (4 Gy)</td>
<td>12.24 ± 0.04</td>
<td>20.37 ± 1.25</td>
<td>6.42 ± 0.30</td>
</tr>
<tr>
<td>Irradiation (6 Gy)</td>
<td>12.82 ± 0.17</td>
<td>13.90 ± 0.66</td>
<td>37.80 ± 0.79</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.
* Significantly different from normal group at p ≤ 0.05.
Fig. (1): Effect of a single exposure to γ radiation (2, 4 and 6 Gy) on prothrombin time (PT), partial thromboplastin time (PTT) and thrombin time (TT) in rats 1 day following the exposure
* Significantly different from normal group at p ≤ 0.05.

Fig. (2): Effect of a single exposure to γ radiation (2, 4 or 6 Gy) on MDA (a), GSH (b) and NO (c) in rats 1 day following the exposure
* Significantly different from normal group at p ≤ 0.05.
It was found that a daily oral administration of warfarin in a dose of 0.45 mg/kg for one week before irradiation (4 Gy) markedly elevated PT, INR, PTT, TT as well as plasma fibrinogen level by 87, 113, 33 and 89, respectively relative to normal values. Also, it caused a significant increase in plasma MDA and NO levels by 14 and 122 % respectively, as well as a reduction in blood GSH level by 19 % as compared to normal group value (Figure 3). Based on the obtained results, 0.45 mg/kg was selected to provide a significant blood disorder.

![Fig. (3): Effect of warfarin administration (0.27 or 0.45 mg/kg) for one week followed by exposure to \( \gamma \) radiation (4 Gy) on coagulation parameters (a) and oxidative stress biomarkers (b) in rats](image-url)

*Significantly different from normal group at p ≤ 0.05

Pretreatment with garlic at a dose of 80 mg/kg, ameliorated irradiation–induced elevation in PT, INR, PTT, TT and fibrinogen levels by 36, 43, 44, 41 and 65 %, respectively relative to irradiated group. Interestingly, it normalized MDA, GSH and NO levels (Table 3,4 and Figure 4,5).

It was observed that combined therapy of garlic and warfarin one week before irradiation completely protected against irradiation-induced elevation in PTT, TT, plasma fibrinogen, MDA, NO levels as well as against reduction in GSH level. On the other hand, a significant elevation was detected in PT and INR values (Table 3,4 and Figure 4,5).

A daily treatment with fenugreek at a dose of 0.8 g/kg, for 7 successive days prior to irradiation provided 39, 46, 48, 45 and 59 % protections against irradiation-induced elevation in PT, INR, PTT, TT, fibrinogen levels, respectively. Moreover, it masked the elevation in MDA and NO levels as well as the reduction in GSH level induced by irradiation by 26, 73 and 57 %, respectively, relative to irradiated group (Table 3,4 and Figure 4,5).

A combined administration of fenugreek and warfarin one week before irradiation afforded significant protection against irradiation–induced elevation in PTT, TT and fibrinogen levels recording 45, 41 and 66 % protection, respectively (Table 3,4 and Figure 4,5). Furthermore, it resulted in modification of oxidative stress parameters as MDA, GSH and NO levels by 24, 55 and 63 % as compared to irradiated group. On the contrary, it significantly elevated PT and INR values by 68 and 96 %, respectively, when compared to the normal values.
Treatment with green tea extract at a dose of 45 mg/kg, 7 successive days prior to irradiation, ameliorated irradiation-induced elevation in PT, INR, PTT, TT and fibrinogen levels by 36, 43, 49, 41 and 66 %, respectively relative to irradiated group. Similarly, it attenuated the increase in MDA (by 25%) and NO (by 64 %) as well as the decrease in GSH (by 61 %) (Table 3,4 and Figure 4,5).

Administration of green tea with warfarin (0.45 mg/kg) one week before exposure to γ radiation normalized PTT, TT and plasma fibrinogen level. In addition, it modified the elevation in MDA (by 26 %) and NO (by 68 %) as well as the reduction in GSH (by 65 %) relative to irradiated group. However, it markedly increased PT as well as INR values by 153 and 216 %, respectively, relative to normal values (Table 3,4 and Figure 4,5).
Fig. (4): Effect of administration of warfarin (0.45 mg/kg) alone or in combination with garlic (80 mg/kg), green tea (45 mg/kg) or fenugreek (0.8 g/kg) against blood disorders induced by γ irradiation (4 Gy) in rats

* Significantly different from normal group at p ≤ 0.05.
Fig. (5): Effect of administration of warfarin (0.45 mg/kg) alone or in combination with garlic (80 mg/kg), green tea (45 mg/kg) or fenugreek (0.8 g/kg) against oxidative stress disorders induced by γ irradiation (4 Gy) in rats

* Significantly different from normal group at p ≤ 0.05
Table (3): Effect of administration of warfarin (0.45 mg/kg) alone or in combination with garlic (80 mg/kg), green tea (45 mg/kg) or fenugreek (0.8 g/kg) against blood disorders induced by γ irradiation (4 Gy) in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PT (Sec.)</th>
<th>INR (Sec.)</th>
<th>PTT (Sec.)</th>
<th>TT (Sec.)</th>
<th>Fibrinogen (g/l)</th>
<th>Platelet count (unit/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21.55 ± 0.51</td>
<td>1.72 ± 0.05</td>
<td>18.28 ± 0.88</td>
<td>27.10 ± 0.41</td>
<td>12.38 ± 1.15</td>
<td>666200 ± 32523</td>
</tr>
<tr>
<td>Irradiation</td>
<td>32.11* ± 0.25</td>
<td>2.88* ± 0.03</td>
<td>36.78* ± 1.05</td>
<td>44.20* ± 1.06</td>
<td>33.99* ± 0.15</td>
<td>654800 ± 33727</td>
</tr>
<tr>
<td>Warfarin</td>
<td>34.68* ± 1.77</td>
<td>3.20* ± 0.21</td>
<td>18.86* ± 1.26</td>
<td>24.37* ± 0.61</td>
<td>11.28* ± 1.21</td>
<td>588400 ± 39709</td>
</tr>
<tr>
<td>Warfarin + Irradiation</td>
<td>42.40** ± 1.90</td>
<td>4.05** ± 0.23</td>
<td>35.00** ± 1.00</td>
<td>36.60** ± 0.98</td>
<td>22.86** ± 1.94</td>
<td>604000 ± 34210</td>
</tr>
<tr>
<td>Garlic</td>
<td>20.48** ± 0.40</td>
<td>1.61** ± 0.04</td>
<td>20.60** ± 0.44</td>
<td>25.93** ± 0.66</td>
<td>12.04** ± 1.67</td>
<td>585600 ± 40690</td>
</tr>
<tr>
<td>Fenugreek + Irradiation</td>
<td>20.28** ± 0.57</td>
<td>1.61** ± 0.06</td>
<td>20.18** ± 0.15</td>
<td>24.33** ± 0.83</td>
<td>13.83** ± 1.81</td>
<td>605250 ± 36102</td>
</tr>
<tr>
<td>Green tea + Irradiation</td>
<td>22.53** ± 0.24</td>
<td>1.82** ± 0.03</td>
<td>18.58** ± 0.42</td>
<td>26.08** ± 0.63</td>
<td>11.60** ± 0.96</td>
<td>540000 ± 40786</td>
</tr>
<tr>
<td>Garlic + Warfarin + Irradiation</td>
<td>41.80** ± 0.32</td>
<td>4.00** ± 0.04</td>
<td>20.43** ± 0.42</td>
<td>27.27** ± 0.41</td>
<td>13.06** ± 1.46</td>
<td>575800 ± 39920</td>
</tr>
<tr>
<td>Fenugreek + Warfarin + Irradiation</td>
<td>36.30** ± 1.35</td>
<td>3.38** ± 0.16</td>
<td>20.43** ± 0.42</td>
<td>26.00** ± 0.71</td>
<td>11.45** ± 1.62</td>
<td>650200 ± 30064</td>
</tr>
<tr>
<td>Green tea + Warfarin + Irradiation</td>
<td>54.53** ± 1.89</td>
<td>5.44** ± 0.21</td>
<td>18.28** ± 0.79</td>
<td>27.10** ± 0.20</td>
<td>10.53** ± 1.46</td>
<td>572000 ± 38853</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.
* Significantly different from normal group at p ≤ 0.05.
@ Significantly different from irradiated group at p ≤ 0.05.
@b Significantly different from warfarin treated group at p ≤ 0.05.

Table (4): Effect of administration of warfarin alone or in combination with garlic (80 mg/kg), green tea (45 mg/kg) or fenugreek (0.8 g/kg) against oxidative stress disorders induced by γ irradiation (4 Gy) in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (nmol/l)</th>
<th>GSH (µmol/l)</th>
<th>NO (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.56 ± 0.23</td>
<td>24.83 ± 2.64</td>
<td>2.033 ± 0.38</td>
</tr>
<tr>
<td>Irradiation</td>
<td>14.18* ± 1.04</td>
<td>14.79* ± 2.76</td>
<td>6.10* ± 0.20</td>
</tr>
<tr>
<td>Warfarin</td>
<td>9.78* ± 0.26</td>
<td>22.76* ± 2.73</td>
<td>2.28* ± 0.46</td>
</tr>
<tr>
<td>Warfarin + Irradiation</td>
<td>12.95** ± 0.50</td>
<td>15.43** ± 2.52</td>
<td>5.09** ± 1.00</td>
</tr>
<tr>
<td>Garlic + Irradiation</td>
<td>10.58* ± 0.46</td>
<td>20.99* ± 0.55</td>
<td>2.28* ± 0.45</td>
</tr>
<tr>
<td>Fenugreek + Irradiation</td>
<td>10.43* ± 0.18</td>
<td>23.28* ± 0.66</td>
<td>1.67* ± 0.29</td>
</tr>
<tr>
<td>Green tea + Irradiation</td>
<td>10.66* ± 0.22</td>
<td>23.86* ± 0.38</td>
<td>2.22* ± 0.33</td>
</tr>
<tr>
<td>Garlic + Warfarin + Irradiation</td>
<td>10.45* ± 0.22</td>
<td>23.80* ± 0.58</td>
<td>2.32* ± 0.54</td>
</tr>
<tr>
<td>Fenugreek + Warfarin + Irradiation</td>
<td>10.76* ± 0.19</td>
<td>22.91* ± 0.92</td>
<td>2.25* ± 0.22</td>
</tr>
<tr>
<td>Green tea + Warfarin + Irradiation</td>
<td>10.50* ± 0.28</td>
<td>24.44* ± 0.63</td>
<td>1.92* ± 0.33</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.
* Significantly different from normal group at p ≤ 0.05.
@ Significantly different from irradiated group at p ≤ 0.05.
@b Significantly different from warfarin treated group at p ≤ 0.05.
DISCUSSION

Earlier studies showed that hematopoietic tissues are the most radiosensitive tissues to ionizing radiation through induction of oxidative damage to cellular macromolecules, leading to demise of the hematopoietic system (26). The current study demonstrated a significant increase in the prothrombin time (PT), international normalized ratio (INR), partial thromboplastin time (PTT), thrombin time (TT) as well as plasma fibrinogen level one day following the exposure to γ radiation at a dose level of 4 Gy. The present results were in agreement with the findings reported in previous publications (27,28). The prolongation in the prothrombin time might be due to the decrease in the secretory function of the liver of factors II, V and VII after radiation exposure, while, the prolongation in the activated partial thromboplastin time might be due to the decrease in the secretory function of the liver after radiation exposure of the factors VIII, IX, XI and XII (29). In addition, the increase in the plasma fibrinogen level might be due to increasing its expression as a result of whole body gamma irradiation and also might be due to up regulation. This expressed fibrinogen is dysfunctional so it resulted in prolongation of thrombin time (29).

Data of the present investigation showed that γ irradiation (4 Gy) had no effect on platelet count one day following the irradiation. Results were in accordance with earlier observation reported elsewhere (30,31).

The results of the present study demonstrated that plasma level of malondialdehyde (MDA) increased one day following the exposure to γ radiation at dose levels of 2, 4 and 6 Gy. This result was consistent with the observation reported in previous publications (32, 33, 34). The elevated level of MDA might be due to the effect of reactive oxygen species (ROS) that are generated from the interaction between radiation and water molecules in cells which subsequently leads to cell damage through oxidation of cell membrane biomolecules such as lipids, proteins and DNA (35).

The current work showed that the exposure to γ radiation at dose levels of 4 and 6 Gy reduced blood glutathione (GSH) level one day following the exposure. These results corroborated with that findings reported in previous studies (36, 37, 38). The significant decrease in reduced glutathione might be attributed to the fact that, GSH scavenges oxygen radicals and protects protein thiol groups from oxidation, so plasma GSH level and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of GSH-redox cycle, significantly reduced due to oxidative stress caused by gamma irradiation (35).

The findings of this study revealed that plasma level of nitric oxide (NO) increased one day following the exposure to γ radiation at dose levels of 4 and 6 Gy. Data were in agreement with the findings of previous studies (33, 34, 37, 39, 40). Elevated level of nitric oxide could be ascribed to the exposure of ionizing radiation which stimulated NO synthases (NOS) to synthesize NO from L-arginine and thus nitric oxide (NO) was formed in higher amounts in response to ionizing radiation (41).

The obtained results showed that prothrombin time and INR were increased following the daily oral administration of warfarin at doses of 0.27 mg/kg and 0.45 mg/kg for one week before irradiation (4 Gy). These findings are consistent with the results of previous studies in which the warfarin daily administration at a dose of 0.2 mg/kg in rats for 5 days (42) and at a dose of 20 mg/kg in mice for 3 consecutive days (43) prolonged prothrombin time and increased INR. This prolongation in PT might be due to the fact that warfarin inhibits vitamin K-dependent synthesis of biologically active forms of calcium-dependent clotting factors II (prothrombin), VII, IX and X, as well as regulatory factors protein C, protein S, and protein Z. Other proteins not involved in blood clotting, such as osteocalcin, or matrix Gla protein, might also be affected (6).

A daily intake of garlic powder at a dose of 80 mg/kg for one week ameliorated irradiation-induced elevation in oxidative stress biomarkers as well as coagulation parameters measured in this study. The present results were in agreement with the findings of earlier studies (44, 45, 46, 47). This might be due to the antioxidants contents that garlic is rich in, which help destroying free radicals particles that can damage cell membrane and DNA and may contribute to the aging process as well as the development of a number of conditions, including heart disease, cancer and ionizing radiation (47, 48, 49). The antioxidant properties of garlic compounds represent the four main chemical classes, alliin, allyl...
cysteine, allyl disulfide, and allicin. Alliin scavenged superoxide, while allyl cysteine and allyl disulfide did not react with superoxide. Allicin suppressed the formation of superoxide by the xanthine/xanthine oxidase system, probably via a thiol exchange mechanism. Alliin, allyl cysteine, and allyl disulfide all scavenged hydroxyl radicals (50).

Herbal medicines follow modern pharmacological principles. Hence, herb–drug interactions are based on the same pharmacokinetic and pharmacodynamic mechanisms as drug–drug interactions (7). Herbs can affect the response of anticoagulant therapy by increasing or reducing PT and INR (51). It is noteworthy to mention that L. Vaes et al (52) reported an interaction between warfarin and garlic that may affect bleeding time through interference with platelet function, so patients using warfarin are cautioned regarding possible risk of bleeding with ingestion of garlic. The current work showed that a daily concomitant intake of garlic powder with warfarin for one week prolonged PT and increased INR. These results were in agreement with earlier studies which reported that heavy use of garlic supplement was associated with spontaneous spinal epidural hematoma (53) and associated with prolonged clotting time with increased post-operative bleeding (54,55). Also it was reported that the coagulation system was considerably reduced after administration of garlic in rats (56). This prolongation in PT could be attributed to antiplatelet effects and the effect on platelet aggregation is proposed to be due to inhibition of cyclooxygenase that plays a key role in arachidonic acid metabolism which results in decreasing the synthesis of thromboxane B2 and the production of potent vasoconstrictors leukotriene C4 and prostaglandin E2 by platelets. Some of the sulfur-containing compounds such as allicin, ajoene, S-allylcysteine, S-methylcytsteine, diallyl disulfide and sulfoxides might be responsible for antithrombotic activity of garlic. It may also be proposed that moderate anti-aggregatory effect of garlic may be due to regulation of membrane phospholipases activity that prevents liberation of arachidonic acid from phospholipids (57).

Interestingly, a daily intake of fenugreek dried ground seeds ameliorated irradiation–induced elevation in the oxidative stress biomarkers as well as the coagulation parameters measured in this study. The present results were consistent with previous reports mentioned in some previous studies (58,59). It seems that mice receiving fenugreek seeds extract prior to whole body gamma irradiation exhibited protective effect against radiation-induced damaging effects (59). Furthermore, Thirunavukkarasu et al. (58) revealed that the administration of aqueous extract of fenugreek seeds inhibited enzymatic leakage and the elevation in lipid peroxidation as well as enhanced antioxidant potential in rats. This protective effect might be ascribed to the high content of flavonoids and polyphenols present in fenugreek which have an antioxidant effect. Furthermore, it might be due to the presence of antioxidant carotenoids (60).

The findings of this study revealed that daily concomitant intake of fenugreek dried ground seeds with warfarin for one week prolonged PT and increased INR. The present results were in agreement with the observation of Lambert and Cormier (61) who reported that the administration of fenugreek for few days in patient treated with warfarin for atrial fibrillation prolonged PT and increased INR. This effect might be attributed to the antiplatelet properties of fenugreek, also it might be due to its content of coumarinic derivatives which, in an additive or synergistic way, could produce such interaction (11).

The daily intake of green tea extract, attenuated irradiation–induced elevation in oxidative stress biomarkers as well as coagulation parameters measured in this work. The present results were in accordance with earlier observations of Hewala et al. (62) who confirmed that Ehrlich carcinoma-bearing mice supplemented with dried extract of green tea and exposed to whole body γ-radiation (4 Gy) after 30 min from the last dose ameliorated γ radiation damaging effect on the body as well as Hewala and El-Feky. (63) who reported that mice receiving 1.5% dried extract of green tea for 14 days then exposed to γ radiation (4 Gy) provided a good protective effect against radiation-induced damage to liver and hematopoietic system. This might be due to green tea polyphenols which could be direct antioxidants by scavenging reactive oxygen species or chelating transition metals. Alternatively, they may act indirectly by up-regulating phase II antioxidant enzymes (64).

The current work showed that the combined administration of dried extract of green tea with warfarin prolonged PT and increased INR. The present results are in agreement with previous studies.
which reported that rats coadministered warfarin with green tea extract at a dose of 10 to 100 mg/kg had prolonged the clotting time. The study by Son et al. (15) supported the hypothesis that this effect might be due to green tea extract catechins (GTC) which have antiplatelet effect. GTC significantly inhibited thromboxane A2 (TXA2) and prostaglandin D2 (PGD2) generations induced through inhibition of TXA2 synthase activity. Moreover, adenosine triphosphate (ATP) released from dense granule was inhibited by GTC.

**CONCLUSION**

It is of value to mention that when patients who take warfarin or awaiting surgery start or discontinue one of these dietary supplements, it would be advisable to monitor the INR and bleeding time to be on safer side. Although dietary supplements are not drugs as defined by the current US law, dietary supplements are not innocuous and may potentially affect the efficacy and safety of concurrent therapies. The true risks of these effects may be difficult to characterize due to the limited number and nature of existing reports.

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