Role of Some Natural Plant to Enhance the Immune System Against Exposure of Ionizing Radiation in Experimental Animals

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Received: 10/1/2013 Accepted: 20/2/2013

ABSTRACT

Tumor necrosis factor–alpha (TNF-α), Transforming growth factor (TGF-1β), Interleukin-5 (IL-5), liver enzymes and Malondialdehyde were determined in 50 male albino rats which are divided into 5 groups: control and 4 irradiated groups, all are exposed to the following γ-radiation doses: 0.5, 1, 1.5, and 2 Gy double dose for two successive weeks, once weekly. All groups except control, 0.5 and 1 Gy groups were treated with Ambrosin extract (natural product) with 20 mg/kg body weight 8 times once weekly. All animals were sacrificed after one week of the last dose of Ambrosin. The results revealed that all immuno response TNF-alpha, TGF-1β and IL-5 showed a highly amelioration after treatment by Ambrosin in the groups which exposed to 1.5, and 2 Gy in comparison with the untreated groups.

Key words: TNF–alpha, TGF-1β, IL-5

INTRODUCTION

Tumor necrosis factor (TNF-α), typical of cytokine, serves as an intermediate of the immune system, protecting the host from the attacks of various infections and cancer cells, and induces the death of cancer cells by causing apoptosis [1]. Recent studies have reported that radiation increases the expression of specific proteins including TNF-α [9].

Transforming growth factor (TGF-1β), which is produced mainly from astrocytes and macrophages, is a polypeptide with various biological actions in the human body and the expression of it is also increased after irradiation [2,27].

Interleukin-5 was originally found as T-cell replacing factor’ that is secreted from T cells to stimulate antibody production from activated B cells. IL-5 induces terminal differentiation of activated B cells into antibody-forming cells in mice and enhances proliferation and differentiation of eosinophil precursors into mature eosinophils in mice and humans. [44].

In the last decade, several studies have shown that protocols using low-dose radiation (LDR) are more effective in providing local tumor control with negligible normal tissue toxicity. LDR stimulates antioxidant capacity, repair of DNA damage, apoptosis and induction of immune responses, which might be collectively responsible for providing effective local tumor control.[43]

People may be exposed to ionizing radiation during radiotherapy or following exposure to radionuclides in nuclear medicine. Radioprotective agents have been used to reduce morbidity or mortality produced by ionizing irradiation. Early developments of such agents focused on thiol synthetic compounds, such as amifostine,[4,5] This compound reduced mortality; however, there were difficulties in administering aminothiols that led to adverse effects. Hence, the development of radioprotective agents with lower toxicity and an extended window of protection has attracted much attention. Natural compounds have been evaluated as radioprotectants and they seem to exert their effect through antioxidant and immunostimulant activities. Although recent agents have lower
efficacy, they have lower toxicity, more favorable administration routes and improved pharmacokinetics compared to the older thiol compounds [5,6].

Radioprotective agents are synthetic compounds or natural products that are immediately administrated before irradiation to reduce injuries caused by ionizing radiation [4,5]. Over the past 60 years, as a result of the great clinical need for effective radioprotectant agents, many have been prepared and tested to find more effective, less toxic, drugs. Initial attempts were focused on synthetic thiol compounds. These agents are highly effective at reducing lethality induced by irradiation [8,26]. Of this class, amifostine is the only radioprotector that has been clinically approved by the Food and Drug Administration (FDA) for mitigating side effects (xerostomia) in patients undergoing radiotherapy [6,24]. This drug offers good protection, but is relatively toxic (nausea, vomiting and hypotension being some of the most common adverse effects) [7,25]. In view of this, the search continues for less toxic, more effective radioprotectors that can be easily self-administered. In recent years, radioprotective agents with a novel mode of action have been investigated; in particular, compounds that can affect haematopoietic stem cell regeneration have attracted significant interest. The aim of this strategy is to increase survival rate by stimulating the function and regeneration of the stem cell population that is decreased, due to radiation induced damage [3,4]. Immunomodulators and cytokines represent the bulk of agents in this category. Naturally occurring compounds that function as antioxidants and immunostimulants are another strategy for the development of radioprotective agents with low toxicity[9]. Therapeutic agents that can be administered following irradiation are another strategy for reducing side effects induced by ionizing radiation; cytokines and immunomodulators, through induction of bone marrow recovery and extra hematological tissue regeneration [9,10].

Ambrosia maritima L. (Damsissa) is one of the wild plants present in Egypt and different African countries of the Nile valley. It belongs to the subfamily tubuliflora which is a branch of the family Compositae of flowering plants. It contains important sesquiterpene lactones and flavonoids which showed molluscicidal and cytotoxic activities [12,22]. The most active ingredients of this plant are ambrosin and damsin [13]. Ambrosin belongs to a group of natural products known as pseudoguaianolides, it was totally synthesized and described by Grieco et al at 1982[14]. It also showed an antioxidant activity against gamma-radiation, cancer, hyperglycemia, diabetes mellitus, mutagenesis, hypercholesteolaemia and arteriosclerosis [14,23].

This study is presented to investigate the possible protective effects of Ambrosin against irradiation-induced immunomodulators and immunoresponses with biochemical changes in experimental animals.

MATERIALS AND METHOD

1- Experimental animals :

The study include fifty male albino rats, aged 1-2 months (110-120 g). Rats were kept in cages under hygienic conditions, fed on standard rodents chow and supplied with water. Rats were divided into 5 groups: control and 4 irradiated with 0.5, 1, 1.5, and 2 Gy double dose of γ-radiation for two successive weeks, once weekly. All groups except control, 0.5 and 1 Gy groups were treated with Ambrosin extract (natural product) with 20 mg /kg body weight 8 times twice weekly. All animals were sacrificed after one week of the last dose of Ambrosin

2-Biochemical study :

2.1- The activities of AST and ALT were determined according to the method of Henry and Frankle[38].

2.2- Malondialdehyde (MDA) determination was carried out according to the method adopted by Draper and Hardley[39]. The method implies the measurement of MDA as one of the main products of lipid peroxidation by the thiobarbituric acid method. The principle of the method
is based on the reaction of MDA with thiobarbituric acid (TBA) with the resulting pink colored tri-methyl complex with a maximum absorption at 530-532 nm. The samples were analyzed by spectrophotometer (Milton Roy spectronic 3000 ARRAY double beam spectrometer, USA)

3-Immunological measurements:

Cytokine IL-5 was determined as described by Banks[40] and measurements of Cytokine TNF–α and TGF-1β were performed according to Whiteside[41]

4-Natural product preparation:

The sesquiterpenes: damsin, ambrosin, chloroambrosin and neoambrosin, the dried powdered whole herb of Ambrosia maritima, L. family Compositae was successively and exhaustively extracted with light petroleum ether according to Abadome [42]. The extracts were concentrated and evaporated from traces of water under rotator evaporator apparatus and were kept at a temperature not exceeding 45°C until injected. The chemical formula of ambrosin is 11(13)-dien-12-oic acid, 6.beta.-hydroxy-4-oxo-.γ.lactone.

5-Irradiation facilities:

Whole body γ–irradiation was performed at the National Centre for Radiation Research and Technology, Atomic Energy Authority (NCRRT), Cairo, Egypt, using caesium-137 in a Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). Animal groups were irradiated at an acute successive double doses levels of 0.5 ,1, 1.5, and 2 Gy delivered at a dose rate of 0.65 Gy min–1.

6-Statistical analysis:

The results obtained in the present study were expressed as mean ± SEM. The statistical difference between various groups were analysed by the Student’s t-test and the significance was observed at the p < 0,01 and p < 0,001 levels[45].

RESULTS AND DISCUSSION

Evaluation of ALT level

As shown in table (1) whole body gamma irradiation at dose level of 0.5Gy and 1Gy caused significant elevation in plasma ALT with percent change of 62.1% and 65.2% in comparison with control value. On the other hand, rats exposed to 1.5Gy and 2Gy doses gamma irradiation and treated with Ambrosin showed the significant decreased in ALT at percent change 22.1% and 27.2% (p < 0,001 ) respectively in comparison with control.

Evaluation of AST level

As shown in table (1) whole body gamma irradiation at dose level of 0.5Gy and 1Gy caused significant elevation in plasma AST with percent change of 43.3% and 65.2% in comparison with control value. On the other hand, rats exposed to 1.5Gy and 2Gy doses gamma irradiation and treated with Ambrosin showed the significant decreased in AST at percent change 24.3% and 15.7% (p < 0,001 and p < 0,01) respectively in comparison with control value.
Evaluation of MDA level

As shown in table (1) whole body gamma irradiation at dose level of 0.5 Gy and 1 Gy caused significant elevation in plasma MDA with percent change of 70.6% and 80.2% in comparison with control value. On the other hand, rats exposed to 1.5 Gy and 2 Gy doses gamma irradiation and treated with Ambrosin showed the significant decreased in MDA at percent change 40.4% and 50.2% (p < 0.001) respectively in comparison with control value.

Evaluation of IL-5 level

IL-5 was showed in table (2) have non significance at dose 0.5 and 1 Gy whole body irradiation. In contrast in those groups which exposure to a dose 1.5 and 2 Gy and treated with Ambrosin was showed a significant increased in serum IL-5 with percent change 69.5% and 59.7% respectively (p < 0.001) in comparison with control value.

Evaluation of TNF-α level

TNF-α was showed in table (2) have significance increased at dose 0.5 and 1 Gy whole body irradiation with percent change 32.4% and 41.1% respectively. On the other hand groups which exposure to a dose 1.5 and 2 Gy and treated with Ambrosin was showed a significant increased in serum TNF-α with percent change 52.8% and 60.2% respectively (p < 0.001) in comparison with control value.

Evaluation of TGF-1β level

In table (2) showed the Gamma irradiation (0.5 Gy and 1 Gy) induced an increase in the activity levels TGF-1β with percent change 49.9% and 63.5%. On the other hand groups which exposure to a dose 1.5 and 2 Gy and treated with Ambrosin was showed a significant increased in serum TGF-1β with percent change 79.8 and 83.6% respectively (p < 0.001) in comparison with control value.

Table (1): Effect of natural product on liver function and Malondialdehyde level in experimental rat after exposure to ionizing radiation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5 Gy</th>
<th>1 Gy</th>
<th>1.5 with Ambrosin</th>
<th>2 Gy with Ambrosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT U/L</td>
<td>42.88±1.1</td>
<td>69.48±1.5***</td>
<td>70.78±1.8***</td>
<td>52.36±1.1***</td>
<td>54.47±1.7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.1%***</td>
<td>65.2%***</td>
<td>22.1%***</td>
<td>27.2%**</td>
</tr>
<tr>
<td>AST U/L</td>
<td>45.66±2.3</td>
<td>65.47±1.9***</td>
<td>75.47±2.6***</td>
<td>56.78±0.9***</td>
<td>52.86±2.3**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43.3%***</td>
<td>65.2%***</td>
<td>24.3%***</td>
<td>15.7%**</td>
</tr>
<tr>
<td>MDA mmol/L</td>
<td>18.32 ± 1.1</td>
<td>31.27 ± 2.4**</td>
<td>33.03 ± 1.2***</td>
<td>25.75 ± 2.1***</td>
<td>27.57 ± 2.5***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70.6%**</td>
<td>80.2%***</td>
<td>40.4%***</td>
<td>50.2%***</td>
</tr>
</tbody>
</table>
Figure (1): Effect of Natural product on IL-5, TNF-α and TGF-1β level in experimental rat after exposure to ionizing radiation

Table (2): Comparison between IL-5, TNF-α and TGF-1β level as regard to Effect of Natural product after exposure to ionizing radiation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5 Gy</th>
<th>1 Gy</th>
<th>1.5 with Ambrosin</th>
<th>2 Gy with Ambrosin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-5 (pg/ml)</strong></td>
<td>225.64 ± 5.2</td>
<td>220.68 ± 4.5</td>
<td>240.68 ± 5.1</td>
<td>382.68 ± 6.2</td>
<td>360.44 ± 5.5</td>
</tr>
<tr>
<td></td>
<td><strong>NS 2.2%</strong></td>
<td><strong>NS 6.6%</strong></td>
<td><strong>NS 69.5%</strong></td>
<td>***** 69.5%**</td>
<td>***** 59.7%**</td>
</tr>
<tr>
<td><strong>TNF-α (pg/ml)</strong></td>
<td>340.45 ± 5.3</td>
<td>450.87 ± 6.3</td>
<td>480.26 ± 6.5</td>
<td>520.36 ± 4.1</td>
<td>545.68 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>***** 32.4%**</td>
<td>***** 41.1%**</td>
<td>***** 52.8%**</td>
<td>***** 60.2%**</td>
<td></td>
</tr>
<tr>
<td><strong>TGF-1β (pg/ml)</strong></td>
<td>220.63 ± 2.4</td>
<td>330.74 ± 3.4</td>
<td>360.25 ± 3.7</td>
<td>395.26 ± 4.1</td>
<td>405.18 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>***** 49.9%**</td>
<td>***** 63.5%**</td>
<td>***** 79.8%**</td>
<td>***** 83.6%**</td>
<td></td>
</tr>
</tbody>
</table>

** Significantly different from control at p < 0.01
*** Highly Significantly different from control at p < 0.001
NS Non Significantly different from control

Sesquiterpene lactones ambrosin, isabelin, psilostachyn, cumanin and peruvin triterpenoids of type α and β-amarine and derivatives of caffeic acid have all been identified in the Ambrosia maritima L [14] Many favonoids and lignans are already known for their antioxidant action and anti-apoptotic potential, and thus contribute towards radioprotection to achieve radioprotection, recovery of damaged cells after radiation exposure and minimization of cell death by inhibition of apoptosis is an inescapable necessity[13]
The results of the present study showed that whole-body gamma irradiation (0.5 Gy and 1 Gy) caused a marked increase in the activity of aminotransferase (ALT and AST) in the rats serum. These data agree with that reported by Roushy et al. [16] who found that irradiation caused a significant increase in ALT and AST activities after 5 and 10 days. The increase in the serum aminotransferase activities could be due to liver damage induced by free radicals. On the other hand, groups exposed to whole body gamma irradiation (1.5 Gy and 2 Gy) and treated with Ambrosin 20 mg/kg body showed an ameliorative in AST and ALT activities. These data agree with that reported by Feliste [17] who suggests that there are several pathways of radioprotection that have been suggested against the damaging effects of ionizing radiation in mammalian cells. The mechanisms that radio-protections implicate include free radical scavenging that protects against ionizing radiation-generated reactive oxygen species (ROS) or chemotherapeutic agents and hydrogen atom donation that facilitates direct chemical repair at sites of DNA damage in liver cells.

The present study also demonstrates a significant increase in serum MDA activity after radiation exposure at dose (0.5 Gy and 1 Gy). These data agree with Diplock [18] who reported that MDA elevation as a result of oxidative stress take place through the decrease of total antioxidant capacity, GSH level, and antioxidant enzyme activities. The MDA elevation has been well accepted as a reliable marker of lipid peroxidation [20]. The oxidative stress occurs when the generation of ROS overrides the ability of the endogenous antioxidant system to remove excess ROS [18]. This overproduction of ROS subsequently leads to cell damage through oxidation of cell membrane biomolecules such as lipids, proteins, and DNA [21, 18]. On the other hand, MDA showed significant decrease in groups treated with Ambrosin 40.5% and 50.2% in comparison with control group, these results were in agreement with Gillesen [19] who suggested that the role of Sesquiterpene lactones and Flavonoids in Ambrosin and Damsine act as an antioxidant regulators and initiators for indigenous antioxidant factors in liver cells, so ambrosin induce the antioxidant enzymes such as superoxide dismutase (SOD) which are important in providing protection from radiation exposure. Balance of the enzymes in the whole organism are required for maximum radioprotection [19].

Our results recorded that IL-5 level showed no significant change in groups exposed to 0.5 and 1 Gy, but on the other hand, groups exposed to 1.5 and 2 Gy and treated by Ambrosin recorded highly significant increase in serum IL-5 with percent change 69.5% and 56.7% (p < 0.001). These results corroborate the findings of MacVitty[37] that Sesquiterpenes elevate the level of cytokins, IL-1, IL-5, IL-6, IL-7, and promote the production of TNF-α and TGF-1β [34, 35].

In the present study, we found that the expressions of TNF-α and TGF-1β in the irradiated groups at 0.5 Gy and 1 Gy were significantly increased with percent change 32.4% and 41.1% in TNF-α serum levels, also in TGF-1β the percent change reached 49.9% and 63.5% (p<0.001) compared to control group. These results were consistent with Lover et al. [29] who reported that the functions of TNF-α and TGF-1β are not fixed but have a multidirectional nature depending on given cells, tissues, and their conditions, through a cascade and interaction with other cytokines. In this manner, they relate to the proliferation of astrocytes, vascular endothelial cells, and the destruction of blood-brain barriers usually shown after irradiation and contribute to the radiation injury including vascular injury, and necrosis [29, 30]. Also, our results demonstrated that TNF-α and TGF-1β were significantly increased in groups that were exposed to 1.5 Gy & 2 Gy and treated with Ambrosin where percent change in TNF-α serum levels was 52.8% and 60.2% respectively. Also, in TGF-1β serum levels were recorded. Their percent change were 79.8% and 83.6% respectively (p<0.001) as compared to control group. These results coincided with Schooltink[32] who stated that Ambrosin stimulates the endogenous production of cytokines, such as IL-5, IL-6, TGF-1β and TNF-α. Also, Ambrosin increased the number of bone marrow cells, spleen cells, GM-CFC, circulating neutrophils, lymphocytes and platelets in irradiated mice [33, 36]. The number of granulocyte–monocyte progenitors in bone marrow increased with Ambrosin treatment [23].

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CONCLUSIONS

The radiation injuries after a single high-dose radiation that issued for various malignant tumors in recent days consist of radiation necrosis and apoptosis, which were associated with the expression of TNF-α and TGF-1β. If we can clarify the roles of cytokines in relation to radiation injury, we can develop methods of restricting or accelerating the expression of the cytokines, which will contribute to the prevention of complications after radiation therapy.

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