Protective Effect of Rhoifolin on Gamma Irradiation Induced Cardiac Dysfunctions in Albino Mice

Omama E. El-Shawi\textsuperscript{1} and *Omayma A. Eldahshan\textsuperscript{2}

\textsuperscript{1}Clinical Biochemistry Lab. Health Radiation Research Department, National Centre for Radiation Research and Technology, Atomic Authority, Cairo, Egypt
\textsuperscript{2}Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

*Corresponding author: Omayma A. Eldahshan  Mail: omiahm@hotmail.com Tell: 002 010 11 84 19 51

Received: 15/2/2013  Accepted: 15/7/2013

ABSTRACT

Rhoifolin (apigenin 7 neohesperidoside) is a flavone glycoside, a natural product belonging to the flavonoid. This study aimed to estimate the optimum dose level of rhoifolin against the lethality of 10Gy irradiation dose in mice. Also, to evaluate the modulator effect of rhoifolin against 10 Gy irradiation induced certain haematological and cardiac biochemical abnormalities. Six groups of mice (n =15) were used. The 1\textsuperscript{st} group subjected to a single dose (10 Gy) irradiation and used as control. The other five groups administered different doses of rhoifolin (12, 24, 30, 36, and 40 mg/kg) po for 7 consecutive days pre irradiation (10 gray). The optimum dose of rhoifolin against radiation induced mortality and body weight loss was evaluated and found to be 36 mg/kg body weight. Further, rhoifolin was administered to mice 36 mg/kg for 7 consecutive days pre-irradiation (10Gy). Mice were sacrificed 24 hours post irradiation; the effect of rhoifolin against irradiation induced certain haematological and cardiac biochemical alterations were estimated. Treatment of mice with rhoifolin pre irradiation resulted in a significant increase of body weight compared to those irradiated. Biochemical analysis revealed that rhoifolin diminished the toxic effect of radiation by decreasing the level of lipid peroxides measured as malondialdehyde, ameliorating the alterations in nitric oxide, lactate dehydrogenase, creatine kinase in plasma and tissue and the plasma lipid profile. Also, the blood platelets count, the content of glutathione and the activity of superoxide dismutase and catalase showed a significant elevation compared to those irradiated. Conclusion: The present study suggests a radioprotective effect of rhoifolin against radiation-induced decrease of blood platelets and cardiac biochemical lesions in whole body irradiated mice.

Key words: Radiation protection/ Rhoifolin/ Swiss albino mice/ heart.

INTRODUCTION

Radiation exposure and the associated risks are of important concerns in medical radiotherapy\textsuperscript{(1)}, occupational exposure\textsuperscript{(2)} and manned spaceflight\textsuperscript{(3)}. Undesirable complications would occur owing to radiation injury to the surrounding normal tissues and to the skin, brain, heart, lung, kidney, liver, or gastrointestinal system of the cancer patient\textsuperscript{(4)}. High doses of radiation applied to the heart during radiotherapy used in breast cancer, Hodgkin’s disease or childhood cancers increase cardiovascular incidence and mortality. Epidemiological studies indicate that much lower irradiation doses typical of occupational, medical or environmental exposures also increase the risk of cardiovascular disease (CVD) several decades after the exposure\textsuperscript{(5)}. 
Excess production of ROS and other radicals have been implicated as inducers of tissue injury in several pathological conditions including cardiovascular injuries and atherosclerosis. Exposure to ionizing radiation provokes oxidative stress characterized by an excess production of oxidants associated to a decrease of antioxidants. A number of medicinal plants have been evaluated for their radioprotective efficacy against the damaging effects of ionizing radiation. Mainly flavones have been reported to render protection against radiation-induced biochemical and haematological alterations in experimental animals.

Rhoifolin (apigenin 7 neohesperidoside) is a flavone glycoside, belonging to the flavonoid family. This natural product extracted from the leaves of Chorisia crispiflora, was reported to have many biological effects including anti-diabetic, anti-inflammatory and antitumor activity.

So far, nothing has been traced in literature about the protective effect of rhoifolin against whole body irradiation-induced cardiac injury and oxidative damage, so the aim of the present study was to determine the optimum dose of rhoifolin against whole body exposure to gamma radiation (10 Gy) and to evaluate its modulator effects on some biochemical alterations and on the blood platelet count.

**MATERIALS AND METHODS**

**Animal care and handling:**
All experimental trials were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. Swiss albino mice weighing 25-30 g, obtained from animal house of the National Cancer Institute (NCI), Cairo University, were used during the present study. The animals were maintained under controlled conditions of temperature and light (Light: dark, 14 hrs: 10 hrs), and provided with standard mice feed and tap water ad libitum.

**Irradiation:**
The animals were whole body exposed to gamma radiation by the use of a Canadian Gamma Cell-40 (137Cs) at the National Centre for Radiation Research and Technology (Cairo, Egypt). The dose rate was 0.670 Gy/min as calibrated during the experimental periods.

**Isolation and purification of rhoifolin (Rf.):**
Powder of air dried leaves of Chorisia crispiflora (1 kg) was extracted with 70 % ethanol at room temperature. The extract was entirely dried and dissolved in a small amount of water and portioned with n-hexane, ethyl acetate and butanol successively. The aqueous water residue was totally dried and extracted with methanol at 40°C. The methanolic extract upon concentration yielded yellow crystals of rhoifolin (8.3 g). Purification to the crystals was achieved by crystallization.

**Identification of the rhoifolin:**
In continuation to our data published before through it’s chromatographic behaviour, UV, NMR and HMBC correlation, HSQC was measured on a Varian Mercury VX-500 spectrometers in DMSO. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference.

**Preparation of Rhoifolin (Rf.) for treatment:**
Rhoifolin was dissolved in saline and 0.05% dimethyl sulfoxide (DMSO) immediately before use and administrated by gavages (po).
EXPERIMENTAL DESIGN

Determination of the optimum dose of rhoifolin:

Mice were divided into 6 groups each of 15 animals. Animals of the 1st group were administered 7 consecutive doses of saline + 0.05% DMSO (1ml/day) (po) and served as irradiated control. The other five groups received different concentrations of Rf. (12, 24, 30, 36 and 40 mg/kg b. wt. /day) dissolved in 1ml (saline + 0.05% DMSO) for 7 consecutive days. Thirty minutes after the last administration, all six groups were subjected to whole body 10 Gy of gamma rays, and were monitored till 30 days for estimation of the most effective (optimum) dose of rf. against 10 Gy of gamma irradiation which caused mortality and induced reduction in body weight along the entire experimental period. The estimated optimum dose was then used for the further hematological and biochemical analysis.

Estimation of the modulator effect of rhoifolin:

Twenty four healthy male Swiss albino mice were randomly assigned into four groups (6 mice / group): i. Control group (C); animals received seven doses of 1ml (saline + 0.05% DMSO) /day, ii. rhoifolin treated normal mice (Rf), received 7 doses of Rf. (36mg/ kg/ day) dissolved in 1ml (saline + 0.05% DMSO) for seven consecutive days. iii. Irradiated group (IRR): mice received seven doses of 1ml (saline + 0.05% DMSO) /day for seven consecutive days, 30 minutes after last administration, animals exposed to 10 Gy of gamma rays. iv. Experimental group (Rf. +IRR). Mice received Rf. as in group ii, 30 minutes after last administration mice were exposed to gamma radiation as in group iii.

Samples collection:

At the end of the experiment, animals were kept overnight fastened and allowed free access to water only. Mice were sacrificed 24 hours post irradiation exposure under diethyl ether anaesthesia. Blood samples were withdrawn from the heart directly into heparinised test tubes. The heart tissues were dissected for the biochemical studies. A portion of the whole blood sample was used for blood platelets counts; glutathione (GSH) superoxide dismutase (SOD) and catalase (CAT) estimations. Another portion of blood sample was centrifuged for separation of blood plasma. The separated plasma from heparinised blood was used for determination of lipid peroxidation (LPO) as malondialdehyde (MDA) and estimation of lipid profile. Heart tissues were weighed (10% heart tissue homogenized in 50 mM phosphate buffer, pH 7.4. The cell debris was removed by centrifugation at 3000 rpm for 15 at 4°C using refrigerated centrifuge. The supernatant was used for the estimation of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Blood samples were centrifuged at 3000g for 15 min at 4°C; the supernatant was collected and stored into aliquots in eppendorff tubes, kept at -20°C till biochemical analysis.

Biochemical assays:

The lipid peroxidation (LPO) product (MDA) was assayed as a marker of oxidative stress in blood plasma and tissue homogenate according to Yoshioka et al. (12), GSH was estimated based on the method of Beutler et al. (13), SOD estimation was carried out according to Minami and Yoshikawa (14). CAT activity was estimated based on (15), nitric oxide (NO) level in blood plasma and cardiac tissue was performed according to Johansson and Borg (16). Lipid profile parameters were estimated in plasma of mice by the use of the commercial available kits from (bio-diagnostic). Cholesterol was estimated according to Allian et al (17). Estimation of Triglycerides was performed according to Fossati and Principe (18). High density lipoprotein-cholesterol (HDL-C) was assayed based on the method of Lopez-Virella et al. (19). Low density lipoprotein (LDL -c) according to Friedewald et al. (20). Lactate dehydrogenase (LDH) and Creatine kinase (KC) activities were estimated by standard kits (Spinreact) according to (21).
STATISTICAL ANALYSIS

All data obtained were performed with the SPSS software package for Windows (Version 15.0), differences between groups were assessed by one-way analysis of variance (ANOVA). The values are expressed as mean ± SE for five animals in each group. The significance was set at $P < 0.05$ level.

RESULTS

In continuation to our previous work about rhoifolin (11), concerning its chemistry, the heteronuclear Single Quantum Correlation (HSQC) was measured and illustrated in fig (1).

The current biological studies revealed that daily oral administration of mice with the various doses (12, 24, 30, 36 and 40 mg/kg b wt/day) of rhoifolin for seven consecutive days pre-irradiation (10 Gy) rendered protection to survival at different levels (23.3%, 40%, 46.6%, 77.3% and 60%) respectively. Based on these observations, the optimum effective dose of rhoifolin was found to be 36 mg/kg body weight (fig. 2).

A significant decrease in body weight was recorded in the animal group exposed only to whole body irradiation as a single fraction of 10 Gy. Moreover; a significant gain in animals’ weight was observed in experimental group (rhoifolin + Irradiation) compared to those only irradiated (Table 1).

No significant difference was observed neither in lipid peroxidation content as measured by the formation of MDA nor in NO level in blood and heart tissue in rhoifolin alone treated mice with respect to normal mice (G1) ($p < 0.05$). A highly significant increase ($p < 0.05$) in lipid peroxidation was observed in blood and heart tissue of animals exposed to 10 Gy of gamma irradiation (G3) as compared to the normal animals (G1). However, a significant decrease in NO level was observed in blood of irradiated mice ($p < 0.05$) concomitant with significant elevation of NO in heart tissue in the same group in comparison with the normal control. Unlike, rhoifolin pretreated irradiated animals showed a significant improvement in such parameters with respect to irradiated animals (table 2).

Table (3) demonstrates the antioxidant status in different treated groups, almost normal levels of GSH content, SOD and CAT activity were recorded in blood and heart homogenate in rhoifolin alone treated group. Meanwhile, a significant decrease was observed in GSH content, SOD and CAT ($p < 0.05$) activities as compared to irradiated group. On the contrary, the values of these parameters in rhoifolin treatment pre-irradiation recorded higher values ($p < 0.05$) compared to irradiated values.

In the present investigation, irradiated animals showed a significant decrease in LDH and CK activity ($p < 0.05$) in the heart associated to a significant increase ($p < 0.05$) in plasma compared to normal control values. These values appeared significantly higher ($P<0.05$) in rhoifolin treatment pre-irradiation (G6) than the irradiated ones. The present results also revealed a marked decline in total platelets count. In rhoifolin-treated pre-irradiated mice a significant improvement was observed in such parameters and the platelets count was increased ($P<0.05$) (table 4).

Table (5) shows a marked elevation in the level of total cholesterol (TC), total triglycerides (TG), and low density lipoproteins-cholesterol (LDL-C) in plasma of irradiated mice. Meanwhile, a significant decrease was observed in HDL-C level compared to that of the normal group. Administration of rhoifolin prior to irradiation exposure ameliorated the change in these fractions.
Fig. (1): HSQC NMR spectrum of rhoifolin

Fig. (2): Thirty days survival of mice and selection of the optimum tolerated dose of rhoifolin against lethal dose (10 Gy) irradiation.

Irradiated group received 10Gy gamma rays and experimental (Rf-pre-treated irradiated) groups were administered 12, 24, 30, 36 and 40 mg/kg then exposed to whole body gamma radiation at 10Gy and were checked daily for 30 days.

Table (1): Changes in body weight of mice post exposure to irradiation with rhoifolin (experimental) or without.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Times post- irradiation</th>
<th>% change (from normal untreated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hrs</td>
</tr>
<tr>
<td>Control</td>
<td>23.06±0.63</td>
<td>28.78±0.89</td>
</tr>
<tr>
<td>Rhoifolin (Rf.)</td>
<td>22.66±0.13</td>
<td>29.33±0.67</td>
</tr>
<tr>
<td>Irradiation (IRR)</td>
<td>22.05±0.16</td>
<td>21.51±0.45^a</td>
</tr>
<tr>
<td>Rf + IRR</td>
<td>22.41±0.38</td>
<td>26.43±0.94^a,b</td>
</tr>
</tbody>
</table>

n = 5 animals/group. Values are represented by mean ± SE. The significance was set at P < 0.05 level: a: Significant from control. b: Significant from irradiated.
Table (2): Malondialdehyde (MDA) and nitric oxide (NO) level in plasma and heart of mice post irradiation with rhoifolin or without.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>MDA</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (µmol/ml)</td>
<td>Heart (nmol/g)</td>
</tr>
<tr>
<td>Control</td>
<td>1.12±0.14</td>
<td>0.80±0.08</td>
</tr>
<tr>
<td>Rhoifolin (Rf.)</td>
<td>1.05±0.08</td>
<td>0.74±0.03</td>
</tr>
<tr>
<td>Irradiation (IRR)</td>
<td>1.91±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rf + IRR</td>
<td>1.20±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n = 5 animals/group. Values are represented by mean ± SE. $= %$ change from the normal values. # = % change from irradiated values. a: Significant from control at p< 0.05. b: Significant from irradiated at p< 0.05.

Table (3): Changes in antioxidant activities in blood, and heart of mice post irradiation with rhoifolin or without.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>GSH</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood mg/dl</td>
<td>Heart mg/g</td>
<td>Blood µg/mL</td>
</tr>
<tr>
<td>Control</td>
<td>26.31±1.52</td>
<td>14.37±0.59</td>
<td>12.98±0.87</td>
</tr>
<tr>
<td>Rhoifolin (Rf)</td>
<td>26.18±1.58</td>
<td>13.1±0.51</td>
<td>12.51±0.61</td>
</tr>
<tr>
<td>Irradiation (IRR)</td>
<td>20.05±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.93±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.77±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rf + IRR</td>
<td>23.90±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.49±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.77±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n = 5 animals/group. Values are represented by mean ± SE. $= %$ change from the normal values. # = % change from irradiated values. a: Significant from control at p< 0.05. b: Significant from irradiated at p< 0.05.
Table (4): Lactate dehydrogenase (LDH) and creatine kinase (CK) activities in plasma and heart tissue and platelets count of mice irradiation with rhoifolin (experimental) or without.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>LDH</th>
<th>CK</th>
<th>Platelets count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma IU/L</td>
<td>Heart IU/mg</td>
<td>Plasma ng/ml</td>
</tr>
<tr>
<td>Control</td>
<td>355±35</td>
<td>288±24</td>
<td>228±20</td>
</tr>
<tr>
<td>Rhoifolin (Rf.)</td>
<td>338±16</td>
<td>295±20</td>
<td>225±15</td>
</tr>
<tr>
<td>Irradiation (IRR)</td>
<td>568±23a 60%</td>
<td>193±14a -32%</td>
<td>333±5a 46%</td>
</tr>
<tr>
<td>Rf + IRR</td>
<td>430±22ab 18%</td>
<td>249±21ab -13%</td>
<td>259±8ab 13%</td>
</tr>
</tbody>
</table>

n = 5 animals/group. Values are represented by mean ± SE. = % change from the normal values. # = % change from irradiated values. a: Significant from control at p < 0.05. b: Significant from irradiated at p < 0.05.

Table (5): Plasma lipid profile of mice after exposure to 10 Gy gamma irradiation with Rhoifolin or without.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>T-Cholesterol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C mg/dl</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115.14±6.05</td>
<td>74.13±3.21</td>
<td>70.96±3.75</td>
<td>67.29±3.18</td>
</tr>
<tr>
<td>Rhoifolin (Rf.)</td>
<td>115.35±7.09</td>
<td>71.61±5.02</td>
<td>69.49±2.76</td>
<td>64.41±3.47</td>
</tr>
<tr>
<td>Irradiation (IRR)</td>
<td>250.65±8.46a 117.69%</td>
<td>158.56±3.81a 113.89%</td>
<td>33.12±3.30a -53.32%</td>
<td>104.04±2.04a 54.61%</td>
</tr>
<tr>
<td>Rf + IRR</td>
<td>191.34±6.78ab 66%</td>
<td>121.61±4.01ab 63%</td>
<td>50.48±2.78ab 62%</td>
<td>79.70±2.88ab 5%</td>
</tr>
</tbody>
</table>

n = 5 animals/group. Values are represented by mean ± SE. = % change from the normal values. # = % change from irradiated values. a: Significant from control at p < 0.05. b: Significant from irradiated at p < 0.0

DISCUSSION

Rhoifolin isolated from Chorisia cripiflora leaves. It has been investigated for its biological effects as anti-inflammatory and anticancer (10), (11). In continuation to this and in trying to build a new block in drug synthesis from natural products, this courage us to investigate if rhoifolin exhibited a radioprotective effect. Here we demonstrated its protective effect on gamma irradiation induced cardiac dysfunction on gamma irradiation induced cardiac dysfunctions in albino mice.

Scavenging of free radicals is known to offer protection against hazardous effects of radiation (22). There is a continued interest in and need for the identification and development of non-toxic and effective radioprotective compounds that can reduce the effect of radiation. In the current study, exposure of animals to 10Gy gamma rays resulted in radiation sickness weight loss and elevated mortality. The results corroborate the findings of Soyal et al. (23) and Fajardo et al. (24). The decrease in body weight post exposure to 10 Gy γ-irradiation observed in the current study is consistent with
the findings of Moccia et al.\(^{(25)}\) who reported that anorexia and weight loss are common side effects of exposure to ionizing radiation in rodents.

Administration of rhoifolin pre-exposure to irradiation has significantly ameliorated the decrease in body weight and reduced the mortality percent indicating the prophylactic action of rhoifolin against radiation-induced metabolic disorder, and reveal its role in protecting against acute whole body gamma-irradiation. The results are consistent with the findings of Andrade et al\(^{(26)}\) and Begum et al.\(^{(8)}\). The later explained the protective effect of apigenin against gamma rays induced weight loss in experimental animals and attributed this to its protective effect on intestinal epithelium, which allowed proper absorption of the nutrients\(^{(8)}\).

Blood biochemical analysis and investigations on heart tissues revealed a significant increase in MDA concentration in irradiated mice. The results corroborate the findings of Cheng et al\(^{(27)}\) who postulated that MDA is often seen as an indicator of the oxidation status in cells or tissues. Therefore, the high level of MDA is detrimental to cells and tissues, and leads to loss of their normal bio-function. Pre-administration of mice with rhoifolin significantly modulates the level of MDA in blood and heart. Our findings are in agreement with Begum et al.\(^{(8)}\) who reported that apigenin decreased LPO in the irradiated mice. Sinha et al.\(^{(28)}\) concluded that free radical scavengers may neutralize the effect of radiation induced free radicals, thus prevent lipid peroxidation. This could be due to the scavenging radical capacity of rhoifolin including both super-oxide and hydroxyl radicals.

Oxidative stress and reactive oxygen species generation leads to a reduction in NO activity and subsequent endothelial dysfunction. This imbalance is a known effect of established CVD risk factors\(^{(29)}\). In the present study a remarkable increase of NO was observed in heart homogenate concomitant with a significant decrease of NO in the blood of irradiated mice, indicating a high level of oxidative stress and free radicals generation. Similar observations were reported previously\(^{(30)}\). They attributed the increase of NO in cardiac tissues to lethal injury of irradiation. Other investigators\(^{(31)}\) attributed the decrease of NO in blood to a decrease in its synthesis resulting from a decrease in NO synthase expression.

GSH is a versatile protector and executes its radio protective function through free radical scavenging restoration of the damage molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the damage state. In the present study, the data displayed a significant reduction in heart and blood GSH following radiation exposure. This result is in accordance with others\(^{(32), (33)}\). They concluded that this reduction in GSH content could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Rhoifolin treatment pre-exposure of mice to gamma rays restored GSH content significantly in blood and cardiac tissue of mice which agree with the findings of Singh et al.\(^{(32)}\), they reported that flavones pre-treatment lower depletion of GSH in liver and blood of irradiated animals.

The endogenous free radical scavenging enzyme, SOD specifically dismutates superoxide radicals in tissues. It is commonly accepted that SOD protects against the free radical injury by converting superoxide anion (O\(^2-\)) to hydrogen peroxide (H\(_2\)O\(_2\)) and thus prevents the formation of hydroxyl radical (OH\(^-\)), and the H\(_2\)O\(_2\) can be removed by catalase. CAT is one of three families of primary antioxidant enzymes in mammalian cells which are critical to peroxide removal. In the current study, significant depletion in SOD and CAT activity in blood and heart homogenate was observed. This could be attributed to an increase in hydroxyl radical and hydrogen peroxide levels.\(^{(34)}\) The decrease in CAT activity could be due to oxidative inactivation of enzyme protein caused by ROS generation as mentioned by Ohta et al.\(^{(30)}\), or may be due to the increased utilization of this antioxidant to counteract lipid peroxidation production\(^{(35)}\).

Administration of rhoifolin to mice pre-irradiation restored GSH content and SOD and CAT activities and in blood as well as heart tissue to a significant extent suggesting that rhoifolin possess a potential antioxidative activity in mitigating oxidative stress resulting from irradiation of mice. Our
findings are consistent with those of Begum et al. (8) who reported that apigenin administration prior to irradiation inhibited the decline in the intracellular antioxidant enzyme.

Radiation is associated with an increase in oxidant free radicals and a decrease in antioxidants and resulting in increased oxidative stress which is followed by development of a variety of subcellular changes in the myocardium, typical of radiation induced cardiac injury (36). Concerning to this, our results demonstrated an alteration in levels of LDH and CK enzyme activities in heart tissue and serum in irradiated mice, indicating cardiac malfunction. Others reported the same trend of results (37), (38). Sridharan and Shyamaladevi (38) reported that LDH enzyme is released from damaged heart tissue into the blood stream due to alterations in dynamic permeability of cardiac cell membranes due to the excessive production of free radicals and lipid peroxides that caused cellular membrane damage and leakage of cytosolic enzymes.

Concerning the toxic effect of ionizing radiation on platelets count, a marked decrease in platelets counts was recorded compared to normal control. Our results are in agreement with those of other authors. (31), (39). They found that doses of ionizing radiation (1-10 Gy) in rodents poses a risk of damage to the hematopoietic system, leading to decreases in blood cells and platelet counts. The increase in blood platelets in rhoifolin pre treatment irradiated mice could be an indicative to its protective potential against gamma irradiation induced decrease in blood cells.

The oxidative stress and other errors of lipid and protein metabolism are involved in pathology of some diseases (40). It is well known that high plasma TC, TG and LDL-C levels are primary risk factors for vascular diseases, and high plasma level of HDL-C confers a protective effect against its development. The present study showed a significant increase in lipid fractions total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL-c) concomitant with a significant decrease in high density lipoprotein (HDL-C) level of irradiated mice compared to the normal values of the control group. These alterations in lipid fractions could be attributed to oxidative stress induced by ionizing radiation that might alter lipid metabolism and serum lipoproteins (40),(41). Other investigators (42) attributed the elevation in total cholesterol in irradiated mice to increased synthesis as an early reaction necessary for the restoration of biomembranes.

Rhoifolin treatment pre-irradiation of mice ameliorated the changes in plasma lipid fractions. The results suggest a hypolipidemic activity of rhoifolin. It is worthy to mention that flavonoids are potential antioxidants, (43) and due to their ability to scavenge ROS; they are capable of inhibiting the process of LDL-C oxidation and subsequently decreasing the risk of CVD (44).

The above results clearly point out that rhoifolin from Chorisia crispiflora has a potential activity to protect against radiation induced cardiac lesions and oxidative stress by stimulating the activity of antioxidant enzymes and scavenging free radicals directly or indirectly, and resulting in the protection against the radiation induced- biochemical disorders.

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