Rhubarb extract ameliorates some biochemical disorders induced by gamma irradiation in male rats

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ABSTRACT

The current study was conducted to evaluate the possible modulating efficacy of prolonged oral administration of rhubarb extract against gamma irradiation-induced some biochemical disorders in male rats. Rhubarb extract was orally administrated at a concentration of 100 mg/kg body wt daily for 21 days before irradiation at a single dose of 7 Gy and for 7 days post exposure. Transaminases (AST & ALT), urea, creatinine, alkaline phosphatase (ALP), lipid profile; cholesterol, triglycerides (T.G.) and low density lipoprotein (LDL) as well as serum glucose level were determined. Also, liver reduced glutathione (GSH) content and lipid peroxidation were estimated. Rats exposed to ionizing radiation revealed transaminases disorders, lipid abnormalities, elevation in serum glucose, ALP activity as well as liver TBARS. Whereas a sharp drop in glutathione was recorded. The obtained data showed that rats treated with rhubarb extract before and after whole body gamma irradiation exhibited a significant amelioration in liver and renal marker enzymes, serum glucose and lipids as well as noticeable improvement in liver glutathione contents. Rhubarb extract was also effective in minimizing lipid proxidation induced by irradiation. It could be concluded that rhubarb extract exerts a beneficial protective role against gamma irradiation.

Keywords, rhubarb, gamma rays, lipid peroxidation, antioxidants, rats.

INTRODUCTION

Ionizing radiation consists of energetic particles and electromagnetic radiation, which can penetrate living tissue or cells and result in transfer of the radiation energy to the biological materials. The absorbed energy of ionizing radiation can break chemical bonds and cause ionization of different atoms and molecules, including water and different biologically important molecules,
which can produce reactive oxygen species (ROS) such as superoxide anion radical (\( \cdot \text{O}_2^- \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), hydroxyl radical (\( \cdot \text{OH} \)) and nitrogen dioxide (\( \text{NO}_2 \)), through the decomposition of the cellular water\(^{(1)}\). These ROS play a critical role in cell damage by causing DNA strand breaks, lipid peroxidation, and protein modification and also by initiating a variety of cellular single transduction pathways\(^{(2)}\) which eventually result in physical and chemical damage to tissues if not scavenged\(^{(3)}\).

Many natural and synthetic compounds have been investigated for their efficacy to protect against irradiation damage\(^{(4)}\). Previous studies developed radioprotective and radiorecovering agents to protect from the indirect effects of radiation by eliminating free radicals produced in response to radiation\(^{(5,6)}\). Supplementary phytochemicals including polyphenols, flavonoids, sulfhydryl compounds, plant extracts and immunomodulators are antioxidants and radioprotective in experimental systems\(^{(7,8)}\).

Rhubarb (Da Huang), the root of the rheum plant species (polygonaceae) is one of the most frequently employed herbs in treating hyperlipidemia and hyperglycemia\(^{(9)}\). The major constituents of rhubarb rhizomes are anthraquinones. Rhubarb is used as a laxative, diuretic to treat kidney stones, gout and liver diseases characterized by jaundance\(^{(10)}\). The biological activity of rhubarb has been investigated in several purposes including antioxidant and anticancer potentiality\(^{(11-13)}\), free radical scavenging potential ability\(^{(14)}\), rinoprotective\(^{(15)}\), hepatoprotective\(^{(10)}\), it has antifungal properties\(^{(16)}\), DNA protection ability\(^{(17)}\), anti-inflammatory\(^{(18)}\), anti-lipid peroxidation capacity\(^{(19)}\).

The present work was devoted to investigate the antioxidant effect of rhubarb extract and its hepatic- and rinoprotective effects in rats exposed to gamma radiation.

**MATERIAL AND METHODS**

*Experimental animals*

Male albino rats (Sprague Dawely Strain) weighing 120 ± 15 g were obtained from the Egyptian holding company for Biological Products and Vaccines. Animals were kept under good ventilation and illumination condition and allowed balanced standard diet and free access to tap water.

*Radiation Processing*

Whole body \( \gamma \)-irradiation of rats was performed using 137Cesium
biological irradiator source, Canadian γ-Cell-40, located at the NCRRT, Egypt. Animals were exposed to a single dose of whole body gamma-rays at a dose level of 7 Gy at dose rate 0.79 rad/sec.

Preparation of aqueous extract

Five grams of rhubarb was immersed in 200mL of distilled water. The materials were boiled until half of the original water remained, and then the extract was filtered before using.

Experimental design

After adaptation period of one week, animals were divided into four groups, each of eight rats, Group 1, control, non-irradiated rats. Group 2, rats received rhubarb extract 100 mg /kg body wt daily for 28 successive days. Group 3, rats were exposed to whole body 7 Gy gamma-irradiation as a single dose. Group 4, rats received treatment (rhubarb extract) as group 2 for 21 days, then exposed to gamma-radiation 7 Gy, followed by treatment with rhubarb extract for 7 days after irradiation. Rats were sacrificed 7 days post γ-irradiation.

Biochemical analysis:

Serum AST & ALT were determined according to Reitman and Frankel, serum alkaline phosphatase (ALP) was determined according to King and Armstrong, serum cholesterol, T.G. and LDL were measured according to Allian et al., Fossati and Principe and Demacker et al., respectively, and serum glucose was determined according to the method of Trinder. The lipid peroxidation products were estimated in the liver as TBARS according to Yoshioka et al. and GSH content was estimated according to Tietz. The biochemical assay was achieved using Helios γ UV/VIS Spectrophotometers. Trace elements (Fe, Cu, Zn, Mn, Ca, Mg & Se) were measured by using Atomic Absorption Spectrometer, SOLAR System Unicam 939, England, after digestion of plant roots in pure nitric acid and H2O2 (4:1) by microwave sample preparation Lab Station, MLS-1200 MEGA, Italy.

Statistical analysis

Student's t-test was applied for the statistical analysis of collected data to determine the probable level of significance. The differences were considered significant at P< 0.05.
RESULTS

A) Effect of radiation (7 Gy) and rhubarb extract (100 mg/kg body wt.) on serum hepatic and renal enzymes (Table 1):

Compared with control, rhubarb extract has no significant effect on hepatic and renal enzymes levels. There is a significant increase (p≤0.05) in these parameters after 7 days of radiation exposure or in irradiated treated group. The percentage change from control after irradiation were for ALT: 31.3, for AST: 82, for ALP: 57, for urea: 19 and for creatinine: 56%, while in case of irradiated treated group were for ALT: 6.3, for AST: 7.2, for ALP: 11, for urea: 7.8 and for creatinine: 14%.

Compared with irradiated group, irradiated treated group exhibited a significant decrease (p≤0.05) in ALT, AST, ALP levels. Urea and creatinine concentration declined towards the control levels.

Table 1: Effect of rhubarb extract on hepatic and renal enzymes activity in different animal groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT U/ml</th>
<th>AST U/ml</th>
<th>ALP U/ml</th>
<th>Urea mg/dl</th>
<th>Creatinin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.63±1.28</td>
<td>41.40±1.40</td>
<td>159±1.80</td>
<td>34.00±1.60</td>
<td>0.64±0.01</td>
</tr>
<tr>
<td>Rhubarb ex. %</td>
<td>31.75±1.40</td>
<td>40.63±1.50</td>
<td>157±1.50</td>
<td>32.50±1.14</td>
<td>0.63±0.01</td>
</tr>
<tr>
<td>% change</td>
<td>+0.4</td>
<td>-1.8</td>
<td>-0.31</td>
<td>-2.0</td>
<td>-0.6</td>
</tr>
<tr>
<td>Radiation %</td>
<td>42.00±1.53*</td>
<td>75.40±1.60*</td>
<td>249±3.2*</td>
<td>53.00±1.70*</td>
<td>1.06±0.04*</td>
</tr>
<tr>
<td>% change</td>
<td>+31.3</td>
<td>+82</td>
<td>+57</td>
<td>+19</td>
<td>+56</td>
</tr>
<tr>
<td>Rad+Rhub ex. %</td>
<td>33.80±1.25#</td>
<td>44.90±2.00#</td>
<td>176±2.50#</td>
<td>40.00±1.70*#</td>
<td>0.73±0.03*#</td>
</tr>
<tr>
<td>% change</td>
<td>+6.3</td>
<td>+7.2</td>
<td>+11</td>
<td>+7.8</td>
<td>+14</td>
</tr>
</tbody>
</table>

* The % change from control.
* Each value represents the mean ± SE of 8 observations.
* Significant difference compared to the values of control rats.
* Significant difference compared to the values of irradiated rats.

B) Effect of radiation (7 Gy) and rhubarb extract (100 mg/kg body wt.) on serum lipid metabolites (Table 2):

Compared to control, there was non-significant effect of rhubarb extract group on cholesterol, T.G. and LDL levels but irradiation and irradiated treated group resulted in a significant increase in cholesterol, T.G. and LDL levels from control. The percentage change from control after irradiation were for cholesterol: 83, for T.G: 170 and for LDL: 40%, while in case of irradiated treated group were for cholesterol: 23, for T.G: 51 and for LDL: 14%.

Compared to irradiated group, irradiated treated group exhibited a
significant decrease in cholesterol, T.G. and LDL levels.

Table (2): Effect of rhubarb extract on serum lipid metabolites in different animal groups:-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol mg/dl</th>
<th>T.G. mg/dl</th>
<th>LDL mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.00 ± 1.32</td>
<td>40.40 ± 1.10</td>
<td>30.00 ± 1.15</td>
</tr>
<tr>
<td>Rhubarb ex. % change</td>
<td>48.00 ± 1.82</td>
<td>42.00 ± 1.40</td>
<td>29.40 ± 1.20</td>
</tr>
<tr>
<td>Radiation % change</td>
<td>96.40 ± 2.53*</td>
<td>111 ± 2.10*</td>
<td>41.50 ± 1.32*</td>
</tr>
<tr>
<td>Rad + Rhub ex. % change</td>
<td>65.00 ± 1.53*#</td>
<td>61.00 ± 2.15*#</td>
<td>34.00 ± 0.98*#</td>
</tr>
</tbody>
</table>

Legends are as in Table (1).

C) Effect of radiation (7 Gy) and rhubarb extract (100 mg/kg body wt) on serum glucose levels, liver GSH and liver MDA (Table 3):-

Compared to control, there was non-significant effect of rhubarb extract group on serum glucose levels, liver GSH and liver MDA levels but in irradiated and irradiated treated group results revealed a significant decrease in GSH levels (-40% & -17.6% respectively) as well as a significant increase in TBARS levels (52% & 11% respectively) and serum glucose levels (59% & 9.9% respectively) in comparison with control values.

Compared to irradiated group, irradiated treated group exhibited a significant increase in GSH and a significant decrease in glucose and TBARS levels.

Table (3): Effect of rhubarb extract on serum glucose levels, liver GSH and liver MDA in different animal groups:-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose mg/dl</th>
<th>GSH mg/g</th>
<th>TBARS nmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 ± 2.12</td>
<td>33.80 ± 1.40</td>
<td>167 ± 1.65</td>
</tr>
<tr>
<td>Rhubarb ex. % change</td>
<td>98.00 ± 2.60</td>
<td>32.9 ± 1.32</td>
<td>169 ± 1.81</td>
</tr>
<tr>
<td>Radiation % change</td>
<td>162 ± 3.03*</td>
<td>20.13 ± 0.79*</td>
<td>255 ± 3.54*</td>
</tr>
<tr>
<td>Rad + Rhub ex. % change</td>
<td>112 ± 3.10*#</td>
<td>27.90 ± 0.96*#</td>
<td>185 ± 2.30*#</td>
</tr>
</tbody>
</table>

Legends are as in Table (1).
Table (4): concentration levels of some essential trace elements in rhubarb plant (mg/g):

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration</th>
<th>Element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>8.500 ± 0.83</td>
<td>Ca</td>
<td>2228 ± 19.6</td>
</tr>
<tr>
<td>Cu</td>
<td>4.580 ± 0.29</td>
<td>Mg</td>
<td>1029 ± 26.0</td>
</tr>
<tr>
<td>Zn</td>
<td>38.48 ± 3.63</td>
<td>Se</td>
<td>11.75 ± 1.58</td>
</tr>
<tr>
<td>Mn</td>
<td>14.49 ± 0.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each value represents the mean ± SD of 4 samples.

Considerable amounts of essential trace elements were identified in rhubarb plant roots.

**DISCUSSION**

In the present study, whole body gamma exposure of rats to 7 Gy single shot has induced a significant elevation in hepatic and renal enzymes, lipid profile, LPOX in addition to blood glucose level, while noticeable decline in hepatic glutathione content was recorded. Administration of rhubarb extract to rats for 21 days before irradiation and 7 days post exposure has significantly minimized the severity of radiation induced biochemical changes.

In the current study, it was noticed that ionizing radiation induced a significant increase in serum ALT, AST and ALP, indicating liver damage. The change in the tissue permeability due to irradiation could enhance the release of transaminases enzymes from their subcellular sites of production to extracellular process and consequently to blood circulation\(^{(31)}\). Radiation exposure caused damage to the cell membrane that increased the ALP activity. This change in ALP activity might be due to radiation induced changes in the amino acid residue and catalytic activity of ALP\(^{(32)}\) and due to destruction of this enzyme by radiation\(^{(33)}\). Furthermore, liver responds to hepatobiliary disease or injury by synthetizing more enzymes which inter the circulation, raising the enzyme level in serum\(^{(34)}\). Treatment with rhubarb extract pre- and post-irradiation significantly decreased these enzyme levels. The ability of rhubarb extract to reduce the injurious effects or to preserve normal hepatic function disturbed by γ-irradiation might attribute to the presence of antioxidants in rhubarb which contain phenolic compounds. Ngoc et al\(^{(35)}\) identified several antioxidant components in the roots of rhubarb particularly anthraquinones which includes: chrysophenol, physcion emodin, glucopyranesoid, stilbense, desoxyrhaponticin, rhaponticin, resveratrol, rhapontigenin, glucopyranoside and ampelopsin B. These components might contribute to the prophylactic and curative efficacy of rhubarb in maintaining the integrity and functional status of hepatocytes.
Rhubarb extract has been reported to possess potent antioxidant and hepatoprotective activities\(^{(10)}\).

The current study revealed an elevation in urea and creatinine levels in response to whole body $\gamma$-irradiation. According to Konnova et al\(^{(36)}\) and Yildiz et al\(^{(37)}\), the elevation of creatinine post irradiation might be due to the back leakage of the filtered creatinine, which may occur through the damaged tubular epithelium along the concentration gradient established by salt and water reabsorption, while the elevation of serum urea occurred via the increased oxidative deamination of amino acids in liver. The impaired detoxification function of the liver caused by irradiation could also contribute to the increase of blood urea\(^{(38)}\). Accumulating evidences suggested that carbamoyl phosphate synthetase which initiate the controlling step in urea biosynthesis act in conjunction with mitochondrial glutamate dehydrogenase to channel nitrogen from glutamate into carbamoyl phosphate and thus into urea. The activity of glutamate dehydrogenase was shown to increase after radiation exposure\(^{(39)}\), the oral administration of rhubarb extract to rats clarified that serum concentration of urea and creatinine were significantly decreased. The decrease in creatinine might cause a decrease in urinary protein extraction, attenuation of lipid derangements, decreased oxygen consumption and the hypertrophy of the kidney\(^{(40)}\). Amin and Nagy\(^{(41)}\) concluded that rhubarb extract retains the balance between lipogenesis and lipolysis in the kidney to counteract the hyperlipidemia associated renal damage in addition to maintaining cellular hydration due to laxative effect of senna, which is associated with diuretic effect of rhubarb. Also the tannins ingredients in rhubarb might have uremic-toxin decreasing action via improved blood urea nitrogen, creatinine, glumular filtration rate and renal blood flow\(^{(42)}\). Rhubarb has proven effective as a diuretic models by blocking Na/K ATPase in renal medulla\(^{(15)}\), leading to improvement of kidney function.

Significant increase was observed in serum cholesterol, T.G. and LDL of irradiated rats. Markevich and Kolomitseva\(^{(43)}\) and Baker et al\(^{(44)}\) reported that stimulation of cholesterol synthesis in the liver after $\gamma$-irradiation leads to hyperlipidemic condition. Also the increase in the activation of $\beta$-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase, the key regulatory enzyme of reaction process of cholesterol biosynthesis, results in reduction of lipoprotein catabolism\(^{(45)}\). While Chaialo et al\(^{(46)}\) and Feurgard et al\(^{(47)}\) suggested that the degeneration effect on hepatic cell and biomembranes led to acceleration in lipid metabolism after irradiation resulting in releasing of structural phospholipids. Also the increase in serum triglycerides level after irradiation
might result from inhibition of lipoprotein lipase activity, leading to reduction in the uptake of triacylglycerols\(^{48}\) in addition to decreased fatty acid oxidation\(^{49}\). Anthraquinone glycosides from rhubarb have lipid-lowering effects, resulting in depression of lipid accumulation\(^{42}\). It consequently has anti-atherosclerotic properties\(^{18}\). Dietary products rich in flavonoids and polyphenols have been reported to correlate with lower plasma triglyceride concentrations and plasma LDL cholesterol\(^{50}\). Tseng et al\(^{20}\) identified the existence of significant amounts of polyphenols and flavonoids, these compounds may contribute to the hypolipidemic as well as hypoglycemic effect of rhubarb.

There was a significant increase in the serum glucose level due to radiation exposure. The hyperglycemia condition induced by irradiation could be attributed to the inhibition of insulin secretion, diminished utilization of glucose by irradiated tissue or to increased blood amino acids level which are considered as an important source for glucose formation through the processes of deamination and transamination\(^{51}\). Oral administration of rhubarb extract is associated with hypoglycemia, as it promotes insulin sensitivity, thus lowering insulin resistance in irradiated rats by regulating the cell energy metabolism or reducing free radical fatty acids\(^{52}\). The hypoglycemic action of rhubarb extract might be attributed to the emodin which is a derivative of anthraquinone. It may help in preservation of \(\beta\)-cell integrity of the pancreatic islets, causing a significant increase in insulin secretion. It also sensitized hepatocytes to the action of insulin, hence decreasing oxidative stress and preserving pancreatic \(\beta\)-cell integrity. Gong et al\(^{53}\) has proved that emodin in rhubarb is a potent agent in the management of clinical and experimental acute pancreatic diseases. Wu et al\(^{54}\) proved the hypoglycemic mechanisms of emodin that included modulation of abnormal eicosanoid metabolism, promotion of pancreatic cytoprotection and improvement of microcirculation.

The present data revealed significant acceleration in lipid peroxidation, associated with depletion in GSH content after radiation exposure. This increase in LPOX is attributed to the peroxidation of the membrane unsaturated fatty acids due to free radical propagation concomitant with the inhibition in bio-oxidase activities\(^{55}\). Excessive lipid peroxidation can cause increased glutathione consumption\(^{56}\). GSH is the most abundant non-protein sulphhydryl-containing compound and constitute the largest components of the endogenous thiol buffer\(^{57}\). The depletion in GSH post gamma exposure may be due to the inactivation of glutathione reductase and peroxidase activities with subsequent production of GSSG\(^{58}\), also the deficiency of NADPH which is necessary to
change oxidized glutathione to its reduced form\(^{(59)}\), or due to its utilization by the enhanced production of ROS\(^{(60)}\). On the other hand, treatment with rhubarb extract significant by modulated the increase in LPOX and the decrease in GSH. The literature attributed the efficacy of rhubarb to its rich flavonoids polyphenols in reducing LPOX and enhancement of antioxidants\(^{(11,12,19)}\). Also considerable amounts of essential trace elements were identified in rhubarb. These metalloelements are involved in multiple biological processes as constituents of enzyme system. These metals increased the antioxidants capacities and the induction of metalloelements dependent enzymes (Cu-, Zn- and Mn-SOD, CAT, metallothioniens, GSST, GSPX, GSH and several selenoproteins). These enzymes play an important role in preventing the accumulation of pathological concentration of oxygen radicals or in repairing damaged caused by irradiation injury\(^{(61)}\). The ability of the extract to quench hydroxyl radicals might be attributed to the prevention of ROS from damaging biomolecules such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars in biological system\(^{(17)}\). In conclusion, the results suggest that rhubarb extract might exert beneficial effects in the management of hypolipidemia, hypoglycemia and radio-protectivity, further mechanistic and clinical studies are needed to confirm the clinical usefulness of rhubarb.

REFERENCES


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التأثير التحسيني لمستخلص جذور نبات الراوند لبعض الاختلالات البيوكيميائية
التي يحدثها الإشعاع في ذكور الجرذان

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قسم البحوث الدودانية - المركز القومي لبحوث وتقنية الإشعاع - هيئة الطاقة الذرية.

تهدف هذه الدراسة إلى تقييم الدور الوقائي لمستخلص جذور نبات الراوند ضد بعض التغيرات البيوكيميائية التي تحدث نتيجة التعرض الإشعاعي لذكور الجرذان. وقد تم تجريع الجرذان بجرعة مقدارها 100 ملليجرام/كيلوجرام من وزن الفأر في اليوم لمدة 21 يوم قبل التعرض للإشعاع ثم 7 أيام بعد التعرض بجرعة مقدارها 7 جرائ. وقد تم ذبح الفئران بعد 7 أيام من التعرض للإشعاع. وقد تم قياس مستوى إنزيمات الناقل الأميني (AST, ALT, ALP)، إنزيم الفوسفاتي القلوى والكلي، والكレビتين وكذلك مستوى الدهون (الكوليسترول والدهون الثلاثية و الكوليسترول منخفض الكثافة) والجلوكوز في مصل الدم، بينما تم قياس إنزيم الجلوتاتيون المختزل (GSH) والتغيرات التي تحدث في نوائح الأکسدة الفوقية للدهون (المواد المتفاعلة مع حمض الثيوريتول) في نسيج الكبد.

وتضمن النتائج أن الجرذان التي تعرضت للإشعاع قد أظهرت إرتفاعاً ملحوظاً في المعيار البيوكيميائي الذي تمت دراستها جداً الجلوتاتيون الذي ارتبط بانخفاضاً ملحوظاً. كما تشير النتائج أيضاً أن الجرذان المجريع بمستخلص جذور نبات الراوند قبل وبعد التعرض الإشعاعي قد أظهرت تحسناً ملحوظاً في الحد من الاختلالات البيوكيميائية التي حدثت نتيجة التعرض الإشعاعي.

وبناءً على ما سبق يمكن إفراض أن مستخلص جذور نبات الراوند له القدرة على الحد من عمليات الفوق تأكسد للدهون، والمحافظة على التركيز الطبيعي للجلوتاتيون المختزل. هذا بالإضافة إلى ضبط بعض الإزيمات الكبدية والكلوية وخفض مستوي الدهون والجلوكوز في الدم وبالتالي له قدرة وقائية على الحد من الضرر الناتج عن التعرض للإشعاع الجامس.