Protective Effect of Hawthorn (Crataegus Linn) against Radiation-Induced Damage in Rats.

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ABSTRACT

Crataegus Linn., commonly known as Hawthorn, is one of the most widely used herbal heart tonic. The objective of this work is to investigate the radioprotective and antioxidant effect of hawthorn (H) extract against γ-irradiation induced biochemical disorders in rats. Twenty four animals were randomly divided into equal four groups as follows: Group 1: control group rats Group 2: irradiated rats whole body exposed to 7Gy gamma-rays, Group 3: treated, rats in this group received freshly prepared Hawthorn (H) at dose (10mg/kg body wt/ day) by gavages for 28 consecutive days. Group4: rats received freshly Hawthorn for 7 consecutive days then exposed to 7Gy whole body gamma irradiation and treated with Hawthorn for 21 consecutive days after irradiation. Exposure to γ- irradiation induced a significant increase of aminotransferases (AST, ALT), and alkaline phosphatase (ALP) activites and total cholesterol (TC), triglycerides (TG) and Low density lipoprotein cholesterol (LDL-C) contents. While, High density lipoprotein-cholesterol (HDL-C) content showed a decrease. Metabolic disorders were associated to significant increases in serum and liver thiobarbituric acid reactive substances (TBARS) and protein carbonyl content (PCC) and marked reduction in glutathione (GSH) content and Catalase (CAT) and Superoxide dismutase (SOD) activities in blood and liver compared with controls. Administration of Hawthorn prior and after radiation exposure was found to offer protection against γ-irradiation induced oxidative stress in rats. Accordingly, it could be concluded that consumption of Hawthorn could modulate the oxidative stress caused by radiation exposure and that due to its antioxidant activity.

Keywords: Hawthorn, liver, blood, γ-rays, rats.

INTRODUCTION

The inevitable exposure of the increasing numbers of workers in the radiation field necessitates further studies to evaluate functional and physiological disorders evoked by unanticipated irradiation exposure on some physiological parameters and suggest specific preventive care measurements [1]. Ionizing radiation passing through living tissues generates free radicals that can induce DNA damage, cell death and are associated with an increased risk for numerous diseases. Reactive oxygen species (ROS) can cause cellular damages leading to a number of pathological situations and dysfunction of cells and tissues [2].

Flavonoids are a family of polyphenolic compounds found ubiquitously in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, [3] As evidenced in the most recent literature, flavonoids have been reviewed for their wide biological properties, including hepatoprotective, antithrombotic, antibacterial, antiviral, anticancer and immunostimulant activities [4].
*Crataegus* has acquired a prominent status in modern herbal literature as an important cardiac tonic. Its common name is Hawthorn, extract of both flowers and berries have been recommended to treat cardiac failure, atherosclerosis, hyperlipidemia, hypertension, angina and a variety of geriatric conditions[3]. *Crataegus* Plant contains mixtures of chlorogenic acid and flavonoids such as querin hyperoside, vitexin and vitexin rhamnoside. Part of mechanism for anti-hyperlipidemic effects of hawthorn fruit might involve the direct protection to human LDL from oxidation [5]. Since there is a direct relation between the lipid profile and the coronary heart disease (CHD), the consumption of flavonoids could lower the risk of (CHD) via a number of mechanisms. Firstly: the antioxidant capacity of flavonoids may improve endothelial function by lowering oxidative stress. Better endothelial function impacts on vasomotor tone, platelet activity, leukocyte adhesion and vascular smooth muscle cell function [6]. Human studies have shown that Hawthorn flavonoids improve coronary circulation and attenuate endothelial dysfunction [7], although the latter may be influenced by individual variation in flavonoid metabolism [8]. Secondly: Hawthorn flavonoids have also been shown to reduce LDL cholesterol [9]. In addition Hawthorn decreased the oxidation of LDL-C [10]. Previous investigations studied the beneficial effects of that hawthorn in decreasing serum TC, LDL-cholesterol and TG in hyperlipidimic humans [11], [12], [13]. Thirdly: *in vitro* and animal studies have revealed effects that go beyond antioxidant capacity, for example, reduced expression of endothelial adhesion molecules [14], stimulation of an anti-inflammatory response [15], and gene expression favoring improved smooth muscle function [16].

As oxidation is part of normal biological reactions, over loading the cells with free radicals could initiate the pathogenesis of many diseases [17]. Therefore, the aim of the present investigation is to modulate the results against oxidative stress developed in rat model by exposure to γ-rays by effectiveness of hawthorn.

**MATERIAL AND METHODS**

**Animals**

Male Wistar rats (130-150 g) purchased from the Egyptian Organization for Biological Products and Vaccines, were used as experimental animals. Animals were maintained under 12 h light/ dark cycle at a constant temperature of 25 °C with free access to standard rat pellet food and tap water and maintained under good ventilation and humidity conditions.

**Plant Material**

100 g of good quality well dried Hawthorn were purchased from a local market of Herbs and Medicinal plants, Cairo, Egypt. Water soluble extract was prepared as previously described [18]. Briefly, the powder (100 g) was stirred in one liter distilled water for 20 minutes at 80 °C followed by rapid filtration through a crude cheese cloth and then Wattman No. 1 filter paper. The resultant filtrate was lyophilized and then stored at -20°C in desiccants until used. The average (w/w) yield was 11.5%.

**Radiation process**

Whole-body irradiation was performed using a Canadian gamma cell-40, (137Cs), at the NCRRT, Cairo, Egypt. Rats were exposed to a single dose of 7 Gy at the dose rate 0.58 Gy/ min.
Experimental design

The animals selected for this experiment were divided into four groups each of six rats. Group 1 (control). Group 2 (irradiated rats): Rats were exposed to whole-body γ -irradiation (7 Gy) . Group 3 (Hawthorn treated animals): Animals were administered hawthorn by gavages (10 mg/kg body wt) for 4 weeks. Group 4 (H+ I+ H.): Animals received Hawthorn as in group3 for seven consecutive days before whole-body irradiation and Hawthorn treatment was continued for other 21 days after irradiation.

At the end of the experiment,(28 days ) animals were sacrificed following anaesthesia with ether. Blood samples were collected by heart puncture in heparinized centrifuge tubes . The liver was removed immediately by dissection, washed in ice-cold isotonic saline and stored. A 10 % (w/ v), liver homogenates were prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5 using Branson sonifier (250, VWR Scientific, USA)

Biochemical analysis

Stored serum samples were analyzed for liver indices represented by the activities of ALT, AST using the method of Reitman and Frankel [19]. Serum activity of ALP was estimated according to Belfield and Goldberg [20]. Serum TC, TG, HDL-C were determined according to Allairi et al [21], Fossati and Prencipe [22] and Demacker et al [23], respectively. The content of serum LDL-C was calculated according to Friedwald and Fredrickson [24]. TBARS were measured in serum and Liver homogenates by using the method of Yoshioka et al [25]. GSH was determined in blood and liver according to Beutler et al [26]. CAT activity was estimated in blood and liver by the method of Johansson and Borg [27]. SOD was assayed utilizing in blood and liver by the method of Kakkar et al [28]. PCC was determined in serum and liver to evaluate protein oxidation as described by Reznick and Rarker [29].In all enzymatic determinants the proteins were evaluated according to Lowry et al [30]and blood Hb content was determined colorimetrically as cyanmethaemoglobin following the method described by Tietz [31] using Spectrum Diagnostic Kit.

Statistical analysis

The data were analyzed using one way of ANOVA for comparison between the groups. The values are expressed as mean± SE. Significance level was computed at p < 0.05.

RESULTS

The results presented in Table (1) showed that γ -irradiation leads to significant (P<0.05) increase in the serum levels of AST, ALT and ALP compared to normal control rats. The administration of Hawthorn for seven days before whole body γ - irradiation and twenty one days post irradiation maintained the activities of these enzymes close to their normal levels as compared to control group (Table 1).
Table 1. Effect of Hawthorn administration for 7 days pre-irradiation (7 Gy) and 21 days post-irradiation on the activities of serum AST, ALT and ALP enzymes of male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.8±1.9</td>
<td>43.0±1.2</td>
<td>19.4±0.21</td>
</tr>
<tr>
<td>Irradiation</td>
<td>101.8±2.4</td>
<td>57.2±1.4</td>
<td>23.9±0.37</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>80.2±2.1</td>
<td>43.4±1.3</td>
<td>19.7±0.39</td>
</tr>
<tr>
<td>H+ Ir.+H</td>
<td>88.5±2.4</td>
<td>47.1±1.9</td>
<td>20.9±0.55</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E for six rats in each group. Data are significant at P< 0.05. a: significantly different compared to control, b: significantly different compared to irradiated group. c: significantly different compared to H. group.

In the present study the level of TBARS and PCC in serum and hepatic tissues were significantly (P<0.05) increased, while the activities of CAT and SOD and the content of GSH were significantly (P<0.05) decreased in irradiated rats group, when compared to control. The TBARS levels and the content of PCC in serum and hepatic tissues showed a significant (P<0.05) decrease in rats treated with Hawthorn prior and post whole body γ - irradiation, when compared to irradiated group. In addition, significant (P<0.05) increases in the GSH content, CAT and SOD activities were observed in rats administrated Hawthorn prior and post irradiation as compared to irradiated rats (Tables2&3).

Table 2. Effect of Hawthorn administration for 7 days Pre irradiation (7 Gy) and 21 days Post-irradiation on the TBARS and PCC in serum and liver.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS</th>
<th>PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum (n)</td>
<td>Liver (n mol/g fresh)</td>
</tr>
<tr>
<td>Control</td>
<td>64.3±1.8</td>
<td>190.9±1.2</td>
</tr>
<tr>
<td>Irradiation</td>
<td>108.9±1.6</td>
<td>345.9±2.4</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>62.1±1.2</td>
<td>192.1±1.7</td>
</tr>
<tr>
<td>H+ Ir.+H</td>
<td>81.9±1.4</td>
<td>225.9±2.3</td>
</tr>
</tbody>
</table>

Legend as in Table 1.

Table 3. Effect of Hawthorn administration for 7 days pre-irradiation (7 Gy) and 21 days post-irradiation on the Catalase (CAT), Superoxide dismutase (SOD) Activities and Glutathione content (GSH) in blood and liver.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (U/g)</th>
<th>Blood (mg)</th>
<th>Liver (mg/g fresh)</th>
<th>Blood (mg)</th>
<th>Liver (mg/g fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.4±1.3</td>
<td>14.11±0.3</td>
<td>51.31±1</td>
<td>23.32±1.9</td>
<td>43.40±1.8</td>
</tr>
<tr>
<td>Irradiation</td>
<td>39.50±1.2</td>
<td>12.90±0.7</td>
<td>32.50±2.3</td>
<td>12.66±2.7</td>
<td>24.50±0.7</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>35.30±0.7</td>
<td>13.90±0.17</td>
<td>52.90±1.9</td>
<td>22.8±1.8</td>
<td>39.90±1.8</td>
</tr>
<tr>
<td>H</td>
<td>32.5±1.6</td>
<td>13.14±0.1</td>
<td>40.90±1.8</td>
<td>19.81±1.9</td>
<td>34.90±1.8</td>
</tr>
</tbody>
</table>

Legend as in Table 1.

As shown in (Table 4), γ - irradiation significantly altered serum lipid profile. The levels of total cholesterol (TC), triglycerides(TG) and LDL-C were significantly (P<0.05) increased and HDL-C was significantly decreased in the irradiated rats group when compared to control group. The prolonged administration of Hawthorn prior and post exposure to single shot dose body γ - irradiation significantly (P<0.05) improved the alteration in the lipid profile when compared to the irradiated rats.
Table 4: Effect of Hawthorn administration for 7 days pre-irradiation (7Gy) and 21 days post-irradiation on serum lipid profile in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL.C (mg/dl)</th>
<th>HDL.C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.21±1.77</td>
<td>54.3±1.32</td>
<td>36.71±0.92</td>
<td>38±5.78</td>
</tr>
<tr>
<td>Irradiation</td>
<td>95.58±2.36</td>
<td>84.24±1.96</td>
<td>45.93±0.85</td>
<td>33±2.28</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>63.24±1.95</td>
<td>56.83±1.95</td>
<td>38.13±0.79</td>
<td>37±3.93</td>
</tr>
<tr>
<td>H + Ir. +H</td>
<td>84.52±1.39</td>
<td>58.16±1.19</td>
<td>42.1±0.93</td>
<td>36±2.29</td>
</tr>
</tbody>
</table>

Legend as table (1)

DISCUSSION

Radiation is one of the most widespread sources of environmental stress in living environment. Ionizing radiation is known to induce various physiological, and biochemical changes in humans and animals. Several molecular mechanisms of ionizing-radiation have been proposed, including cumulative damage by ROS, dislocation in replicative cells, genome instability, mutation or altered expression of specific enzymes and cell death [32]. Oxidative stress refers to the cytotoxic consequence of oxygen free radicals: superoxide anions, hydroxyl radicals and hydrogen peroxide, which are generated as by-products of normal and abnormal metabolic processes induced by irradiation and may lead to DNA damage and mutagenesis, protein and carbohydrate oxidation and metabolic disorders [33].

The oxidative stress due to free radical-formation was greatly augmented during ionizing-radiation exposure [34]. Coping with irradiation stressing conditions required a complex adjustment of the physiological and biochemical metabolic pathways to ensure survival by minimizing intracellular damage. It was likely that animal particular antioxidants generally decrease the level of oxidation in such systems by transferring hydrogen atoms to the free radical structure [35]. Gamma irradiation showed an increase in the level of serum AST, ALT and ALP activities indicative to the toxicity induced by radiation exposure (Table1). These results are in agreement with those previously reported by Franken, et al [36]. The increase in serum levels of these enzymes may be due to alteration in the dynamic permeability of membranes by ionizing radiation, allowing leakage of biological active material out of the injured cell, which may be associated with cell death or injuries [37]. The peroxidative products caused the cell membrane to become leaky with the consequent release of these enzymes into the serum. This agrees with the work of Masayuki, et al. [38], who reported that lipid peroxidation is recognized to be a major factor in the liver injury. The decreased level of AST and ALT observed after consumption of the hawthorn which indicate the release of these enzymes had been inhibited. Probably, a chemical component in the hawthorn is stabilizing the integrity of the cell membrane, keeping the membrane intact and the enzymes enclosed [39].

The present study revealed an increase in serum ALP activity after irradiation. A similar increase in the activity of alkaline phosphatase after irradiation has also been reported at sub lethal doses as reported by others [40], [41]. In the current study, the discharge of enzymes from lysosomes may be due to activation of preexisting latent enzymes or due to synthesis of new lysosomes as a consequence of irradiation [42]. It is already known that radiation enhances the permeability of membranes of several cellular organelles, and hence increase in serum alkaline phosphatase activity [43]. It was observed that Hawthorn shows radioprotection in rats against lethal dose of gamma
radiation. The results of a work by Luo, et al., [44] demonstrating the liver-protecting activity of melanin-like pigment derived from Hawthorn are in line with our findings.

In the present study there was a direct damage to tissues by ionizing radiation yields ROS, which may be diminished by a number of antioxidant defenses [45]. These anti-oxidant systems are widely distributed in cells, underlying their importance in preventing the damaging effects of ROS in γ-irradiated tissues [46]. It could be observed that exposure to γ-irradiation decreased the activities of CAT and SOD in serum and hepatic tissues (Table 3).

CAT is the most potent catalysis known conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. This decrease is thought to occur as a result of accumulation of hydrogen peroxide, which is a product of peroxidation in tissues. As hydrogen peroxide concentration increases, more and more of the enzyme will be used up in an attempt to clear off the accumulating hydrogen peroxide [47]. An increase in CAT activity of irradiated rats consumed Hawthorn was observed. These results are in harmony with Oluwatosin et al [48].

It is well documented that SOD, CAT and GSH have complementary activities in the antioxidative defence system. The increase in CAT and SOD activities in rats reseveing Hawthorn before and after irradiation may be a result of reduced extent of lipid peroxidation which in turn reduces the concentration of hydrogen peroxide. GSH and SOD offer protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation [49]. GSH and SOD are versatile protector and executes a radioprotective function through free radical scavenging, restoration of the damage molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state [50]. The present study demonstrates a significant reduction in serum GSH following exposure to γ-irradiation. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation [51]. An increased content of serum GSH and SOD in the Hawthorn pre and post-treated irradiated animals was observed. These results are in accordance with earlier observation that Hawthorn bio flavonoids have antioxidant properties [52] and are a powerful chemopreventor of oxidative damage caused by free radicals [53]. This ability of Hawthorn protect the liver from radiation-induced damage may be attributed to its ability to restore the activity of antioxidative enzymes and possibly could reduce the generation of free radicals and hepatocellular damage.

In the present study, the recorded significant increase of PCC in irradiated rats group may be due to the interaction of protein with reactive oxygen species produced after exposure to γ-irradiation [54]. The significantly decreased levels of protein carbonyl content observed in the Hawthorn group indicate that Hawthorn administration is as efficient in protecting serum proteins from oxidation as in protecting serum lipids [12]. The fact that Hawthorn improves serum antioxidant capacity has been documented by Shahat, et al., [55], who showed that Hawthorn supplementation significantly improves serum antioxidant capacity, and Da Silva et al [56], who found that Hawthorn supplementation delays α-tocopherol depletion in Cu-oxidized serum.

Besides the alteration of antioxidant enzymes after irradiation, the excessive ROS produced can cause tissue injury through lipid peroxidation. Malondialdehyde (MDA) is an end' product derived from the breakdown of polyunsaturated fatty acids and related esters. The measurement of this
aldehyde provides a convenient index of lipid peroxidation. In the present study, we demonstrated that γ -irradiation increased the TBARS levels significantly. However, the TBARS Levels decreased in the irradiated rats consuming Hawthorn, this may ascribe to the antioxidant activities by Hawthorn, which consequently confront the cell membrane lipid peroxidation damage. Rigelsky, et al., [6] found that consumption of Hawthorn produced a dose dependent decrease in the rate of formation of MDA in the serum, liver and the kidney. This is evident in the decrease in the resultant rate of formation of TBARS observed in rats administered with Hawthorn. This corroborates the findings of Walker, et al., [57] who concluded that Hawthorn has an antioxidant property against lipid peroxidation in rats. Moreover, Schussler, et al.,[58] sustained that rats supplemented with Hawthorn extract for 60 days evidenced an increase body antioxidant activity as their liver microsomes were less susceptible against challenge induced oxidative degradation of lipids as compared to those of non treated control animals. Human consuming Hawthorn has also been reported to have increased plasma antioxidant activity [59]. The significant decreased levels of serum and hepatic PCC and MDA observed in the irradiated rats treated with hawthorn suggest that, Hawthorn administration exerts an antioxidant effect on serum molecules either by directly protecting plasma lipids and proteins from oxidation or by improving the existing anti-oxidant defences in plasma as mentioned by(Chu, et al 2003) [8].

Serum cholesterol al et and triglyceride levels in rats exposed to γ -irradiation were significantly increased (p< 0.05) compared to the control group. However in the group treated with Hawthorn prior and post-irradiation, both cholesterol and triglyceride values recorded a significant decrease (p< 0.05) compared to the irradiated group. The significant elevation of total cholesterol in serum of irradiated animals was explained by Bok, et al.,[60], who mentioned that irradiation increases activation of HMG-CoA reductase enzyme, the key regulatory enzyme in the reaction of the cholesterol synthesis process.El-Khaffif, et al.,[61] reported that increased level of serum cholesterol fractions was probably due to its release from tissues, destruction of cell membrane and increase rate of cholesterol biosynthesis in the liver and other tissues. The increase in plasma triglycerides in rats exposed to γ -irradiation may be attributed to inhibition of the activity of lipoprotein lipase. These results are consistent with the observation of Sedlakova, et al.,[62], who mentioned that lipoprotein lipase activity decreases post irradiation exposure in adipose tissues giving rise to hypertriglyceridemia.

It has been reported that lipid lowering effect of Hawthorn administration in hyper lipidaemic rats through reactivation of lipoprotein lipases, increased faecal excretion, of cholesterol and bile acids [63]. Lipid lowering effect of Hawthorn consumption has also been reported and assumed to be due to increased intestinal fermentation and formation of volatile fatty acids and acetates in caecum and colon [64]. These alterations in turn stimulated the secretion of the humoral factors from large intestine or central nervous system modifying cholesterol metabolism [65].

CONCLUSION

These observations show that Hawthorn protects from γ -irradiation induced oxidative stress, hepatic injury and alteration in lipid profiles changes. Since oxidation sters simulates many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in Hawthorn might be effective as plant hepatoprotectors as well as, of some obvious therapeutic implications.
REFERENCES