Effect of Red Beetroot (*Beta vulgaris L.*) And its Fresh Juice Against Carbon Tetrachloride Induced Hepatotoxicity in Rats

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Abstract: The present study aimed to investigate the effect of dried, fresh juice and waste (pulp) after juicing of red beetroot against carbon tetrachloride induced hepatotoxicity in rats. Total phenol, total flavonoids and antioxidant activity of the three mentioned samples were determined. The results from chemical analysis revealed that dried beet root have the highest amount of total phenols, total flavonoids and antioxidant activity followed by beet juice then beet waste respectively. Thirty male albino rats were divided into 5 groups; group (1) control negative, while the other rats were administered a dose of (2 mL CcL₄/kg b.wt.) twice a week for two weeks to induce chronic damage in the liver then classified into 4 subgroups as follow, the 1st subgroup served as control positive, the 2nd subgroup was fed on basal diet supplemented with dried red beetroot at the level of 10%, the 3rd subgroups was given orally (10 ml/Kg b.wt./day) fresh juice from beetroot (divided into 3 doses /day), the 4th subgroup was fed on basal diet and supplemented with the waste (pulp) from beetroot at the level of 10% for 8 weeks. The results from serum analysis indicated that supplementation with dried beetroot, juice and waste (pulp) of beetroot significantly (P<0.05) restored the enzyme activities of the liver AST, ALT and ALP to normal level. The mean values of MDA and serum total bilirubin were also significantly reduced (P< 0.05), while the mean value of total protein, albumin, CAT, SOD and GSH was significantly (P<0.05) increased as compared to control positive. Due to the presence of both total flavonoids and total phenols in beet root, juice and the waste of beetroot, these materials represent a rich sources of antioxidants and protect the liver cells from CcL4 induced liver damage. So, it is advice to add beet root powder and the waste (pulp) of beet root to bakery product, candies and yogurt and consume it as a routine diet to hepatic disease patients. Also, patients suffering from liver diseases may drink beet root juice to enhancing liver functions and increase antioxidant enzymes.

Keywords: Hepatotoxicity • Beetroot • Carbon Tetrachloride • Rats • Antioxidants • Liver Functions

INTRODUCTION

Chemical-induced liver injury depends mostly on the oxidative stress in hepatic tissue and underlies the pathology of numerous diseases. There is still a lack of effective therapeutics; hence, a treatment with antioxidants has been proposed for prevention and/or attenuation of injury. Such an approach has been defined as chemoprevention and a large body of evidence from various experiments has supported its efficacy [1]. Foods rich in antioxidants have been proposed as a tool to prevent and cure liver damage [2].

Red beet (*Beta vulgaris L.*) is cultivated throughout the world for its roots, which are used as a food and as a source of natural dye [3]. Beets are small herbaceous plants with broad dark green leaves. Beet pigments, betalains, have been examined as natural colorants in food products such as processed meat, ice cream, baked goods, candies and yogurt. Beetroot is a rich source of potent nutrients including magnesium, sodium, potassium, vitamin C and betaine. Results from several in vitro studies have demonstrated that betalains from beetroots possess powerful antiradical and antioxidant activity [4]. Medicinally, the roots and leaves of the beet have been employed as a folk remedy to treat a wide variety of ailments including immune system stimulation and liver and kidney diseases [5].
Besides other active chemicals, beetroots contain a unique class of water-soluble, nonphenolic antioxidants, the betalains, including two classes of compounds, red betacyanins (principally betanin) and yellow betaxanthines. The antioxidant effects of betalains have been demonstrated mainly in various in vitro experiments [6]. Kapadia et al., [5] showed that co-administration of betanin with model carcinogens exhibited a chemopreventive effect on experimental carcinogenesis. The effect was evident as a significant reduction in tumor incidence, multiplicity and delay in tumor latency period.

Agarwal et al., [7] reported the hepatoprotective activity of beetroot ethanolic extract against CcL\textsubscript{4} induced liver injury in rats assessed on the basis of routine serum markers of liver function, alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and the concentration of cholesterol and triglycerides. Also, Kujawaska, et al., [8] investigated the potential protective effect of beetroot juice (8 mL/kg/day for 28 days) in a model of oxidative stress induced by N-nitrosodimethylamine and carbon tetrachloride and mentioned that, pretreatment with beetroot juice can counteract, to some extent, xenobiotic-induced oxidative stress in rats.

Residue from the processing of fruits and vegetables, traditionally considered as an environmental problem, are being increasingly recognized as sources for obtaining high-phenolic products. The polyphenolics from waste materials, being derived from agro-industrial production, may be used as functional food ingredients and as natural antioxidants to replace their synthetic equivalents that have experienced growing rejection [9]. Conventional drugs and synthetic antioxidants used in the treatment of liver diseases are generally inadequate and have some serious adverse effects. The interest for searching of alternative drugs and natural antioxidants of plant origin for the treatment of liver disease has increased. So, the aim of this work is to investigate the protective effect of dried, fresh juice and waste after juicing of red beetroot (Beta vulgaris L.) against carbon tetrachloride induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Chemicals:** All chemicals including Carbon tetrachloride (CcL\textsubscript{4}) and Kits were purchased from local distributor (Sigma chemical) Cairo, Egypt.

**Plant:** Red beetroot (Beta vulgaris L.) was purchased from a local market, Cairo, Egypt.

**Rats:** Thirty male rats of Sprague Dawley strain weighing (180 ±5 g.) were obtained from the National research Center, Dokki, Cairo. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in stainless steel cages one per cage at temperature 22±3 °C and relative humidity 30-70 %. The animal diet was given ad libitum. Animals were acclimatized for one week prior to experiment.

**Preparation of Dried Beetroot, Juice and Waste of Beetroot:** Red beetroots were washed with tap water, chopped into small pieces and then dried at Solar Energy Center, National research Center, Dokki, Cairo. The dried material was reduced into powder form as far as possible.

The juice was prepared from chopped beetroots by household juice extractor then filtered through stainless steel refinery. The pulp left after juice extraction was dried in an oven at 50 ± 1°C and powdered using a lab grinder and stored at 4°C till use.

**Determination of Antioxidant Content in Dried and Waste of Beetroot after Juicing:** Total phenolic content was determined according to the Folin-Ciocalteu procedure [10] and expressed as mg of gallic acid equivalent (GAE) per g of sample. Total flavonoid content was determined according to Zilic et al. [10] and expressed as mg of catechin equivalent (CE) per g of sample. Determination of radical DPPH scavenging activity (Antioxidant activity) was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH•) according to Hwang and Do Thi [11]. The same methods were used for determination of antioxidant content in juice of beetroot with some modifications (using the juice as an extract).

**Experimental Design:** The basal standard diet was prepared in accordance with AIN-93 formulation [12]. After a period of adaptation on basal diet, thirty rat were divided into five groups (6 rats each ); Group (1): was fed on basal diet only and served as a negative control group, while the other rats were administered a dose of (2 mL CcL\textsubscript{4}/kg b.wt.) twice a week for two weeks to induce chronic damage in the liver according to Sundaresan and Subramanian, [13] then classified into four subgroups as follow, subgroup (1) served as control positive group, subgroup (2) was fed on basal diet supplemented with dried red beetroot at 10%, subgroup (3) was given orally (10 ml/Kg Body weight/day) fresh juice from beetroot (divided to 3 doses /day), subgroup (4) was fed on basal diet supplemented with the waste from beetroot juice at 10%.
Biochemical Analysis: At the end of the experiment (eight weeks), the animals were fasted overnight, and then they were anaesthetized and sacrificed to obtain blood samples. Each blood sample was placed in dried clean centrifuge tube and then centrifuged for 10 minutes at 3000 (rpm) to separate the serum. Serum was carefully separated into clean dried Wassermann tubes by using a Pasteur pipette and kept frozen until analysis.

Statistical Analysis: The obtained results were statistically analyzed with SPSS Inc. software (version 16.0). One way ANOVA was used to study a significant difference between means of the dietary groups with a significance level at (P<0.05) [14].

RESULTS AND DISCUSSION

Table (1) shows the content of total phenols, total flavonoids and the antioxidant capacity of dried, juice and waste (pulp) of red beetroot. The chemical analysis reveals that all three samples have total phenols and total flavonoids. Dried beet root have the highest amount of total phenols and total flavonoids followed by beet juice then beet waste respectively.

The DPPH is a decolorization assays which measure the relative antioxidant abilities of natural extracts to scavenge free radicals generated in the assay system [15]. Our results demonstrate that dried beetroot have the highest antioxidant activity followed by juice Beetroot then the waste after juicing.

The beet waste (pulp) has a considerable amount of total phenols, total flavonoids and the antioxidant activity, so we have to use this waste in food technology. The above results indicate that red beet roots have good antioxidant properties and were in accordance with Váli et al. [16] who mentioned that beetroots contains important bioactive agents (betaine and polyphenols), which have a wide range of physiologic effects. Pal et al., [17] and Georgiev et al., [18] reported the presence of flavonoids, carbohydrate, betain and anthocyanin pigments in Beta vulgaris root of phytochemical studies. The antioxidant activity determined by the DPPH method exhibited 40% and 78% activity in methanol extracts of carrot and beetroot pulp waste (20 mg) respectively [19]. So, the results from chemical analysis suggest that red beetroot, juice and beetroot wastes can be exploited for their antioxidant components and used for value addition in food formulations.

The effect of treatment with dried beetroot, juice from red beetroot and residue from juice extraction (pulp) on the activity of serum liver enzymes (ALT, AST, ALP and total bilirubin) is recorded in Table (2). Subcutaneous injection of CeL₄ to rats in a dose (2 mL CeL₄/kg b.wt.) twice a week for two weeks significantly (P < 0.05) elevated the mean levels of ALT, AST, ALP and total bilirubin when compared with the negative control group. In this aspect, Mansour et al. [20] reported that a single dose of CeL₄ induced hepatotoxicity manifested biochemically by significant elevation of activities of liver enzymes such as ALT and AST. Hepatic injury can be determined by measuring the leakage of cellular enzymes into plasma or serum. Increased serum enzyme levels, including ALT and AST had been associated with loss of hepatocyte membrane integrity, or more specifically necrosis in the case of ALT elevation [21]. It is well established that CeL₄ induces hepato-toxicity by cytochrome P450 mediated reactions to produce CeL₄-derived radicals [22].

On the other hand, bilirubin is a breakdown product of hemoglobin which will conjugate with glucuronic acid in hepatocytes to increase its water solubility. Thus, release of unconjugated bilirubin from damaged or dead liver cells is also one of the major markers for liver damage [23].

The ALP is the prototype of these enzymes that reflects the pathological alteration in biliary flow. The CeL₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubins content [24], this mechanism was in harmony with our results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>Total Phenols (GAE mg/g)</th>
<th>Total Flavonoids (CE mg/g)</th>
<th>DPPH (AAE mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dried beetroot</td>
<td>4.96</td>
<td>4.99</td>
<td>4.14</td>
</tr>
<tr>
<td></td>
<td>Beetroot juice</td>
<td>0.879</td>
<td>1.329</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Beet waste (pulp)</td>
<td>0.233</td>
<td>0.287</td>
<td>0.105</td>
</tr>
</tbody>
</table>

GAE: Gallic acid equivalent, CE: Catchin equivalent, AAE: Ascorbic acid equivalent
Table 2: Effect of dried, fresh juice and waste (pulp) from red beetroot on liver functions in carbon tetrachloride injected rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>ALT (µ/L)</th>
<th>AST (µ/L)</th>
<th>ALP (µ/L)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>29.86 ± 2.12</td>
<td>38.20 ± 1.42</td>
<td>69.41 ± 2.44</td>
<td>4.60 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>53.88 ± 1.78</td>
<td>69.15 ± 2.28</td>
<td>93.00 ± 1.93</td>
<td>8.50 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Dried Beetroot</td>
<td>34.86 ± 7.79</td>
<td>44.70 ± 1.57</td>
<td>69.16 ± 1.93</td>
<td>5.73 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Beetroot Juice</td>
<td>36.50 ± 2.23</td>
<td>47.66 ± 1.56</td>
<td>71.00 ± 1.86</td>
<td>7.19 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Beetroot waste (pulp)</td>
<td>38.91 ± 2.04</td>
<td>49.16 ± 2.00</td>
<td>73.50 ± 1.82</td>
<td>6.66 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

*Values were expressed as Means ± SE, Values at the same column with different litters are significant at P<0.05.

Rats supplemented with either dried beetroot, juice and waste (pulp) from juice extraction had significantly decreased (P<0.05) serum AST and ALT as compared to positive control group with the most significant (P < 0.05) effect was observed in rats fed with dried beet root. Also, these treatments induced suppression of the increased ALP activity (P < 0.05) with the concurrent depletion of raised bilirubins suggests the possibility of these materials to have ability to stabilize biliary dysfunction in rat liver during hepatotoxicity by CcL4.

Our results helped to stabilize the plasma membrane and suppressed the leakage of enzymes through cellular membrane. This hepatoprotective effect may be contributed by its antioxidant capacity [25].

There were no significant differences in serum AST, ALT and ALP among the three tested groups unless the mean level of total bilirubin there was a significant (P < 0.05) difference between the rats fed dried beet and the other both group fed on either beet juice or the residue of juice extraction (pulp). It was also clear that, there was no significant difference in the mean value of serum ALP among the three tested group and the control negative group.

Treatment with dried red beetroot, beet root juice and waste from juice extraction (pulp) significantly (P<0.05) restored the enzyme activities of the liver AST, ALT, ALP, total bilirubin and total protein to normal level, these results were in accordance with results of Kapadia et al., [5] who recorded that red beetroot (Beta vulgaris L.) is used as a popular folk remedy for liver and kidney diseases, for stimulation of the immune and hematopoietic systems and as a special diet in the treatment of cancer. Also, Agarwal et al. [7] mentioned the hepatoprotective activity of beetroot ethanolic extract against CcL4-induced liver injury in rats assessed on the basis of routine serum markers of liver function, ALT and ALP activities and the concentration of cholesterol and triglycerides.

Table 3: Effect of dried, fresh juice and waste (pulp) from red beetroot on serum total protein and albumin in carbon tetrachloride injected rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>T. protein (g/dL)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>7.34 ± 0.20</td>
<td>3.61 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>5.79 ± 0.23</td>
<td>2.23 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Dried Beetroot</td>
<td>7.01 ± 0.22</td>
<td>3.30 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Beetroot Juice</td>
<td>6.68 ± 0.25</td>
<td>2.88 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Beet waste (pulp)</td>
<td>6.51 ± 0.18</td>
<td>2.68 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

*Values were expressed as Means ± SE, Values at the same column with different litters are significant at P<0.05.

Also, our results of the current study were in accordance with those of Pal et al [17] who reported that, the treatment with ethanolic extract of Beta vulgaris caused significant reduction of CcL 4 induced elevated serum levels of enzyme activities and bilirubin with parallel significant increase in total protein, indicating that, the extract could preserve the normal functional status of the liver. The hepatoprotective effect of beet root may be due to presence of its chemical contents. The results were in agreement with Sadeek, [26] who reported that, treatment with beetroot (oral dose of 8ml/Kg/day/ rat) for 28 days significantly (P<0.05) restored the enzyme activities of the liver AST, ALT and ALP to normal level. The mean values of total bilirubin were also significantly reduced (P< 0.05).

From the results in Table (3) it could be observed that, administration of CcL4 caused a significant (P<0.05) decrease in the serum levels of total protein and albumin as compared to the normal rats. Administration with dried beetroot, beet juice and waste from juice extraction (pulp) caused a significant (P<0.05) increase in serum level of total protein as compared to the positive control group. Also the treatment with either dried beet root or beet juice significantly (P<0.05) increase the mean level of serum albumin, while beetroot waste (pulp) caused non significant differences as compared to control positive
Table 4: Effect of dried, fresh juice and waste (pulp) from red beetroot on the activity of CAT, SOD, GSH enzymes and serum concentrations of MDA in carbon tetrachloride injected rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>CAT (mmol/dl)</th>
<th>SOD (µ/dl)</th>
<th>GSH (µmol/dl)</th>
<th>MDA (µmol/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>68.25±1.75a</td>
<td>99.75±1.79a</td>
<td>43.17±0.13a</td>
<td>1.82±0.13a</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>44.01±1.58d</td>
<td>66.24±1.60d</td>
<td>23.43±0.22d</td>
<td>3.42±0.22d</td>
<td></td>
</tr>
<tr>
<td>Beetroot</td>
<td>61.65±2.13b</td>
<td>89.71±1.54d</td>
<td>35.73±0.09d</td>
<td>1.99±0.09d</td>
<td></td>
</tr>
<tr>
<td>Beet Juice</td>
<td>58.00±3.24b</td>
<td>85.30±1.88b</td>
<td>32.94±0.17b</td>
<td>2.11±0.17b</td>
<td></td>
</tr>
<tr>
<td>Beet waste (pulp)</td>
<td>50.50±1.55c</td>
<td>72.37±0.94c</td>
<td>28.41±0.12c</td>
<td>2.61±0.12c</td>
<td></td>
</tr>
</tbody>
</table>

CAT: catalase; SOD: superoxide dismutase; GSH: reduced glutathione; MDA: malondialdehyde.

*Values were expressed as Mean±SE, Values at the same column with different litters are significant at P<0.05.

Results presented in table (4) demonstrate the effect of dried beetroot, fresh juice and waste (pulp) from red beetroot on the activity of catalase (CAT), superoxide dismutase (SOD), GSH enzymes and serum concentrations of malondialdehyde in carbon tetrachloride injected rats. The mean Values of serum CAT, SOD and GSH level in untreated hepatotoxicity rats (control positive) group were significantly lower at (P<0.05) as compared with those of the normal rats as a result of induction with CcL₄. These results were in agreement with Augustyniak et al., [32] and Khan et al., [33] whom found that CcL₄ initiates lipid peroxidation and reduces tissue CAT and SOD activities. Also, these results were confirmed by Ruidong et al., [34] and Wang et al., [35] who demonstrated that MDA levels in the CcL₄ treated group as indicators of lipid peroxidation were significantly higher than that in the normal control group.

Glutathione (GSH) is an important endogenous antioxidant substance. The decrease of GSH content may be due to increased GSH consumption as it participates in the detoxification system for the metabolism of CcL₄ and results in an enhanced susceptibility of hepatocytes to CcL₄ toxicity [36]. Our results showed that CcL₄ obviously decreased GSH content in the hepatocytes, but treatment with either dried beetroot, fresh juice and waste (pulp) from red beetroot significantly (P<0.05) reverse it. This suggested that the nature of supplementation with either dried beetroot, fresh juice and waste (pulp) from red beetroot protecting-SH compounds (such as GSH) from CcL₄ injury may be a mechanism of its hepatoprotection.

Supplemented diets with dried beetroot, fresh juice and waste (pulp) from red beetroot caused significant increase (P<0.05) in the mean values of serum CAT, SOD and GSH level as compared with those of positive control groups. It is clear that, there were no significant differences in the mean values of serum CAT, SOD and GSH level among groups fed on either dried beetroot and beet juice group. But, there were a significant differences (P<0.05) among groups fed on dried beetroot and group fed on beet waste (pulp).

Liver cells possess a number of compensatory mechanisms to deal with reactive oxygen species (ROS) and their effects; among these are the induction of a number of antioxidant proteins such as SOD, CAT, glutathione peroxidase (GSHPx) and GSH. Therefore, oxidative stress, caused mainly by ROS, is also associated with hepatic diseases [37]. SOD is an effective defense enzyme that protects biological macromolecules from oxidative stress [38].
On the other hand, the elevations in the levels of end products of lipid peroxidation (MDA) in the liver of rats treated with CcL₄ were significantly (P<0.05) observed as compared with those of the negative control rats. MDA is an indicator to evaluate the levels of lipid peroxidation and inflammation during liver damage [39]. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [24].

Treatment with dried beetroot, fresh juice and waste (pulp) from red beetroot significantly (P<0.05) reversed these changes in serum levels of MDA compared with positive control group. It is obvious that, there were no significant differences in the mean values of serum MDA level among groups fed on either dried beetroot and beet juice group and control negative group. These results were in agreement with Sadeek, [26] who reported that, treatment with beetroot (oral dose of 8ml/Kg/day/ rat) for 28 days significantly (P<0.05) decrease the mean values of MDA and significantly increase (P< 0.05) serum total protein, erythrocyte SOD as compared with the respective mean values for control positive group. Also, The current results were confirmed with Lima et al., [40] who mentioned that, the antioxidant mechanisms in biological systems include direct quenching free radicals to terminate the radical chain reaction, chelating transition metals, acting as reducing agents, or stimulating the antioxidative enzyme activities. Other compounds with antioxidant activity include glutathione and flavonoids which protect cells against oxidative stress [41].

CONCLUSIONS

Due to the presence of both total flavonoids and total phenols, beet root, juice and the beetroot was represent rich sources of antioxidants. So, it is advice to add beet root powder and the waste (pulp) of beet root to bakery product and consume it as a routine diet to hepatic disease patients. Also, patients suffering from liver diseases may drink beet root juice to enhancing liver functions and increase antioxidant enzymes.

REFERENCES


