Effects of dietary supplementation with doum and selenium on liver injury in experimental rats

Huda Ahmad AL-amer, Nabila M. Rashwan

Nutrition and Food Science Dep. Princess Nora Bint Abdul-Rahman University, Riyadh. Home Economics Dept, Faculty of Education, Suez University, Ismailia Egypt.

ABSTRACT

Forty-two adult male albino rats Sprague-Dawley strain were classified into six groups (7 rats each). Rats were subcutaneously administered a single dose of CCl4 in paraffin oil in dose 1ml/kg for two days from the start of the experimental period for inducing rats liver injuries. Then, rats classified into control (+ve), doum powder, doum extract, selenium, doum powder with selenium and doum extract with selenium. The period of the study was 60 days. In compared with control (+ve), the values of liver total lipid and cholesterol were significantly decreased but final weight and liver superoxide dismutase were significantly increased in doum powder, doum extract, doum powder with selenium and doum extract with selenium rat groups. The value of nitric oxide was decreased but food efficiency ratio and liver triglyceride were increased in doum extract, doum powder with selenium and doum extract with selenium rat groups. Values of weight gain, food intake, and serum glutathione peroxidase & superoxide dismutase in addition of liver glutathione peroxidase, glutathione transferase, catalase and glycogen were significantly increased in all treated groups. However, values of ALT, AST, ALP, γ GT and liver malondialdehyde were significantly decreased in all treated groups. Hence, results revealed that administration of doum can improve liver functions especially doum extract. The combination of doum extract and selenium showed the most desirable effects in lowering side effects of liver toxicity. Values of serum

Key words: CCl4-liver – doum – selenium – rats.

Introduction

The liver is a vital organ has many functions for the body as converting nutrients derived from food into essential blood components, storing vitamins and minerals, regulating blood clotting, producing proteins and enzymes, maintaining hormone balances, and metabolizing and detoxifying substances that would otherwise be harmful to the body. The liver also makes factors that help the human immune system fight infection, removes bacteria from the blood, and makes bile, which is essential for digestion. Liver disease is any condition that causes liver inflammation or tissue damage and affects liver function (Ward and Daly 1999).

Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes. Selenium is an essential mineral found in small amounts in the body. This mineral is required by every living cell on this planet for proper function and structure. Most people should have enough selenium in their bodies if they are healthy and have a well-balanced diet. However, those who choose alternative lifestyles such as smoking cigarettes or drinking alcohol may have a lower level of selenium. Selenium is found in minute amounts in foods, with the richest sources being from meats, fish, whole grains, and dairy products. The selenium content of vegetables is dependent on the soil in which they are grown. Selenium is an antioxidant mineral required to support the normal function of the immune system. It also has a role to play in maintaining thyroid function and male fertility (Brown and Arthur 2001 and Burk 2002).

Doum palm (Hyphaene thebaica) is a type of palm tree with edible oval fruit. It is native to the Nile valley in Egypt. It was considered sacred by the Ancient Egyptians and the seed was found in many pharaoh's tombs. The fruit is sold by street vendors, and is popular among children, gnawing its sweet yet sour hard fibrous flesh beneath the shiny hard crust. The fruits are oval, shiny, and red to orange in color, with an average length and diameter of 6 and 5 cm. The benefits of doum are including lowering blood pressure in hypertensive patients and changing blood lipids and lipoproteins in a manner that decreases the risk on the cardiovascular system (Lokuruka 1990, Kamis et al., 2000 and Hetta and Yassin 2006).

The aim of this study was to investigate the effect of doum fruit or its extract in combination with selenium to lower the side effect of liver injury.

Corresponding Author: Huda Ahmad AL-amer, Nutrition and Food Science Dep. Princess Nora Bint Abdul-Rahman University, Riyadh.
Materials And Methods

A – Materials:

Carbon tetrachloride (CCl₄) was used to induce experimental acute hepatitis in rats. It was purchased from El Gomhorya Co., Egypt in the form of 40% liquid dispensed in 1 L plastic bottles. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt. Selenium powder (as selenite) was purchased from El-Gomhorya Company, Cairo Egypt. Forty-two Sprague –Dawley adult male rats were purchased from the National Research Center, Giza, Egypt. The average weight was 98±7 g. The standard diet was performed according to NRC (1995)

B- Methods:

1-Experimental design:

Dried doum palm obtained from local market and crushed to powder. The doum powder was added to the standard diet as 10% in substitution of fiber. The methanolic extract of 5kg doum powder was extracted by 70% ethanol on cold until exhaustion. The solvent was distilled in rotary evaporator at 55°C till dryness. The rat dose of the doum extract was 20 mg/kg b.wt. Selenium dose was 4.0 mg per kg diet for 60 days.

Rats were housed in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into six groups (7 rats each). Rats were subcutaneously administered a single dose of CCl4 in paraffin oil in dose 1ml/kg for two days from the start of the experimental period to induce liver injury according to the method described by Lee et al., (2005). The CCl4 rats were classified as following:

Control (+ve) group: fed on the standared diet only.
Doum powder group: fed on the standared diet and doum powder.
Doum extract group: fed on the standared diet and doum extract.
Selenium (Se) group: fed on the standared diet with selenium.
Doum powder with selenium group: fed on the standared diet and doum powder with selenium.
Doum extract with selenium group: fed on the standared diet and doum extract with selenium.

The food intake was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) was determined by Chapman et al., (1950).

At the end of experiment (60 days), rats were anesthetized, blood samples were collected from hepatic portal vein in clean centrifuge tubes. The liver of sacrificing rats was removed and perfused with 50 to 100 of ice cold 0.9%NaCL solution.

Serum alanine and aspartate aminotransferase (ALT&AST), alkaline phosphatase (ALP) and gamma glutamyle peptidase (γGT) activity enzymes were estimated according to Reitman and Frankel (1957), Kind and King (1954) and Henry, (1974), respectively. In addition, blood glutathione peroxidase (GPX), superoxide dismutase (SOD) and nitric oxide (NO) activities were determined as described by the method of Beutler et al., (1963), Dechatelet et al. (1974) and Green et al., (1981), respectively. Liver glycogen, triglyceride, total lipids and cholesterol according to Rerup and Lundquist, (1967), Young and Pestaner (1975) and Abell et al., (1952), respectively. Liver glutathione peroxidase (GPX), glutathione transferase (GST), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) were estimated according to Weiss et al., (1980), Habig et al., (1974), Beuchamp and Fridovich (1971), Luck (1965) and Draper and Hadley,(1990), respectively.

Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry (1987).

Results:

Table 1: Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final Weight(g)</th>
<th>Weight gain (g)</th>
<th>Food intake(g/w)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>155.98±9.14</td>
<td>55.71±5.42</td>
<td>10.32±1.22</td>
<td>0.089±0.001</td>
</tr>
<tr>
<td>Doum powder</td>
<td>185.47±10.11</td>
<td>88.33±8.23</td>
<td>16.32±1.34</td>
<td>0.090±0.002</td>
</tr>
<tr>
<td>Doum extract</td>
<td>196.37±15.49</td>
<td>96.71±9.21</td>
<td>16.99±13.22</td>
<td>0.094±0.01b</td>
</tr>
<tr>
<td>Se</td>
<td>165.62±11.33</td>
<td>65.32±7.11</td>
<td>13.29±1.24</td>
<td>0.081±0.003d</td>
</tr>
<tr>
<td>Doum powder+ Se</td>
<td>194.49±14.24</td>
<td>95.32±8.91</td>
<td>16.83±1.01</td>
<td>0.094±0.004a</td>
</tr>
<tr>
<td>Doum extract+ Se</td>
<td>212.05±17.76</td>
<td>113.28±10.21</td>
<td>16.54±1.19</td>
<td>0.114±0.005e</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant.
The initial weight of the experimental rats was 89±7 g. There was a significant increase in final weight, weight gain and food intake consumed doum powder (P<0.05, 0.01 &0.001), extract, doum powder with selenium and doum extract with selenium (P<0.01&0.001) rat groups compared with control (+ve). FER appeared obviously increased in doum extract, doum powder with selenium and doum extract with selenium rat groups (P<0.05&0.001) compared with control (+ve). Selenium group showed a significant increase in weight gain and food intake but a significant decrease in FER compared with control (+ve). Moreover, selenium group showed a significant decrease in final weight, weight gain, food intake and FER compared with treated groups as in table (1).

### Table 2: The Mean values ± SD of serum ALT, AST, ALP and γ GT of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>ALT (µ/l)</th>
<th>AST (µ/l)</th>
<th>ALP (µ/l)</th>
<th>γ GT (µ/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>115.71±13.21</td>
<td>151.41±13.14</td>
<td>170.87±19.96</td>
<td>14.96±1.49</td>
</tr>
<tr>
<td>Doum powder</td>
<td></td>
<td>78.51±7.17</td>
<td>81.57±8.21</td>
<td>99.11±10.21</td>
<td>9.14±1.59</td>
</tr>
<tr>
<td>Doum extract</td>
<td></td>
<td>58.96±5.11</td>
<td>67.88±6.14</td>
<td>88.32±9.05</td>
<td>7.66±1.41</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>99.61±9.76</td>
<td>104.35±12.83</td>
<td>130.21±15.35</td>
<td>11.32±2.13</td>
</tr>
<tr>
<td>Doum extract+ Se</td>
<td></td>
<td>65.11±6.36</td>
<td>78.32±9.24</td>
<td>71.14±9.21</td>
<td>8.11±1.21</td>
</tr>
<tr>
<td>Doum extract+ Se</td>
<td></td>
<td>59.66±5.01</td>
<td>63.71±6.14</td>
<td>64.63±7.35</td>
<td>6.32±0.66</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Values of serum ALT, AST, ALP and γ GT were significantly decrease in all treated groups (P<0.05, 0.01 &0.001) compared with control (+ve). There were non-significant differences in ALT&AST among doum extract, doum powder with selenium and doum extract with selenium rat groups but there were non-significant differences in ALP between doum powder and doum extract rat groups and also between doum powder with selenium and doum extract with selenium rat groups. The value of γ GT was in non-significant differences among doum powder, selenium and doum extract with selenium rat groups as shown in table (2).

### Table 3: The Mean values ± SD of serum GPX, SOD and NO of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>GPX(µ/ml)</th>
<th>SOD(µ/ml)</th>
<th>NO(µ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>51.14±6.18</td>
<td>19.81±1.76</td>
<td>11.99±2.19</td>
</tr>
<tr>
<td>Doum powder</td>
<td></td>
<td>70.35±7.22</td>
<td>29.28±1.43</td>
<td>9.11±1.61</td>
</tr>
<tr>
<td>Doum extract</td>
<td></td>
<td>88.22±10.11</td>
<td>35.19±3.96</td>
<td>8.11±1.49</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>61.21±5.66</td>
<td>25.11±3.21</td>
<td>9.21±2.40</td>
</tr>
<tr>
<td>Doum powder+ Se</td>
<td></td>
<td>77.14±8.71</td>
<td>27.21±2.78</td>
<td>7.81±1.55</td>
</tr>
<tr>
<td>Doum extract+ Se</td>
<td></td>
<td>90.41±11.12</td>
<td>33.24±4.20</td>
<td>7.12±1.32</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Values of serum GPX and SOD were significantly increase in all treated groups (P<0.05, 0.01 &0.001), but the value of NO was decreased in doum extract, doum powder with selenium and doum extract with selenium rat groups (P<0.05 & 0.01) compared with control (+ve). There were non-significant differences in GPX among doum powder, doum extract and doum powder with selenium rat group. The value of SOD was significantly increased in doum extract and doum extract with selenium rat groups compared with other groups. There was non-significant difference in NO among all treated groups except doum extract with selenium rat group as shown in table (3).

### Table 4: The Mean values ± SD of liver SOD, GPX, catalase, GST and MDA of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>GPX (µ/mg)</th>
<th>GST (µmg)</th>
<th>Catalase (µ/mg protein)</th>
<th>SOD (µ/mg)</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>28.81±4.35</td>
<td>0.31±0.04</td>
<td>21.45±3.24</td>
<td>18.75±4.00</td>
<td>18.89±2.14</td>
</tr>
<tr>
<td>Powder</td>
<td></td>
<td>45.50±5.19</td>
<td>0.98±0.27</td>
<td>38.98±4.15</td>
<td>45.96±6.11</td>
<td>13.95±1.50</td>
</tr>
<tr>
<td>extract</td>
<td></td>
<td>55.32±6.27</td>
<td>1.11±0.53</td>
<td>41.87±5.50</td>
<td>48.37±6.39</td>
<td>14.32±1.81</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>42.11±5.16</td>
<td>0.85±0.09</td>
<td>37.39±4.10</td>
<td>40.76±5.17</td>
<td>12.11±1.99</td>
</tr>
<tr>
<td>Powder+ Se</td>
<td></td>
<td>61.31±7.38</td>
<td>1.05±0.32</td>
<td>58.31±6.17</td>
<td>62.60±7.88</td>
<td>13.29±2.11</td>
</tr>
<tr>
<td>extract+ Se</td>
<td></td>
<td>75.13±6.96</td>
<td>1.96±0.43</td>
<td>71.11±8.81</td>
<td>60.31±7.11</td>
<td>11.23±1.55</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Values of liver GPX, GST and catalase were significantly increase (P<0.05, 0.01 &0.001) but liver MDA was significantly decrease (P< 0.01 &0.001) in all treated groups. However, the value of liver SOD was significantly increased in all treated groups except selenium rat group (P<0.05 & 0.01) compared with control (+ve).

The values of GPX, catalase and SOD were significantly increase in doum powder with selenium and doum extract with selenium rat groups compared with other groups. The value of liver GST was significantly increase
but liver MDA was significantly decreased in doum extract with selenium rat group compared with other groups except MDA in selenium group as shown in table (4).

### Table 5: The Mean values ± SD of liver glycogen, triglyceride, total lipids and cholesterol of the experimental rat groups.

<table>
<thead>
<tr>
<th></th>
<th>Glycogen (mg/100g)</th>
<th>Triglyceride (mg/g)</th>
<th>Total lipids (mg/g)</th>
<th>Cholesterol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>3.98±0.26</td>
<td>1.96±0.11</td>
<td>68.66±7.55</td>
<td>8.11±1.32</td>
</tr>
<tr>
<td>powder</td>
<td>5.01±0.33</td>
<td>1.99±0.32</td>
<td>53.38±6.11</td>
<td>3.87±0.66</td>
</tr>
<tr>
<td>extract</td>
<td>5.14±0.32</td>
<td>2.11±0.24</td>
<td>51.21±5.27</td>
<td>5.30±0.78</td>
</tr>
<tr>
<td>Se</td>
<td>4.87±0.32</td>
<td>1.86±0.43</td>
<td>59.81±7.31</td>
<td>7.11±1.03</td>
</tr>
<tr>
<td>Powder+Se</td>
<td>5.11±9.18</td>
<td>2.21±0.62</td>
<td>48.55±6.20</td>
<td>4.25±0.33</td>
</tr>
<tr>
<td>extract+Se</td>
<td>5.36±0.14</td>
<td>2.44±0.12</td>
<td>42.96±5.11</td>
<td>3.99±0.28</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

The value of liver glycogen was significantly increased in all treated groups (P<0.01 &0.001) but liver triglyceride was significantly increased in doum extract, doum powder with selenium and doum extract with selenium rat groups (P<0.05) compared with control (+ve). The values of liver total lipids and cholesterol were significantly decreased in all treated groups except selenium rat group (P<0.05, 0.01 &0.001) compared with control (+ve). There were non-significant differences in liver glycogen and triglyceride among doum powder, doum extract, doum powder with selenium and doum extract with selenium rat groups. The value of liver total lipids was significantly decreased in doum powder with selenium and doum extract with selenium rat groups compared with other treated groups. Selenium group showed a significant increase in liver cholesterol compared with other treated groups as shown in table (5).

### Discussions:

It is known that diets rich in fruits and vegetables have been associated with decreased risks of several chronic diseases. These protective effects have been attributed partly to the various antioxidant compounds. Plant foods possess outstanding antioxidant such as polyphenols and free radical scavenging properties and their consumption recommended (Abd El-Ghaney et al., 2012).

The results of the present investigation revealed good nutritional results that are might be due to the effect of selenium and doum as antioxidant hence enhances nutrient utilization in rats.

Research on the doum fruit pulp have shown that it contains 4.91% proteins, 5.26% fat, 4.5% ash and 85.33% total carbohydrate with fatty acids and also provided the proximate composition and the content of the minerals calcium, phosphorus and iron in the mesocarp of doum, in particular the nutritionally (Hoebekke 1989 and Bonde et al.,1990). Doum was reported to contain important substances including saponins, tannins, and flavonoids; hence the use of doum, which is rich in flavonoids and saponins, in folk medicine is not surprising (Dosumu et al., 2006). Aqueous ethanolic extract of doum leaves appeared to be a potent scavenger of reactive oxygen species. The extract inhibits (HO.) attack on salicylic acid. The phenolic content of doum extract revealed the presence of four major compounds. An in-depth phytochemical investigation showed the presence of fourteen compounds (Amany 1994 and Omayma et al., 2009).

Liver injury shows extensive metabolic disorders in protein synthesis, as well as abnormalities in the metabolic detoxification process. Abnormally high liver enzyme activity, or liver enzymes spilling out into the bloodstream, signaling liver damage tests will look for ALT and AST enzymes in the bloodstream, as well as an increase in alkaline phosphatase (ALP), and declining levels of aminotransferases (enzymes that cause the chemical change of nitrogen carrying amino). Administration of male rats with CCl4 significantly increased the activity of ALT and AST in plasma and the levels of glutathione in the liver but inhibited the activity of GST. It is known that glutathione and glutathione S-transferase aid in the protection of cells from the lethal effects of toxic and carcinogenic compounds (Foster and Sumar 1997 and Abd El-Ghaney 2006).

On the other hand, selenium is a trace element that acts by several mechanisms, including detoxifying liver enzymes, exerting anti-inflammatory effects, and providing antioxidant defense. Selenium deficiency causes oxidative stress. The presence of selenium helps induce and maintain the glutathione antioxidant system. Low levels of selenium can contribute to heart failure, atherosclerosis and stroke. Selenium provides protection against both hepatitis B and C and liver cancer. Viral hepatitis patients should take selenium on a continuous basis (Yu et al., 1997).

Se research has attracted tremendous interest because of its important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess reactive oxygen species (ROS) and reactive nitrogen species. Cellular and plasma glutathione peroxidase represent a major class of functionally important selenoproteins and the functional parameters used for the assessment of selenium status. Glutathione peroxidases catalyze the reduction of peroxides that can cause cellular damage. Thioredoxin reductase provides reducing power for several biochemical processes and defends against oxidative stress. Selenoprotein P works...
as a transporter of selenium between the liver and other organs. Selenium in the form of selenomethionine can also unspecifically substitute for methionine in other proteins (Holben and Smith 1999, Alexander 2007 and Tinggi 2008).

Our biochemical findings are in agreement with previous investigation of Hetta and Yassin (2006) who reported that doum plant exhibited a highly significant decrease in serum cholesterol and Non-HDL cholesterol. One fraction exhibited a highly significant decrease in cholesterol level but with only a moderately significant effect in decreasing the Non-HDL level. Decreasing Non-HDL, especially LDL, cholesterol, can reduce the risk of atherosclerosis and subsequent cardiovascular diseases. Kamis et al., (2003) reported that the daily oral administration of the suspension of hyphaene thebaica (L) mart for three weeks revealed a significant decreased in the levels of triglycerides, cholesterol and total lipids in addition to total proteins and albumin compared to the control.

The hypolipidemic properties of the aqueous doum pulp suspension could be partly due to the presence of glycosides. Saponins form complexes with cholesterol and bile in the intestine thereby indirectly reducing the cholesterol level in the blood (Milgate and Robert 1995). The reduction of triglycerides level might be due to the decreased lipogenesis or increased lipolysis and subsequent oxidation of fatty acids into acetyl CoA that could alleviate demand for the synthesis of cholesterol and bile acid (Modu et al 2000 and Kamis et al 2000).

It is concluded that the natural, safe and non-toxic doum plant could be administered to liver injured patient to improve liver function.

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