Hepatoprotective activity and antioxidant effects of El Nabka (Zizyphus spina-christi) fruits on rats hepatotoxicity induced by carbon tetrachloride

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Abstract: The present study was designed to evaluate the protective effect of the El Nabka (Zizyphus spina-christi) fruits as an antioxidant against carbon tetrachloride (CCL₄) induced oxidative stress and hepatotoxicity in Albino Wistar rats was investigated. Subcutaneous injection of CCL₄, produced a marked elevation (P<0.05) in the serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Daily dietary containing powder of ZSCF at 2.5, 5, 10, and 15% of basal diet for 6 weeks produced a reduction in the serum levels of liver enzymes. ZSCF has also restored normal levels of malondialdehyde and retained control activities of endogenous antioxidants such as Superoxide Dismutase (SOD), and Glutation Peroxidase (GSH). Therefore, it is concluded that ZSCF can protect the liver against CCL₄-induce oxidative damage in rats, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.


Keywords Zizyphus, hepatotoxicity, antioxidants- and malondialdehyde

1. Introduction:

The liver is one of the most important organs, owing to its biological functions such as drug metabolism, amino acid metabolism, lipid metabolism and glycolysis. Acute and chronic liver diseases constitute a global concern, and medical treatments for these diseases are often difficult to handle, and have limited efficiency (Lee et al., 2007). Therefore, there has been considerable interest in role of complementary and alternative medicines for the treatment of liver disease. Developing therapeutically effective agents from natural products may reduce the risk of toxicity when the drug is used clinically. (Shen et al., 2009). Chemicals such as carbon tetrachloride (CCL₄) catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causes the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatases (ALP) into the circulating blood. (Singh et al., 1998; Xiong et al., 1998). The major component of the antioxidant system in mammalian cells consists of three enzymes, namely, Superoxide Dismutase (SOD), Catalase (CAT) and glutathione peroxidase. These enzymes work in concert to detoxify superoxide anion and hydrogen peroxidase (H₂O₂) in cells. Therefore, reducing oxidative stress may be an effective therapeutic strategy for preventing and treating hepatic fibrosis Amin and Ghoneim (2009).

Zizyphus spina-christi belongs to the family Rhamnaceae and grows throughout Upper Egypt and Sinai. Zizyphus has a common name ‘‘Nabka”, Arabs used it to maintain a healthy lifestyle and used for soothing properties (Adzu et al., 2002). Zizyphus species are commonly used in folklore medicine for the treatment of various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anemia, diarrhea, and insomnia (Han and Park, 1986; Kirtikar and Basu, 1984). The genus Zizyphus is known for its medicinal properties as hypoglycemic, hypotensive anti-inflammatory, antimicrobial, antioxidant, antitumour,
and liver protective agent and as an immune system stimulant (Said et al., 2006). They widespread in the Mediterranean region, Africa, Australia, and tropical America. Previous phytochemical studies on the different species of the genus Zizyphus led to the isolation and characterization of cyclopeptide alkaloids, flavonoids, sterols, tannins, and triterpenoid saponins (Ikram et al., 1981; Nawwar et al., 1984). Phytochemical studies of the genus Zizyphus have revealed that peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid and triterpenoidal saponin glycosides have been isolated and chemically identified. (Ikram et al., 1981; Higuchi et al., 1984; Nawwar et al., 1984; Han et al., 1990; Barboni et al., 1994; Abu-Zarga et al., 1995; Cheng et al., 2000; Shahat et al., 2001; Tripathi et al., 2001).

The aim of this work was to evaluate the role of Zizyphus spina-christi fruits to reduce the hepatotoxicity in rats that induced by CCL4.

2. Material and Methods:

Plant material

The fruits of Zizyphus spina-christi were collected from Sinai, Egypt in January 2010, and the plants were identified by the corresponding author. The fruits were cleaned and washed under tap water. Seeds were separated, dried at 40 °C under-vacuum oven and then crushed to a fine powder.

Total phenolic content

The total phenolic content was estimated using the Folin–Ciocalteu’s reagent (Obanda and Owuor, 1997; Singleton and Rossi, 1965). A calibration curve of gallic acid (ranging from 5 mg/ml to 30 mg/ml) was prepared and the results were determined using gallic acid standard curve, and expressed as milligram of gallic acid equivalents per gram of the extract.

Animals

Thirty six adult male albino rats, weighing (150-170) g each, were obtained from Medical Insects Research Institute, Doki, Cairo, Egypt. Rats were housed in wire cages under normal laboratory conditions and were fed on standard diet for one week as an adaptation period. Diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to rats by glass tubes projecting through the wire cages from an inverted bottle supported to one side of the cage. Food and water were provided ad-labium and checked daily. Standard diet was prepared from fine ingredients according to AIN, (1993).

Experimental groups:

Male Wister rats were randomly divided into two main groups, the first, negative control group (n=6), fed on basal diet and the second hepatotoxic groups (n=30), which were subjected to subcutaneous injection of a single dose of 0.3 ml/kg CC14 mixed with equal volume of corn oil on the 7th day. (Saraswat et al., 1993).

Hepatotoxic groups (n=30) were divided into 5 subgroups 6 rats per group (1) positive control fed on basal diet, group (2) fed on basal diet containing 2.5% of ZSCF, group (3) fed on basal diet containing 5% of ZSCF, group (4) fed on basal diet containing 10% of ZSCF powder and group (5) fed on basal diet containing 15% of ZSCF powder. Food intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by the determination of food intake and body weight gain.

Blood and tissue collection:

At the end of the experiment (8 weeks) rats were starved for 12h then sacrificed under general anesthesia (ether). Blood samples were collected into clean dry centrifuge tubes, stored at room temperature for 15 minutes, put into a refrigerator for one hour; centrifuged at 3000 rpm for 10 minutes to separate serum. Serum was carefully aspirated and transferred into dry clean Wassermann tubes using Pasteur pipette and then kept frozen at - 20° C till analysis.

Biochemical assays:

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamine transferase (GGT), alkaline phosphates(ALP) and total bilirubin (T.B) were determined using commercially available kits (Alkan Medical, Cairo, Egypt) according to the manufacturer’s instructions.

The most prominent product of lipid peroxidation (LP) is malondialdehyde (MDA) which is used as an indirect index of LP in biological system (Sorg, 2004). The method of Uchiyama and Mihara (1978) was used to determine MDA, based on its reaction with thiobarbituric acid to form a pink complex with maximum absorption at 535 nm. The superoxide dismutase (SOD) enzyme activity was determined according to the method described by Sun and
Zigman (1978). This method was based on the ability of SOD to inhibit the auto-oxidation of epinephrine at alkaline pH to adrenochrome and other derivatives, which were easily monitored in the near-UV region of the absorption spectrum.

The glutathione peroxidase (GSH-Px) activity was assayed by the method of Rotruck et al. (1973).

Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL-c) were determined using the methods described by of Allain et al. (1974), Fossati and Prencip (1982) and Lopez-virella (1977) respectively. The determination of low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were carried out according to the methods described by Lee and Nieman (1996) calculation was as follows:

\[
\text{VLDL}_c = \frac{\text{TG}}{5}\quad \text{And } \text{LDL}_c = \text{Total cholesterol} - (\text{HDL}_c + \text{VLDL}_c).
\]

**Statistical analysis**

SPSS (version 10) (SPSS Inc., Chicago, IL, USA) was used to carry out a one-way analysis of variance (ANOVA). When significant differences were detected by ANOVA, analyses of differences between the means of the treated and control groups were performed using Dennett's t-test.

**3. Results and Discussion**

The proximate chemical composition of dry ZSCF was presented in table (1). The results indicated that carbohydrate content (83.18%) was high in dry ZSCF while protein (4.7%), moisture (5.4%) and fat (0.94%) contents were low. These results are in agreement with those obtained Berry-Koch et al. (1990) and Abdelmuti (1991). Also, Saied et al. (2007) observed that the flesh of ZSCF is rich in carbohydrates. Also, Nour et al. (1987) and Abdelmuti (1991) found that one hundred gram dried fruit pulp contains 314 calories, 4.8 g protein and 0.9 g fat.

The present results also indicated that ZSCF contained high level of total phenolic compounds (7.55mg /g as gallic acid). Other investigators reported that ZSCF has many compounds such as peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, butulinic acid and triterpenoidal saponin glycosides (Ikram et al., 1981; Higuchi et al., 1984; Nawwar et al., 1984; Han et al., 1990; Barboni et al., 1994; Abu-Zarga et al., 1995; Cheng et al., 2000; Shahat et al., 2001; Tripathi et al., 2001).

Data in table (2) illustrates the mean value of liver functions for normal and hepatotoxic rats. Levels of ALT and AST in serum were used as biochemical markers to evaluate the hepatic injury. The serum ALT, AST, GGT and ALP levels and T.B content were significantly higher (P<0.05) in hepatotoxic groups than that in normal group. However the values of ALT, AST, GGT and ALP for 10% and 15% ZSCF levels were significantly lower than that of positive control. Serum T.B content level in 5, 10 and 15% ZSCF groups were significantly lower (P<0.05) than that of positive control. Carbon tetrachloride (CCL\(_4\)) induced a severe hepatic damage, which represented in markedly elevating activities of ALT and AST in serum (shen et al., 2009). Similar results were obtained by Amin and Ghoneim (2009) who found that serum AST and ALT levels were significantly increased in the fibrosis group compared to that in the normal group, but were significantly decreased in the ZSCF treated groups. Compared to that of normal rat, serum GGT activity increases 6.90 times after 8 weeks of CCL\(_4\) induction; serum AIP content decreases 66% after 8 weeks of CCL\(_4\) induction. ZSCF restores normal levels of both AIP and GGT in serum reduced the CCL\(_4\)-induced levels of ALT and AST. Shen et al. (2009) concluded that CCL\(_4\) induced a severe hepatic damage, which represented in elevating markedly activities of ALT and AST in serum.

Results in table (3) represent the mean values of lipid profile in normal and hepatotoxic rats. It was clear that serum cholesterol, triglyceride, LDL and VLDL were significant (P<0.05) higher in hepatotoxic positive control groups compared to that in normal group (negative control group). The hepatotoxic groups were significantly improved by addition of ZSCF especially at 15% level which
showed similar in serum cholesterol triglyceride, LDL and VLDL levels to negative control group.

HDL in hepatotoxic groups were improved by addition of ZSCF specially at 15% level. It is important to note that peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, butulinic acid and triterpenoidal saponin glycosides have been isolated and chemically identified from ZSCF (Ikram et al., 1981; Higuchi et al., 1984; Nawwar et al., 1984; Han et al., 1990; Barboni et al., 1994; Abu-Zarga et al., 1995; Cheng et al., 2000; Shahat et al., 2001; Tripathi et al., 2001).

Data in Table (4) indicated the effect of adding different portions of ZSCF to hepatotoxic rats diet on antioxidant defense system.

MDA is commonly used as marker of free radical mediated lipid peroxidation injury Amin and Ghoneim (2009). MDA level in serum of hepatotoxic positive control group was significantly (P<0.05) elevated compared to that in normal group. Addition of ZSCF to hepatotoxic rats diet reduced (P<0.05) MDA levels compared to positive control group. Increasing the levels of addition of ZSCF to the hepatotoxic rats diet groups resulted in significant decrease in MDA levels. Amin and Ghoneim (2009) showed that MDA level in CCL4-induced animals increased significantly by two-fold compared to control level.

SOD is the first line of defense to oxidative stress, and GSH is an intracellular reluctant that play major roles in catalysis, metabolism and transport (Amin and Ghoneim, 2009). The mean values of SOD and GSH activity of liver in hepatotoxic group were significantly lower than that of control group, while the 15% level of ZSCF group was recovered significantly (P<0.05) compared to the other concentration of ZSCF groups, followed by 10%, 5% and 2.5% ZSCF groups respectively. Amin and Ghoneim (2009), reported that pretreatment of ZSCF increased the activity of tested antioxidant enzymes SOD and CAT. The antioxidant properties of flavonoids from various plant extracts reveal their stimulatory action on antioxidative enzymes (Nagata et al., 1999; Sreelatha et al., 2009). Amin and Ghoneim (2009), showed that ZSCF exerts a therapeutic effect on CCL4-induced liver fibrosis in rats, possibly through its antioxidant action. Shen et al. (2009) observed that CCL4-induced generation of peroxy radicals and O2•− results in inactivation of CAT and SOD, and they showed that CCL4 challenge significantly decreased the activities of SOD and CAT in liver.

Zizyphus jujube administration inhibited lipid peroxidation at higher level after CCL4 treatment. Interestingly, FZJ 200mg/kg was able to increase the activities of endogenous antioxidant enzymes (SOD, CAT, and GSH-Px) and levels of GSH in hepatic tissue. FZJ pretreatment demonstrated to inhibit MDA of the reactive oxygen radical production (Shen et al., 2009).

Data in table (5) show the effect of adding different portions of ZSCF to hepatotoxic rats diet on body weight in rats. Before inducing hepatotoxicity, there was no significant difference in the body weight between groups. After the period of treatment it is clear that weight of normal group was increased significantly (P<0.05) compared to the positive control groups, followed by the weight of 15, 10, 5 and 2.5% of ZSCF groups respectively. The relative body weight gains were increased by 33.97, 30.53, 22.32, 21.57% for 15, 10, 5 and 2.5% of ZSCF groups respectively as compared to positive control group which increased by 15.42%.

Table (1). Proximate chemical composition of dry ZSCF

<table>
<thead>
<tr>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate(%)</th>
<th>Ash (%)</th>
<th>T. phenolic mg /g gallic</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7±0.8</td>
<td>7.4±0.2</td>
<td>0.94±0.3</td>
<td>83.18±029</td>
<td>3.79±0.4</td>
<td>7.55±0.33</td>
</tr>
</tbody>
</table>

Values in the table were expressed as means ± SD
Table (2) Effect of adding different portions of ZSCF to hepatotoxic rats diet on liver functions.

<table>
<thead>
<tr>
<th></th>
<th>Control(-)</th>
<th>Control (+)</th>
<th>Levels of ZSCF</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>91.1±4.03</td>
<td>170.75±1.7</td>
<td>125.47±2.98</td>
<td>123.69±2.36</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>44.22±4.03</td>
<td>67.84±1.70</td>
<td>59.75±2.98</td>
<td>57.74±2.36</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>17.36±0.75</td>
<td>65.13±2.76</td>
<td>36.66±3.63</td>
<td>33.72±1.21</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>34.5±2.5</td>
<td>112±7.5</td>
<td>56.4±6.2</td>
<td>50.0±3.3</td>
</tr>
<tr>
<td>T.B (mg/dl)</td>
<td>0.37±0.02</td>
<td>0.625±0.03</td>
<td>0.615±0.02</td>
<td>0.557±0.05</td>
</tr>
</tbody>
</table>

Values in the table are expressed as means ± SD
Different letters in the same row are significantly different (P<0.05)

Table (3). Effect of adding different portions of ZSCF to hepatotoxic rats diet on lipid profile:

<table>
<thead>
<tr>
<th></th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Levels of ZSCF</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>96.15±3.4</td>
<td>143.65±7.7</td>
<td>115.4±9.7</td>
<td>108.35±3.3</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>50.13±4.2</td>
<td>111.1±6.2</td>
<td>70.23±3.1</td>
<td>63.4±7.2</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>52.1±3.7</td>
<td>27.5±2.3</td>
<td>33.33±1.6</td>
<td>38.4±2.2</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>34.1±1.7</td>
<td>93.94±6.8</td>
<td>68±5.9</td>
<td>57.3±4.7</td>
</tr>
<tr>
<td>VLDLc (mg/dl)</td>
<td>10±0.82</td>
<td>22.21±1.24</td>
<td>14±0.78</td>
<td>12.7±1.4</td>
</tr>
</tbody>
</table>

Values in the table were expressed as means ± SD
Different letters in the same row are significantly different (P<0.05)

Table (4). Effect of adding different portions of ZSCF to hepatotoxic rats diet on antioxidant defense system.

<table>
<thead>
<tr>
<th></th>
<th>Control(-)</th>
<th>Control (+)</th>
<th>Levels of ZSCF</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>7.9±0.75</td>
<td>34±2.2</td>
<td>24.75±1.7</td>
<td>23.5±0.56</td>
</tr>
<tr>
<td>SOD (unit/prot)</td>
<td>7.3±0.28</td>
<td>2.71±0.35</td>
<td>3.63±0.75</td>
<td>4.4±0.45</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>31.55±2.9</td>
<td>16.93±2.9</td>
<td>20.2±1.8</td>
<td>22.9±2.5</td>
</tr>
</tbody>
</table>

Values in the table were expressed as means ± SD
Different letters in the same row are significantly different (P<0.05)
MDA (molondialdehyde), SOD(Superoxide dismutase enzymes), GSH(glutathione peroxides)
Table (5). Effect of adding different portions of ZSCF to hepatotoxic rats diet on body weight in rat.

<table>
<thead>
<tr>
<th>Levels of ZSCF</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control (-)</th>
<th>Control(+)</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Relative gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>162.25±2.22</td>
<td>162.25±3.86</td>
<td>157.75±4.19</td>
<td>233.25±4.27</td>
<td>43.75±1.69</td>
</tr>
<tr>
<td>Control(+)</td>
<td>162.25±2.22</td>
<td>187.25±3.30</td>
<td>191.75±4.78</td>
<td>215±4.08</td>
<td>5.06</td>
</tr>
<tr>
<td>2.5%</td>
<td></td>
<td></td>
<td>160.25±2.87</td>
<td>196±3.37</td>
<td>15.42±1.23</td>
</tr>
<tr>
<td>5%</td>
<td></td>
<td></td>
<td>159.75±3.20</td>
<td>308.5±2.65</td>
<td>21.57±2.34</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td>159.75±3.69</td>
<td>33.97±0.84</td>
<td>15.42±1.23</td>
</tr>
<tr>
<td>15%</td>
<td></td>
<td></td>
<td>160.5±3.69</td>
<td>33.97±0.84</td>
<td>22.32±1.78</td>
</tr>
</tbody>
</table>

Values in the table were expressed as means ± SD
Different letters in the same row are significantly different (P<0.05)

References


12/20/2010