Effect of *Hibiscus sabdariffa* calyx extract on carbon tetrachloride induced liver damage

D. Dahiru*, O. J. Obi and H. Umaru

*Department of Biochemistry, School of Pure and Applied Sciences, Federal University of Technology, P.M.B. 2076 Yola, Nigeria.*

Received 4 February 2003

MS/No BKM/2003/008, © 2003 Nigerian Society for Experimental Biology. All rights reserved.

---------------------------------------------------------------------------------------------------------------------------

**Abstract**

The effect of aqueous ethanol (1:1) extract of the calyx of *Hibiscus sabdariffa* on carbon tetrachloride (CCl₄) induced liver damage was investigated. Oral administration of the extract following a single CCl₄ dose promoted the healing of oxidative liver damage as determined by serum aminotransferases, ALT, AST, levels and liver thiobarbituric acid reactive substances levels. It appeared from the study that the extract of *Hibiscus sabdariffa* enhances the recovery from hepatic damage induced by CCl₄.

**Key words:** Carbon tetrachloride, *Hibiscus sabdariffa*, Liver damage.

---------------------------------------------------------------------------------------------------------------------------

*Correspondence Author. E-mail: ddahiru@futy.edu.ng*
INTRODUCTION

_Hibiscus sabdariffa_ Linn (Roselle) belongs to the family Malvaceae, which is native to Old World tropics, probably in the East Indies; now cultivated throughout the tropics (Duke and Archley, 1984). The vegetable is widely grown and commonly used as port herb or soup in the northern part of Nigeria. In Hausa the plant is locally called 'yakuwa', the seed 'Isontea' while the fresh calyx is referred to as 'soboroto'. The Yoruba call the leaves 'Amukan' and the flowers 'Ishapa' (William, 1980). The plant finds various uses in traditional medicine. It was reported to be antiseptic, digestive, diuretic, emollient and purgative (Truswell, 1992). It is a folk remedy for abscesses, billion conditions, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, neurosis, scurvy, and strangury (Duke 1985). Recent scientific research work has established the protective effect of the dried flower extract of _Hibiscus sabdariffa_ (Tseng et al., 1997), anti-inflammatory activity (Dafallah and Mustapha, 1996), antihypertensive effect of the calyx extract (Adegunloye et al., 1996 and Onyeneke et al., 1999) and antimutagenic activity (Chewonarin et al., 1999).

The chemistry of the calyx revealed that per 100g, it contained 49 calories, 84.5% H2O, 1.9g protein, 0.1g fat, 12.3g total carbohydrate, 2.3g fibre, 1.2g ash, 1.72mg Ca, 57mg P, 2.9mg Fe, 300µg β carotene equivalent, and 14mg ascorbic acid (Duke and Atchley, 1984). The presence of saponins, tannins, cyanogenic glycoside had been reported (Akanya et al., 1997). Other phytochemicals are protocatechuic acid a phenol (Lin et al., 2003) and anthocyanins (Wang et al., 2000).

Experimental liver damage produced by carbon tetrachloride (CCl4) has been extensively studied and the profile of damage even after the single administration of this hepatotoxic has been well established (Anand et al., 1992). The mechanisms of cell injury with CCl4 have been divided into two hypotheses. One is the lipid peroxidation theory (Farber and Gerson, 1984) while the other is the covalent binding theory (Hruszkewez et al., 1978). Cell damage by free radicals was reported as the predominant mechanism of hepatotoxicity (Gregus and Klaassen, 1995). The critical process underlying CCl4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990).

It has been shown that CCl4 induced lipid peroxidation can be obstructed by natural antioxidants (Subramanian et al., 1999 and Wang et al., 2000). The identification of naturally occurring inhibitors of peroxidation resulting in cell damage, in the diet could therefore lead to important new strategies for disease prevention (Subramanian et al., 1999), by providing cheaper and affordable sources of drugs.

The present investigation was undertaken to study the effect of feeding the aqueous ethanol (1:1) extract of _Hibiscus sabdariffa_ calyx on CCl4 induced liver damage. This was with the view to establish yet another advantage of ingesting the calyx.

MATERIALS AND METHODS

Plant Material

The plant material (Hibiscus sabdariffa L. Calyx) was obtained in Yola, Adamawa State, Nigeria. The plant was authenticated at the Biological Sciences Department, Federal University of Technology, Yola, Adamawa State. The plant material was dried under the sun prior to grinding into a fine powder using a laboratory mortar.
Preparation of Extract

One hundred-gram (100g) portion of the dried sample was weighed into a Whatman paper thimble and extracted by refluxing with aqueous ethanol (1:1) solvent system in a soxhlet apparatus for six hours. The extract was evaporated to dryness using rotary evaporator. The extract was weighed and stored at 4°C until required.

Animals

Male Wister strain albino rats weighing between 130-140g were purchased from the Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. The animals were housed in a well-ventilated room and fed with Vital feed (Grand Cereals and Oil Mills Ltd. Jos) and water ad libitum.

Animal treatment

The animals were distributed evenly into six (6) groups of five animals each in stainless steel cages under standard conditions (23±2°C, 60-70% relative humidity, and 12h photoperiod). All animals had free access to Vital pellet diet and water throughout the period of study. Carbon tetrachloride (1.0mg/kg) and extracts were administered to the animals orally by gastric intubation. CCl₄ used was of best quality available (Merck, Darmstadt, Germany). Group I received vehicle mineral oil (olive oil) as control. Group II received single dose of CCl₄ in mineral oil (1:1v/v). Group III in addition to CCl₄ received 24h later, a daily dose of the extract, 250mg/kg, for 3 days. Group IV was treated with CCl₄ as in III, but instead given 500mg/kg extract. Groups V and VI received only 250mg and 500mg/kg extracts, respectively, for 3 days.

Collection and preparation of tissue samples for analysis

Rats from the various groups were sacrificed by decapitation 24h after respective treatment period. The blood was collected into a clean centrifuge tube and allowed to stand in a test tube rack for 30 minutes to clot before being centrifuged at 1800rpm for 10 min to collect the serum. The serum was used to estimate the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total protein.

The liver was excised after dissecting the animals with a dissecting blade and placed in ice cold saline in a beaker. Weighed amount of liver tissue was minced and homogenised in Tris HCl buffer solution (1g liver tissue/10ml) using a glass Teflon, motorised Potter-Elveghem homogenizer (Melsungen, Germany). The homogenate was then centrifuged at 3000g for 10min at 4°C.

Analytical Method

The levels of aspartate aminotransferase and alanine aminotransferase were analysed by the method of Reitman and Frankel (1957), while total protein was analysed by the method of Wootton (1964) using three (3) replicates per sample.

Tissue lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) at 532nm by the method of Ferreyra et al., (1977). The assay mixture of 2.0ml contained 1.6ml of Tris HCl buffer (0.15M, pH 7.4), 0.2ml of potassium dihydrogen phosphate (10mM) and 0.2ml of 10% homogenate (Subramanian et al., 1999).

Statistical analysis

The results were expressed as Mean ± S.D for all groups. Student ‘t’ test was used to determine the significance between the Means.

RESULTS

The aqueous ethanol (1:1) extract of Hibiscus sabdariffa calyx gave a yield of 23.31g/100g plant material, representing
23.3% yields. The levels of hepatic lipid peroxidation were estimated in terms of the thiobarbituric acid reactive substances (TBARS) in nmoles/mg protein (Table 1). Hepatic lipid peroxidation was significantly increased (p<0.01) in CC14 group compared to control.

A significant decrease in the level of lipid peroxidation was observed in the two groups administered the extract compared to CC14 group. There was no significant difference between the lipid peroxidation levels in control group and those administered extracts only.

Table 1: Effect of Hibiscus sabdariffa calyx extract on CC14-induced lipid peroxidation in the rat liver.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lipid Peroxide content (n mole TBARS/mg liver protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Olive oil (solvent control)</td>
<td>1.48±0.06</td>
</tr>
<tr>
<td>II</td>
<td>CC14</td>
<td>2.16±0.02a</td>
</tr>
<tr>
<td>III</td>
<td>CC14; calyx extract(250mg)</td>
<td>1.50±0.03b</td>
</tr>
<tr>
<td>IV</td>
<td>CC14; calyx extract(500mg)</td>
<td>1.64±0.05b</td>
</tr>
<tr>
<td>V</td>
<td>Calyx extract(250mg)</td>
<td>1.47±0.05</td>
</tr>
<tr>
<td>VI</td>
<td>Calyx extract(500mg)</td>
<td>1.50±0.07</td>
</tr>
</tbody>
</table>

Values are means ± S.D. n=5.

a - Significantly higher (p<0.01) than solvent control (group I)

b - Significantly lower (p<0.01) than CC14 control (group II).

Table 2: Effect of Hibiscus sabdariffa calyx extract on serum ALT, AST and total protein levels of CC14 treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>ALT (i.u)</th>
<th>AST (iu)</th>
<th>Total protein (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Olive oil (control)</td>
<td>37.00±3.81</td>
<td>132.50±12.76</td>
<td>79.50±2.69</td>
</tr>
<tr>
<td>II</td>
<td>CC14</td>
<td>137.12±8.51*</td>
<td>209.30±12.49</td>
<td>81.00±1.87</td>
</tr>
<tr>
<td>III</td>
<td>CC14; extract(250mg)</td>
<td>49.25±2.17**</td>
<td>137.00±3.26</td>
<td>75.75±4.32</td>
</tr>
<tr>
<td>IV</td>
<td>CC14; extract (500mg)</td>
<td>54.75±4.44**</td>
<td>1.59.50±2.69</td>
<td>82.00±6.82</td>
</tr>
<tr>
<td>V</td>
<td>Extract(500mg)</td>
<td>41.13±4.03</td>
<td>133.10±7.08</td>
<td>78.75±3.96</td>
</tr>
<tr>
<td>VI</td>
<td>Extract(500mg)</td>
<td>43.78±2.86</td>
<td>148.40±6.50</td>
<td>81.75±3.37</td>
</tr>
</tbody>
</table>

* - Significantly higher than group I (p<0.01)

** - Significantly lower than group II (p<0.01).
The levels of ALT, and AST were significantly (p<0.01) elevated in CC1₄ rats by 3.7 fold and 1.58 fold respectively compared to control rats. Treatment with the extract (250mg/kg and 500mg/kg) following experimental liver damage resulted in marked reduction of both ALT and AST enzyme levels compared with control. Administration of 250mg/kg extract restored ALT and AST levels to nearly normal by 75% and 98.5%, respectively, while treatment with 500mg/kg extract restored ALT and AST levels by 67.6% and 83.1%, respectively. Total protein levels of CC1₄ rats and other experimental groups did not show any significant change compared to normal control. The extract at both 250 and 500mg/kg administered alone (GP V and VI) did not show any significant effect on the levels of ALT, AST and total protein compared to control.

DISCUSSION

The hepatic lipid peroxidation level was significantly (p<0.01) increased in CC1₄ rats compared to solvent control. This finding is in accordance with the known hepatic toxic effect of CC1₄ that causes oxidative damage in the liver (Farber and Gerson, 1984). Similarly the elevation of serum ALT and AST is a known effect of CC1₄ toxicity which specifically affects the liver (Anand et al., 1992). That the levels of liver enzymes ALT, AST, in the serum were lower when CC1₄ treated rats are given Hibiscus sabdariffa calyx extract than in untreated CC1₄ control is an evidence that the extract promotes healing of peroxidised liver. Administration of 250mg/kg extract was more effective in lowering the enzymes level compared to 500mg/kg. Thus the peroxidative wound healing of the extract was not dose dependent at the concentrations used. It was earlier suggested that the extract at higher doses, and when given for a long period could be toxic (Akindahunsi and Olaleye, 2002). The protective effect of the extract could be due to the rich Vitamin C content of the extract (Duke and Atchley, 1984 and Akanya, 1997), which serves as an antioxidant and a reductant especially in the conversion of any $\alpha$-tocopheroyl radicals formed, to $\alpha$-tocopherol (Packer and Kagan 1993). The presence of Hibiscus protocatechuric acid (phenol) and Hibiscus anthocyanins both isolated from the flower were reported to have protective effect against tart butyl hydroperoxide induced hepatic toxicity in rats (Tseng et al., 1996; Wang et al., 2000). It might be possible that the calyx also contained these natural antioxidants which produced the observable effects.

In conclusion, CC1₄ induces liver damage. At low doses of Hibiscus sabdariffa calyx extract, wound-healing activity due to hepatic liver damage was highly promoted. It could be possible that moderate ingestion of the calyx in form of juice can be of great advantage.

REFERENCES


Park, J.H.Y; Vanderhoof, J.A; Blackwood S. and Macdonald R.S. (1990) Characterisation of type I and type II insulin-like growth factor receptors in an


