BIOKEMISTRI 15 (1): 27-33 (June 2003)

An international journal published by the
NISEB
Nigerian Society for Experimental Biology

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 (CCl_4) induced liver damage was investigated. Oral administration of the extract following a single CCl_4 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 CCl_4.

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 anti-mutagenic activity (Chewonarin *et al.,* 1999).

The chemistry of the calyx revealed that per 100g, it contained 49 calories, 84.5% H_2O, 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 protocatechuric acid a phenol (Lin *et al.,* 2003) and anthocyanins (Wang *et al.,* 2000).

Experimental liver damage produced by carbon tetrachloride (CCl_4) has been extensively studied and the profile of damage even after the single administration of this hepatotoxin has been well established (Anand *et al.,* 1992). The mechanisms of cell injury with $CC1_4$ 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 CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990).

It has been shown that $CC1_4$ 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 $CC1_4$ 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. CCl_4 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 CCl_4 in mineral oil (1:1v/v). Group III in addition to $CC1_4$ received 24h later, a daily dose of the extract, 250mg/kg, for 3 days. Group IV was treated with CCl_4 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 aspatate 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 $CC1_4$ group compared to control.

A significant decrease in the level of lipid peroxidation was observed in the two groups administered the extract compared to $CC1_4$ 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 $CC1_4$-induced lipid peroxidation in the rat liver.

Group	**Treatment**	**Lipid Peroxide content (nmole TBARS/mg liver protein)**
I	Olive oil (solvent control)	1.48±0.06
II	CCl_4	2.16±0.02a
III	CCl_4; calyx extract(250mg)	1.50±0.03b
IV	CCl_4; calyx extract(500mg)	1.64± 0.05b
V	Calyx extract(250mg)	1.47±0.05
VI	Calyx extract(500mg)	1.50±0.07

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 CCl_4 control (group II).

Table 2: Effect of *Hibiscus sabdariffa* calyx extract on serum ALT, AST and total protein levels of $CC1_4$ treated rats.

Groups	**Treatment**	**ALT (i.u)**	**AST (iu)**	**Total protein (g/l)**
I	Olive oil (control)	37.00±3.81	132.50±12.76	79.50±2.69
II	$CC1_4$	137.12±8.51*	209.30±12.49	81.00±1.87
III	$CC1_4$, extract(250mg)	49.25±2.17**	137.00±3.26	75.75±4.32
IV	$CC1_4$, extract (500mg)	54.75±4.44**	1.59.50±2.69	82.00±6.82
V	Extract(500mg)	41.13±4.03	133.10±7.08	78.75±3.96
VI	Extract(500mg)	43.78±2.86	148.40±6.50	81.75±3.37

** - 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 $CC1_4$ 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 $CC1_4$ 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 $CC1_4$ rats compared to solvent control. This finding is in accordance with the known hepatic toxic effect of $CC1_4$ that causes oxidative damage in the liver (Farber and Gerson, 1984). Similarly the elevation of serum ALT and AST is a known effect of CCl_4 toxicity which specifically affects the liver (Anand et al., 1992). That the levels of liver enzymes ALT, AST, in the serum were lower when CCl_4 treated rats are given *Hibiscus sabdariffa* calyx extract than in untreated CCl_4 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 ∝-tocopheroxyl radicals formed, to ∝-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, CCl_4 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

Adegunloye, B.J, Omoniyi, J.O, Owolabi, O.A; Ajagbonna, O.P; Sofola O.A, Coker H.A. (1996) Mechanisms of blood pressure lowering effect of the calyx of *Hibiscus sabdariffa* in rats.*Afr. J. Med. Sci.* **25**: 235-8.

Akanya, H.O; Oyeleke, S.B, Jigam, A.A. and Lawal, F.F. (1997) Analysis of sorrel drink (Soborodo). *Nig. J. Biochem.Mol. Biol.***12**:77-82.

Akindahunsi, A.A. and Olaleye, M.T. (2002) Toxicological investigation of extract of *Hibiscus sabdariffa* (calyces). A paper presented at the 22nd annual conference of the Nigerian Society of Biochemistry and Molecular Biology, 18th-21st September. Zaria, Nigeria.

Anand, K.K; Signh, B, Chemd, D. and Bhandan, B.K. (1992) An evaluation of *Lawsonia alba* extract as hepatoprotective agent. *Planta Medica* **58**:22-25.

Castilla-Cortazar, I., Garcia M., Begona, M., Jorge, Q., Raquel, P., Santiago, S and Jesus, P. (1997) Hepatoprotective effects of insulin-like growth factor I in rats with carbon tetrachloride-induced cirrhosis. *Gastroenterology* **113**:162-1691.

Chewonarim T; Kinouchi, T; Kataoka K; Arimochi H; Kuwahara, T; vinitketkumuen, U. and Ohnishi, Y. (1999) Effect of roseele (*Hibiscus sabdariffa* Linn) a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and formation of aberrant crypt foci induced by colon carcinogens azoxymethane and 2-amino-1-me-Hyl 6-phenylimidazo (4,5-b) pyridine in F344 rats. *Food Chem.Toxicol* **37**: 591-601.

Dafallah, A.A. and al-Mustafa, Z. (1996) Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibicus sabdariffa*. *Am J. Clin. Med.* **24**:263-9.

Duke, J.A. (1985) Handbook of Medicinal Herbs (7th ed.) Livingstone Group Ltd. Edinburgh 228-9.

Duke, J.A. and Atchley, A.A. (1984) Proximate analysis. In: Christie, B.R. (Ed.), *The Handbook of Plant Science in Agriculture*. CRC Press, Inc., Boca Raton, Florida.

Edmonson, H.A., Peters, R.L. (1985) Pathology In: Kiosane, J.H. (Ed) V. Mosby Co., St. Louis, Toronto 2:1097-1101.

Farber, J.L. and Gerson, R.J. (1984) Mechanism of cell injury of hepatotoxic chemicals. *Pharmacological Reviews* **36**:715-755.

Ferreyra E.C., de Fenos O.M., Bernacchi, a.s., de Castro C.R., Castro, J.A. (1977) Treatment of Carbon tetrachloride induced liver necrosis with chemical compounds. Toxicology and Applied Pharmacology. 42:513-521.

Gregus, Z. and Kiwassen, C. (1995) Mechanism of toxicity. In: Klaassen C.D. (Ed) *The Basic Science of Poisons*, 5th ed. New York, McGraw-Hill 35-74.

Hruskewycz, A.M; Glende, E.A. and Rechnagel, R.O. (1978) Destruction of microsomal cytochrome P_{450} and glucose 6-phosphatase by lipid extracted from peroxiditized microsomes. *Toxicol.Appl. Pharmacol*. **46**:695-702.

Lin, W.L.; Hsieh, Y.J; Chou, F.P. Wang, C.J; Cheng, M.T. and Tseng, T.H. (2003) Hibiscus protocatechuic acid inhibits lipopolysaccharide induced rat hepatic damage. *Arch Toxicol* **77**:42-7.

Masuda, Y. and Nakamura, Y. (1990) Effect of oxygen deficiency and calcium omission on $CC1_4$ in isolated perfused livers from phenobarbutal pretreated rats. *Biochem. Pharm.* **40**:1865-1876.

Onyeneka, P.C., Ajani, E.O., Ameh, D.A. and Gamaniel, K.S. (1999) Anti hypertensive effect of roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and comparison of its toxicity with that in wistar rats. *Cell Biochem Funct* **17**:199-206.

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

intestinal epithelial cell line. *Endocrinology* **126**:2998-3005.

Reitman, S. and Frankel, S. (1957) Colorimetric method for the determination of serum oxaloacetic and glutamine pyruvic transaminases. *Am. J. Clin. Pathol.* **28**:53-56.

Subramanian, L.R. Selvam; A.L. Mudaliar (1999) Prevention of $CC1_4$ induced hepatotoxicity by aqueous extract of Tumeric. *Nutr. Res*. **19**: 429-441.

Truswell, A. S. (1992) ABC of Nutrition 2nd (ed). Tavisteek Square Inc. London 50-93.

Tseng, T.H; Kao, E.S.; C.Y; chou, F.P. Lin wu H.W. and Wang, C.J. (1997) Protective effect of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. *Food Chem. Toxicol* **35**:1159-64.

Wang, C.J; Wang J.M; Lin WL, Chu, CY; Chau, F.P. and Tseng, T.H. (2000) Protective effect of *Hibiscus* anthoyanins against tert-butl hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol* **38**: 411-6.

William, C.N; chew, W.Y. and Rahuraruma, J.A. (1980) Tree and Field Crops of the Welter Regions of the Tropics, U.K. Group, London 88.

Wootton, I.D.P. (1964) In. *Microanalysis in Medical Biochemistry* (4th Ed) J. and A Churchill Ltd. London. 138-140.