BIOKEMISTRI 20(2):47-52 (December 2008)
This article is downloadable online in PDF format at
http://www.bioline.org.br/bk

Printed in Nigeria

An international journal published by the

Nigerian Society for Experimental Biology

The effect of *Hibiscus sabdariffa* calyx extract on cisplatin-induced tissue damage in rats

Tebekeme OKOKO[1*] and Ibiba F. ORUAMBO[2]

[1]*Biochemistry Division, Department of Chemical Sciences, Niger Delta University, P.M.B 71, Wilberforce Island, Bayelsa State, Nigeria*
[2]*Department of Chemistry, Rivers State University of Science and Technology, P.M.B 5080, Port Harcourt, Rivers State, Nigeria*

Received 14 December 2008

MS/No BKM/2008/050,

Abstract

The effect of *Hibiscus sabdariffa* calyx extract on cisplatin-induced tissue damage was studied. A total of twenty rats were used for the study and split into four groups of five rats per group-group I, II, III, and IV. Tissue damage was induced in rats of groups II, III, and IV by a single intraperitoneal administration of cisplatin (5 mg/kg b.w.). Four days later, rats in groups III and IV were given 200 mg/kg b.w. and 300 mg/kg b.w respectively of the *Hibiscus sabdariffa* calyx extract once daily for another five days. Rats in group I were untreated controls. Tissue damage was later assessed in sera by measuring the levels of alanine aminotransferase, aspartate aminotransferase, urea, and creatinine. Thiobarbituric acid reactive species, catalase, and reduced glutathione levels were also measured in the livers and kidneys. The results reveal that the administration of cisplatin alone resulted to a significant increase in the levels of the serum markers over controls ($p < 0.05$). Cisplatin also caused a significant decrease in catalase activity, and also caused a significant reduction in the levels of reduced glutathione in the liver and kidney over controls ($p < 0.05$). Cisplatin also caused a significant increase in the levels of thiobarbituric acid reactive species in the liver ($p < 0.05$). All the markers assessed were brought to near control levels when the *Hibiscus sabdariffa* extract was given to the rats with the dose of 300 mg/kg b.w. possessing a higher activity. The results show that the *Hibiscus sabdariffa* extract ameliorated cisplatin-induced tissue damage in rats which may be dose-dependent.

Keywords: *Hibiscus sabdariffa,* urea, creatinine, cisplatin, glutathione

*To whom correspondence should be addressed. **E-mail:** tebebuddy@yahoo.com , **Tel**: +2348051069342

INTRODUCTION

The consumption of botanicals as complementary/ alternative medicine has been encouraged because they are relatively cheap and coupled with the belief that they could significantly contribute to the improvement of human health in terms of cure and the prevention of various human disorders in addition to the less frequent side effects reported when compared to modern medicine[1]. Among these botanicals of focus is *Hibiscus sabdariffa* Linn which is known as the Red Sorrel in English and the Zobo plant in Nigeria. *Hibiscus sabdariffa* Linn is an annual dicotyledonous herbaceous tropical plant that belongs to the family Malvaceae. The tender leaves of the plant are eaten as salad and curries traditionally and as a remedy for various ailments such as liver disorders, hypertension, pyrexia[2], and as a diuretic, emollient, and purgative[3]. The infusions of the calyces have been shown to reduce intestinal transit, anti-inflammatory and anti-mutagenic effects[4-6] among others. Duke and Atchley[7] revealed that the calyces of the plant (the principally utilized part) contain 14 mg ascorbate, 300 µg β-carotene, 1.9 g protein, 12.3 g total carbohydrate, 2.7 mg Iron per 100 g dry weight. In addition to these nutrients, various phytochemicals have also been identified in the calyces which include saponins, tannins, anthocyanins, cyanogenic glycosides, flavones, and polyphenols[8,9]. Despite these established bioactivities, it is a common belief that the plant is still underutilised as it is obvious that it could also possess other known activities due to the phytochemicals identified. Based of these, this work focused on the effect of the methanolic extract of the calyces of *Hibiscus sabdariffa* on cisplatin-induced oxidative damage in rat. Cisplatin (also called cisplatinum and carboplatin) is an anticancer drug but its cytotoxic potential is a major limiting factor of its chemotherapy as its use causes various tissue injuries notable among them is dose related kidney damage[10].

MATERIALS AND METHODS

Animals

Twenty albino rats (*Rattus novergicus*) of both sexes (100-115 g) were purchased from the Nigerian Institute of Medical Research, Yaba, Lagos and kept in the animal housing facility of the Department of Biological Sciences of the Niger Delta University. They were allowed unlimited access to tap water and growers' mash *ad libitum* housed in standard rat cages.

Plant material and preparation of extract

The calyces of matured *Hibiscus sabdariffa* were collected fresh from a garden and sun-dried. The dried calyces were subsequently ground using a blender to fine powder. Five hundred grams of the powder was extracted using light petroleum in a soxhlet extractor for 24 hours. A second extraction was made using 80 % methanol and concentrated using a rotary evaporator at 40^0C and finally dried in a vacuum dessicator at 40^0C. The resulting residue which weighed 8.13 g (recovery 1.63%) was later stored under -4^0C until required. However, a 10 mg/ml solution of the extract was prepared in distilled water before administration to the rats.

Treatment of animals

The rats were split into four groups of five rats each after matching the weights. They were acclimatized to laboratory conditions for five days before commencement of treatment. Tissue damage was induced in rats of groups II, III, and IV by a single intraperitoneal-administration of cisplatin (5 mg/kg body weight). Four days later, doses of 200 mg/kg b.w., and 300 mg/kg b.w. of the *Hibiscus sabdariffa* calyx extract was administered to rats in group III, and group IV respectively through the oral route using a gavage needle once daily for five days. Rats in group II were given sterile water in place of the *Hibiscus sabdariffa* extract. Rats in group I were untreated controls. They were all allowed unlimited access to tap water and growers' mash.

Collection of tissues

Twenty four hours after the last administration of the extract, each rat was subjected to light anaesthesia in a urethane saturated chamber, and dissected. The thoracic region was exposed and blood was drawn through direct cardiac punctures and delivered into sample containers having no anticoagulant. Blood samples were allowed to clot for 20 minutes and centrifuged at 4000 rpm for 15 minutes.

Sera were collected using micropipettes and analysed. The livers and kidneys were also excised immediately and washed in cold saline. Ten percent tissue homogenates were prepared in phosphate buffer (0.05M, pH 7.25). Perinuclear fractions were obtained after centrifuging homogenates at 1500 rpm for 20 minutes using a centrifuge.

Biochemical analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in sera as described[11]. Creatinine and urea were determined in serum by the modified Jaffe, and the modified Berthelot-Searcy enzymatic methods respectively using assay kits obtained from QCA Spain. Catalase in liver and kidney was measured in tissue homogenates following the method of Aebi[12] with slight modifications. Briefly, 0.1 ml of perinuclear fraction was added to 1.5 ml of phosphate buffer (0.05M, pH 7.25), and 1.4 ml of distilled water. The reaction was initiated by the addition of 0.1 ml of 0.01M H_2O_2. Amount of H_2O_2 consumed was determined by measuring absorbance at 240 nm after 30 s. The enzyme activity was expressed as k/g protein. Thiobarbituric acid reactive species (TBARS) were measured in the perinuclear fractions as reported[13]. Reduced glutathione (GSH) was also measured in the perinuclear fractions as previously described[14].

Statistical analysis

Results of representative values were expressed as mean ± standard error of the mean (S.E.M). Comparisons between two groups were made using a two-tailed Student's *t*-test. P values below 0.05 were considered statistically significant.

RESULTS

The levels of ALT, AST, creatinine and urea levels in sera are shown in Table 1. When the rats were treated with cisplatin alone, there was a significant increase in the levels of ALT, AST, creatinine and urea in group II over control rats ($p < 0.05$), while the levels of these markers in groups III, and IV were significantly reduced close to control levels after administration of the extract ($p < 0.05$), with group IV having the lowest values. Table 2 shows the levels of GSH, catalase, and TBARS in the livers and kidneys of the rats.

Table 1: Effect of pre-treatment with or without extract on some serum enzymes of hepatotoxicity, and non-enzymatic markers of kidney dysfunction

Group	ALT (U/L)	AST (U/L)	Creatinine(mg/L)	Urea (mg/L)
I	15.6±0.02	28.8±0.03	16.4±0.54	126.7±1.65
II	24.8±0.17[a]	52.4±2.82[a]	27.3±1.43[a]	293.6±7.32[a]
III	18.3±0.44[b]	33.5±0.57[ab]	17.8[b] ± 0.21	137.8±11.21[ab]
IV	15.8±1.22[b]	29.2±1.45[bc]	16.2 ± 1.04[b]	128.2±9.21[abc]

Values presented as the means ± S.E.M of readings from 5 individual rat sera ([a] significantly different from Group I; [b] significantly different from group II; [c] significantly different from group III, $p < 0.05$)

Table 2: Effect of pre-treatment with or without extract on the glutathione, catalase, and TBARS levels in liver and kidney

Tissues and Groups	TBARS (µg/mg protein)	GSH (mg/g protein)	Catalase (k/g protein)
Liver I	1.13±0.00	8.93±0.54	31.72±1.42
II	2.67±0.12[a]	6.26±0.07[a]	17.44±0.23[a]
III	1.25±0.01[b]	7.43±0.13[ab]	23.17±0.65[ab]
IV	1.23±0.14[b]	9.53±0.48[bc]	27.56±1.21[abc]
Kidney I	0.50±0.01	2.73±0.06	105.76±8.55
II	0.49±0.05	1.63±0.03[a]	68.12±3.28[a]
III	0.49±0.03	2.74±0.01[b]	87.35± 9.71[ab]
IV	0.51±0.03	2.87±0.22[b]	93.58±7.76[abc]

Values presented as the means ± S.E.M of readings from perinuclear fractions from homogenates from 5 individual rat tissue ([a] significantly different from Group I; [b] significantly different from group II, [c] significantly different from group III, $p < 0.05$)

When the rats were treated with only cisplatin, there was a significant reduction in the levels of GSH and catalase in both liver and kidney over controls ($p < 0.05$), while these reduced levels were increased close to control levels after the extract was given to the rats ($p < 0.05$) with the group given the dose of 300 mg/kg b.w. having the highest elevations. The level of TBARS in liver was significantly higher when the rats were treated with cisplatin alone ($p < 0.05$). However, the level of the marker was significantly reduced when the extract was administered to the rats ($p < 0.05$). The levels of TBARS in the kidneys did not show any significant difference between the groups ($p > 0.05$).

DISCUSSION

Cisplatin is a cell cycle phase non-specific cytotoxic agent[15] and it is this property that has been exploited in the treatment of various cancers. As with other drugs, its lack of specificity for neoplastic cells makes it seriously limiting. A major consequence is the reported nephrotoxicity and hepatotoxicity[16]. Though the toxicokinetics of cisplatin has not been fully known, lipid peroxidation may be involved in its adverse effects. The principal active metabolite is free filterable platinum[17] which has the propensity of adding to double bonds of membrane lipids, form adducts with proteins, DNA, and can also form stable products with other macromolecules.

Chemotherapeutic levels known to induce mild kidney dysfunction in rats is thought to be a single dose of 5 mg/kg body weight which peaks about 3 – 5days[10,18] thus the choice of a single dose of 5 mg/kg body weight, and the four days exposure before the administration of the extract for the present study.

The increase in serum creatinine and urea levels in the rats after the intraperitoneal administration of cisplatin (group II) shows that cisplatin induced mild kidney dysfunction which is in accordance with previous studies[19]. This damage to the kidney was further experienced with the depletion in the levels of GSH and catalase in the kidney.

The increase in the levels of ALT and AST in the sera of rats in group II also show that at that dose (5 mg/kg b.w.), cisplatin also damages the liver which was further evidenced by the elevated level of TBARS, and the reduction in the levels of GSH and catalase in the perinuclear fraction of the rat livers. The antioxidant enzyme catalase and GSH with other peroxidases constitute a supportive team against reactive oxygen species[20,21] hence the depletion of GSH and catalase could enhance lipid peroxidation[22].

The reduction in the levels of TBARS, ALT, AST, and the elevation of catalase and GSH in the liver close to control levels in rats of groups III and IV clearly show that the *Hibiscus sabdariffa* extract reduced the liver damage induced by cisplatin. Also the reduction in the levels of serum creatinine, urea and the elevation of the levels of kidney GSH and catalase in rats of group III, and IV also indicate that the extract also reduced cisplatin induced kidney dysfunction. From the results, it was obvious that the bioactivity of the extract is dependent on the dose administered. The mechanism of this action is not fully understood. However, it could be suggested that the extract reduced the lipid peroxidation induced by cisplatin. It could also be suggested that the various phytochemicals inherent in the extract acted synergistically to sequester the free filterable platinum hence making it less available for cellular damage. The calyces of *Hibiscus sabdariffa* are rich in vitamin C, hence the reduction in the liver and kidney damages may be ascribed partly to this natural antioxidant which also functions in the conversion of α-tocopheroxy radical to α-tocopherol[23]. The extract may also reduce the involvement of Ca^{2+} dependent permeabilisation of renal cortex mitochondria as it has been shown that there is a possible involvement of Ca^{2+} dependent permeabilisation of renal cortex mitochondria in cisplatin nephrotoxicity[19]. Anthocyanins are ubiquitous in the calyces of the plant thus this property of the extract may be attributed partly to these flavonoids which are known to be potent antioxidants and free radical scavengers[25].

Further work should focus on elucidating the actual protective mode of the extract. Since there are various phytochemicals in the calyces, proper fractionation of the extract should be done and each fraction subjected to

both in vitro and in vivo studies since some notable bioactivities of terpenes have also been reported[25] in addition to the fact that flavonoids also possess pro-oxidant activities [26]. Progress in this area will also extend the borders of even cisplatin chemotherapy.

The occurrence of *Hibiscus sabdariffa* Linn in the biosphere is nature's generosity in providing mankind with cheap and natural bioactive materials. Thus the exploitation of this natural gift in the production of neutraceuticals is recommended.

REFERENCES

1. **Hu, X., Sato, J., Oshida, Y., Yu, M., Bajotto, G. and Sato, Y. (2003)** Effect of Goshajinki-gan (Chinese herbal medicine): Niu-che-sen-qi-wan) on insulin resistance in streptozotocin induced diabetic rats. *Diab. Res. Clin. Pract*. **59**:103-111.
2. **Chifundera., K, Balagizi, K. and Kizungu, B. (1994)** Les empoisonnements et leurs antidotes en me´ decine traditionnelle au Bushi, Zaire. *Fitoterapia* **65**:307-313.
3. **Truswell, A. S. (1992)** *ABC of Nutrition*. 2nd (ed). Tavisteek Square Inc., London, pp. 50-53.
4. **Owulade, M. O., Eghianruwa, K. I. and Daramola, F. O. (2004)** Effect of aqueous extracts of *Hibiscus sabdariffa* calyces and ocimum gratissimum leaves on intestinal transit in rats *Afr. J. Biomed. Res*. **7**:31 – 33.
5. **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**:263-269.
6. **Chewonarim, T., Kinouchi, T., Kataoka, K., Arimochi, H., Kuwahara, T., Vinitketkumuen, U. and Ohnishi, Y. (1999)** Effect of roselle (*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.
7. **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, p. 32.
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.
9. **Wang, C. J., Wang, J. M., Lin, W. L., Chu, C. Y., Chou, F. P. and Tseng, T. H. (2000)** Protective effect of *Hibiscus* anthocyanins against tert-butyl Hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol*. **38**:411-416.
10. **Stein, J., Lifschitz, M. and Barnes, L. (1978)** Current concepts on the nephrotoxicity of acute renal failure. *Am. J. Physiol*. **243**:F171-F181.
11. **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.
12. **Aebi, H. E. (1987)** *Catalase*. In: Bergmeyer, HU (Ed.), Methods of Enzymatic Analysis. VCH Verlagsggesellscaft, Weinheim p. 273-285.
13. **Ohkawa, H., Ohishi, N., and Yagi, K. (1979)** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*. **95**:351-358.
14. **Fairbanks, V. F., and Klee, G. G. (1986)** *Biochemical aspects of haematology*. In: Tietz NW (Ed.), Textbook of Clinical Chemistry. Saunders Company, Philadelphia, pp. 1498-1533.
15. **Haskel, C. M. (1990)** *Cancer treatment*. 3rd ed. Philadelphia, W. B. Saunders Co. pp. 55-56.
16. **Kintzel, P. E. (2001)** Anticancer drug-induced kidney disorder. *Drug saf*. **24**:19-38.
17. **Balis, F. M., Holcenberg, J. S. and Bleyer, W. A. (1983)** Clinical pharmacokinetics of commonly used anticancer drugs. *Clin. Pharmacokin*. **8**:202-232.
18. **Singh, J. (1989)** A possible mechanism of cisplatin-induced nephrotoxicity. *Toxicol* **58**:71-80.
19. **Campbell, P. I. and Al-Nasser, I. A. (1996)** Renal insufficiency induced by cisplatin in rats is ameliorated by cyclosporine-A. *Toxicol.* **114**:11-17.
20. **Bandhopadhay, U., Das, D. and Banerjee, K. R. (1999)** Reactive oxygen

species: Oxidative damage and pathogenesis. *Curr. Sci*. **77**:658-665.

21. **Tabatabaie, T. and Floyd, R. A. (1994)** Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents. *Archiv. Biochem. Biophy*. **314**:112-119.
22. **Anandan, R., Deepa-Rekha, R. and Devaki, T. (1999)** Protective effect of *Picrorrhiza kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine- induced hepatitis in rats. *Curr. Sci*. **76**:1543-1545.
23. **Packer, L. and Kagan, V. E. (1993)**. Vitamin E: the antioxidant harvesting centre of membranes and lipoproteins. Marcel Dekker Inc., New York. pp 131-139.
24. **Gilani, A. H. and Janbaz, K. H. (1995)** Preventive and curative effects of *Artemisia absinthium* on acetaminophen and CCl_4– induced hepatotoxicity. *Gen. Pharmacol*. **26**:309-315.
25. **Zhou, Q., Xie, H., Zhang, L., Stewart, J. K., Gu, X. and Ryan, J. J. (2006)** cis-Terpenones as an effective chemopreventive agent against aflatoxin B1-induced cytotoxicity and TCDD-induced P4501A/B activity in HepG2 cells. *Chem. Res. Toxicol*. **19**:1415-1419.
26. **Galati, G. and O'Brien, P. J. (2004)** Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemoprotective and anticancer properties. *Free Rad. Biol. Med*. **37**:287-303.