Vol. 11 (2008) 39-42

Comparative Antioxidant Activity of *Hibiscus sabdariffa* and Ascorbic Acid on Ferrous Sulphate-induced Oxidative Stress in *Clarias gariepinus.*

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Lipid peroxidation is becoming a popular biological marker of oxidative stress. *Hibiscus sabdariffa* has been reported to serve as a herbal remedy for various disease conditions, but studies on its antioxidant activity and the extent to which it acts remain scarce. The antioxidant activity of *H. sabdariffa* aqueous extracts, an indigenous herbal drink, was compared with that of ascorbic acid in *Clarias gariepinus* (African catfish) with ferrous sulphate-induced oxidative stress. Eye tissue and blood samples were collected for the assay of reduced glutathione, malondialdehyde, lipid hydroperoxide and glucose levels. Administration of *H. sabdariffa* aqueous extract (0.27 ml/kg body weight) resulted in a significant reduction ($p<0.05$) in glucose levels (75.48±10.87 mg/dl) as compared with ascorbic acid (88.06±4.44 mg/dl). It was also observed that the aqueous extract significantly reduced ($p<0.05$) the lipid hydroperoxide levels (1.66±2.24 nmol/ml) as compared with ascorbic acid (2.04±2.21 nmol/ml). The results obtained suggest that the *H. sabdariffa* aqueous extract possesses antioxidant potency comparable with that of ascorbic acid.

Keywords: Oxidative stress, antioxidants, *Hibiscus sabdariiffa*, ferrous sulphate, *Clarias gariepinus*.

INTRODUCTION

The interaction between contaminants and biomolecules is the first step in the generation of toxic effects. Thus an understanding of the biochemical alterations induced by pollutants may contribute to the prediction of toxic effects that may occur at higher levels of biological organization [1].

Oxidative stress is the disruption of the pro-oxidant-antioxidant balance in favor of the former leading to potential damage to DNA, proteins and lipids as well as cell apoptosis [2]. Although almost all organisms possess antioxidant defense and repair systems, these protective systems may be insufficient to entirely prevent the stress and accompanying damage induced by oxidative agents including ferrous sulphate [3]. Therefore, research efforts are currently focusing on the protective biochemical complement of naturally occurring plant phytochemicals which have antioxidant activity. These phytochemicals (vitamins, proteins, enzymes, carotenoids, flavonoids, anthocyanins, phenolic compounds and pectin) vary in composition and mechanism of action in protecting tissues against oxidative damage [4].

The present study was aimed at comparing the antioxidant potential of the aqueous extract of *Hibiscus sabdariffa* with vitamin C in reversing or minimizing the oxidative stress and associated damage induced by ferrous sulphate in African catfish.

MATERIALS AND METHODS

Plant material

The dried calyces of *Hibiscus sabdariffa* were purchased from a local market in Abraka, Delta State, Nigeria. The plants were identified at the Botany Department of Delta State University in Nigeria.

Preparation of *Hibiscus sabdariffa*

The aqueous extract of *Hibiscus sabdariffa* (HSEt) was locally prepared by boiling 200 g of

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the calyces in 200 ml of tap water at 100 °C for 15 min. The mixture was pounded with a pestle and then filtered. The filtrate was kept in plastic bottles and stored under refrigeration at 3 °C.

Preparation of vitamin C tablets

White vitamin C tablets (May and Baker, Dagenham, UK) were purchased from Rio Pharmacy, Abraka, Delta state, Nigeria. The tablets were powdered, transferred into a stoppered bottle and stored in a cool dry place.

Purchase and treatment of fish

Forty juvenile sized (15-20 cm long) *Clarias gariepinus* purchased from a local farmer in Obiaruku, Delta state Nigeria, were used for the experiment. The fish were transported in an open plastic water tank to the laboratory and then acclimatized in aerated wide plastic bowls containing fresh water for two weeks, during which the water was changed twice daily. The fish were fed with roughly mashed dried crayfish and fingerlings. At the end of the acclimatization period, the fish were weighed and then divided into four different groups A to D each consisting of 10 fish on the basis of their mean body weight.

Group **A**: The control group. They were given the normal diet of crayfish and fingerlings.

Group **B**: These received 1.05 g ferrous sulphate/10 l of fresh water.

Group **C**: These were treated with 1.43 g ferrous sulphate and 1.43 ml of HSEt/10 litres of fresh water.

Group **D**: These were given 0.95 g ferrous sulphate and 0.95 g of ascorbic acid/10 litres of fresh water.

All the groups were fed on 0.5 g of roughly mashed dried crayfish and fingerlings per 10 litres of fresh water.

Sample collection

After the 21-day exposure period, four fish from each experimental group were randomly selected and sacrificed. Blood and eye tissue samples were collected. The blood sample was analyzed almost immediately for reduced glutathione (GSH) concentration, while the eye tissue sample was homogenized in 4.5 ml of normal saline and centrifuged at 1000 g for 5 min at room temperature. The supernatant was decanted into bijou bottles and stored frozen until analysis.

Biochemical assays

Homogenates of eye tissue were analyzed for GSH, malondialdehyde (MDA), lipid hydroperoxide (LHP) and glucose levels within 48 h. Blood GSH level was determined by the method described by Olga and Kelly [5] while eye tissue GSH level was determine using Ellman's method [6]. The eye tissue level of MDA was assayed by the thiobarbituric acid (TBA) method [7]. The LHP levels were estimated using the iodometric assay method [8]. The chemicals and reagents used for the assay of GSH, MDA and LHP, were Analar Grade and were supplied by BDH (Poole, England). Eye tissue glucose level was determined by the glucose oxidase method [9] using commercial kit (Biosystems S.A. Costa Brava, Barcelona, Spain).

Statistical analysis

Differences between the groups were compared by Analysis of Variance (ANOVA). P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The results obtained from the analysis of samples are shown in table 1. The changes in blood GSH and eye tissue GSH, MDA, LHP and glucose levels induced by ferrous sulphate in *Clarias gariepinus* and the effect of vitamin C and HSEt on these changes is illustrated.

A marked increase in lipid peroxidation was observed in all the treated groups as compared with control group. Treatment with the aqueous

Table 1: The effect of vitamin C and *Hibiscus sabdariffa* on blood GSH, eye GSH, MDA and glucose levels induced by ferrous sulphate in *Clarias gariepinus*.

Experimental Group	Blood	Eye			
	GSH (mg/ml)	GSH (mg/ml)	Glucose (mg/dl)	MDA (nmol/ml)	LHP ($x10^5$ mmol/ml)
A	2.40± 0.50	1.10± 0.36	98.24 ± 1.65	16.69 ± 2.85	1.29 ± 0.72
B	3.28±0.54	1.45± 0.59	100.48 ±14.33	31.25± 10.64	1.06 ± 1.34
C	1.78 ± 0.86	1.63 ± 0.04	75.48 ± 10.87	32.81 ± 8.12	1.66 ± 2.24
D	4.21 ± 0.43	0.70 ± 0.17	88.06 ± 4.44	24.38 ± 7.98	2.04 ± 2.21

GSH = reduced glutathione, MDA = malondialdehyde, LHP = lipid hydroperoxide.

Values are expressed as mean ± standard deviation for four fish per group.

extract of *Hibiscus sabdariffa* at a dose of 0.27 ml/kg body weight significantly increased serum GSH level and decreased the eye tissue MDA and glucose levels. On the other hand, treatment with ascorbic significantly decreased the levels of eye tissue glucose and MDA but increased the LHP levels.

The use of traditional medicine and medicinal plants in most developing countries for the maintenance of good health has been widely observed [3-4, 10]. Free radical-induced lipid peroxidation has been associated with a number of disease processes including oxidative stress [11].

The results obtained indicate that administration of HSEt significantly increased the tissue and serum level of GSH in fish treated at a dose of 0.27 ml/kg body weight comparable to ascorbic acid. The depletion of GSH promotes the generation of reactive oxygen species and oxidative stress through a cascade of events [12]. The increase in GSH upon HSEt administration may be attributed to the phytochemical constituents of the extract which possess antioxidant activity, thereby having a sparing effect on GSH.

The significant increase in MDA and LHP levels in ferrous sulphate treated fish (Table 1) demonstrates the potential toxic effect of ferrous sulphate on biological systems. This is in agreement with the result obtained by Usoh *et al.* [3]. In the present study, ascorbic acid was found not to cause a reduction in LHP levels. This may be due to auto-oxidation of ascorbic acid to generate free radicals thereby exacerbating lipid peroxidation. Alluding to this, Machlin *et al.* [13] stated that "in the presence of transition metals, ascorbic acid can provoke the formation of free radicals". Stuart [14] further confirmed the auto-oxidative effect of ascorbic acid.

Exposure to ferrous sulphate significantly increased the eye tissue glucose concentration as compared with the control. At a dose of 0.27 ml/kg, HSEt significantly decreased eye tissue glucose levels. This may be due to the antidiabetic activity of HSEt. Several workers have previously observed the antidiabetic and hypoglycemic activity of plant extracts [4,10].

CONCLUSION

The use of HSEt was shown to have a counteracting and antiperoxidative effect on free radical generating pollutants such as ferrous sulphate. In this study, *Hibiscus sabdariffa* aqueous extract compared favorably with ascorbic acid in its antioxidant activity. The crude extract should be further purified to isolate the antioxidant constituents of the plant.

ACKNOWLEDGEMENT

The assistance of Dr. I. Onyesom and Dr. E.C. Ochornogor both of Delta State University, Abraka, Delta State, Nigeria are highly appreciated. May God increase your wisdom.

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