Journal of Applied Pharmaceutical Science

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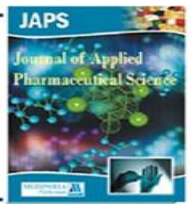

ISSN: 2231-3354
Received on: 16-11-2011
Revised on: 06-07-2012
Accepted on: 22-07-2012
DOI: 10.7324/JAPS.2012.2832

Antioxidant and Hepatoprotective Effects of Dried Flower Extracts of *Hibiscus sabdariffa* L. on Rats Treated with Carbon Tetrachloride

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ABSTRACT

The hypolipidemic, antioxidative and hepatoprotective activities of 200 and 300mg/kg body weight ethanolic extract of dried flower of *Hibiscus sabdariffa* L. (HSE) were assessed in rats treated with 0.25ml/kg body weight (intraperitoneally) of carbon tetrachloride (CCl_4). Hepatic malondialdehyde (MDA), serum lipid profile, serum vitamins A, C and β-carotene, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were measured. The oral administration of the extracts showed a significant ($P<0.05$) dose-dependent decrease in the CCl_4- induced MDA formation in liver. Also HSE pretreatment, showed a significant increase in HDL-C concentration and decrease in the levels of total cholesterol, LDL-C and TG as compared to control. The levels of vitamins A, C, and β-carotene were shown to be significantly ($P<0.05$) decreased and increased respectively in CCl_4 and HSE treated groups when compared with the control. The increase in the levels of these vitamins might not be unconnected with the antioxidant properties possessed by the extract. The extract also displayed a strong hepatoprotective effect as it significantly reduced CCl_4-induced hepatotoxicity in rats, as judged from the serum activities of ALT, AST, and ALP. These results suggest that the ethanolic extract of dried flower of *Hibiscus sabdariffa L.* possesses antioxidant, hepatoprotective and hypolipidemic effects on CCl_4-induced oxidative stress in rats.

Keywords: Carbon tetrachloride, *Hibiscus sabdariffa L*, antioxidant activity, hepatoprotective activity, hypolipidemic activity.

INTRODUCTION

The liver is the major site of intermediary metabolism and synthesis of many important compounds. Liver diseases remain one of the serious health problems. Liver may be damaged by free radicals through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Free radicals cause damage to nucleic acid, proteins, and membrane lipids and have been associated with many aging related problems including carcinogenesis and heart diseases (Halliwell *et al.*, 1992, Wang and Jiao, 2000). Peroxidative damage to cell membranes affects the integrity and function of the membrane, compromising the cell's ability to maintain ion gradients and membrane phospholipid asymmetry.

Liver disease may result in elevated levels of plasma total cholesterol, LDL-C, and TGs. Elevated levels of total serum cholesterol are associated with increased risk of atherosclerosis and cardiovascular disease (CAD) (Dominiczak, 2005). Low density lipoprotein cholesterol (LDL-C) causes the buildup of cholesterol and blockage of arteries.

Almost all organisms possess antioxidant defense and repair systems which quench the production of oxygen – derived species, but these protective systems are insufficient to entirely prevent the damage when there is increased oxidant radical generation (Simic 1988). Osawa *et al.,* (1990) demonstrated that antioxidant agents of plant and animal origin have been found to protect human liver from free radicals damage. Vitamins A, C, and E provide defense against oxidative damage (Padmore, *et al*., 1998)

Hibiscus sabdariffa L. is of the family malvaceac. It has been cultivated in Asia for over 300 years but is now cultivated in many other countries of the world including Nigeria (Tindal, 1983). Studies revealed that the dried flowers of *Hibiscus sabdariffa L.* a Chinese herbal medicine, have been used effectively in folk medicine against hypertension, pyrexia, and liver disorders (Tseng *et al*; 1997). *Hibiscus sabdariffa* L. Is popularly used in Nigeria for preparation of local soft drink called "zobo". The drink is consumed by over 50% percent of Nigerians especially around the Northern part of Nigeria. The present study is aimed at investigating the effects of dried flowers extract of *Hibiscus sabdariffa L.* on lipid peroxidation, serum lipids, β-carotene, vitamins A and C and some liver enzymes in rats treated with carbon tetrachloride.

MATERIALS AND METHODS

Plant Material

The fresh flowers of *Hibiscus sabdariffa* L were purchased at Uyo Market, Akwa Ibom State. They were dried at room temperature and sohxlet extracted with 80% ethanol (three changes), lyophilized, weighed and preserved at 4^0C and used as when required (Rana *et al;* 2000).

Experimental Animals

Twenty female wistar rats (weighting 68-109g) obtained from the department of pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo were used for the experiment. They were maintained and housed in cages in the departmental animal house and fed on commercial rat pellets manufactured by Pfizer Livestock Ltd, Aba but purchased from Uyo main market, Uyo.

Experimental Design

Animals were randomized into five groups with four in each group and put on normal diet. The extract was dissolved in distilled water and administered orally while CCl_4 (0.25ml/kg) was dissolved in liquid paraffin (1:1) and administered intraperitoneally.

Group A (control): only normal diet

Group B: Animals were treated with only 0.25ml/kg of CCl_4

Group C: Animals were treated with 200mg/kg body weight of HSE for 7 days

Group D: Animals were treated for 6 days with 200 mg/kg body weight of HSE and 0.25ml/kg of CCl_4 on the 7^{th} day

Group E: Animals were treated for 6 days with 300mg/kg body weight of HSE and 0.25ml/kg of CCl_4 on the 7^{th} day.

Determination of Lipid Peroxidation

Lipid peroxidation in microsomes prepared from liver was estimated spectrophotometrically by thiobabitturic acid-reacting substances (TBARS) as described by the procedure of Varshney and Kale, (1990).

Determination of Serum Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), and Triglyceride (TG)

Serum was separated and analyzed for TC, HDL-C and TG using Randox kits (Randox Laboratories Ltd; UK). The procedures were as described in the manufacturer's manual. LDL-C was determined using the formula: LDL-C=TC-(HDL-C+Tg/5) (Mckenny, 1999).

Determination of Serum β –Carotene, Vitamins A and C

Serum β-carotene and vitamin A were assayed according to the method of Suzuki and Katoh (1990) as described by Kokcam and Naziroglu (1999). Serum vitamin C was determined chemically according to the procedure described by Erel *et al.*, 1997 using dinitrophenylhydrazine (DNPH).

Determination of Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) Activities

The activities of serum ALT, AST and ALP were estimated using Randox kits (Randox laboratories Ltd; Uk).

Statistical Analysis

The results were reported as means ± SD from four repeated determinations and evaluated with the analysis of student's t-test. Differences were considered to be statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

The study of numerous compounds that could be useful antioxidants has generated increasing interest in the field of nutrition and medicine. Antioxidants have various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Rao *et al.,;* 2004). The hypolipidemic, antioxidative and hepatoprotective effects of dried flower extract of *Hibiscus sabdariffa L. treated* with CCl_4 are shown in table 1-3. The results (table1) revealed a significant ($P<0.05$) dose-dependent increase in serum HDL-C and decrease in hepatic malondialdehyde (MDA), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) in the HSE-treated groups when compared with the control and CCl_4-treated group B. Cholesterol is a general indicator of the level of

atherogenic lipid in the circulation and cholesterol and polyunsaturated fatty acids (PUFA) are the main components of LDL-C. PUFA is the substrate required for MDA formation and the amount of peroxidized lipid formed may be related both to the amount of substrate and to the level of peroxidation. Therefore, the more the lipid, the greater the level of lipid peroxidation activity and the greater the amount of lipid peroxidation products such as MDA (Farombi *et al.,*; 2003). The mechanism is that hydroxyl radical ($OH^{\cdot}$) attacks polyunsaturated fatty acid (PUFA), forming a carbon-centred lipid radical. The radical rearrange to form a conjugate dienyl radical. This radical reacts with ambient oxygen (O_2), forming a hydroperoxyl radical, which then abstracts a hydrogen from a neighbouring lipid, forming a lipid peroxide and starting a chain reaction. This reaction continues until the supply of PUFA is exhausted, unless a termination reaction occurs. Vitamin E is the major chain terminating antioxidant in membranes; it reduces both conjugated dienyl and hydroperoxyl radicals, quenching the chain or cycle or lipid peroxidation reactions. Studies have demonstrated that lipid peroxide levels can reflect the severity of a disease process (Das, *et al* 1993) and increased MDA is an indicator of reactive oxygen species (ROS) generation in the tissue (Halliwell and Hutteridge, 1989).

Since the liver is the major site of cholesterol metabolism with the intestine, adrenal cortex and gonads making lesser contributions, the high serum concentration of total cholesterol evident in CCl_4- treated groups might be attributed to the damage inflicted on the liver hepatocytes by this toxicant. The high HDL-C concentrations evidently shown in our results in the extract treated groups are desirable and the relationship between high HDL-C concentration and reduced cardiovascular riskhave been reported (NCEP 2001, Mckenny, 1999).

It is reported that patients with high triglyceride levels may have a high LDL-C or a low HDL-C levels which predicts coronary heart disease (CHD) risk (Gordon and Rifkind, 1989., Assman and Schulte, 1991., Grundy and Vega, 1992., Reardon, 1985).

The decrease in the levels of TC, LDL-C and TG in the extract treated groups suggests that the extract may prevent atherosclerosis and may also be beneficial in the context of the risk of CHD.

It is evident from the results (table 2) that the serum levels of vitamins A, C and β-carotene were significantly decreased in ($p.<0.05$) CCl_4-treated group B and increased in HSE-treated groups as compared to control. The decrease and increase in the levels of these non-enzymatic antioxidant in group B and extract treated groups could be linked to increased production of ROS and to the ability of HSE to mitigate ROS generated by CCl_4 respectively. βeta-carotene (the precursor of vitamin A) is an antioxidant and may play a role in trapping peroxyl free radicals in tissue at low partial pressures of oxygen. The ability of β-carotene to act as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure. Since β-carotene occurs at low oxygen concentration, it compliments antioxidant properties of vitamin E which is effective at higher oxygen concentrations. Their antioxidant properties account for their hepatoprotective activity in the HSE treated rats (Mascio *et al.,* 1991).

Vitamin C is known to act as an effective antioxidant on its own and it also shows excellent synergistic activity with vitamin E in the inhibition of oxygen radical-induced lipid peroxdation *in vitro* (Stocker, *et al.,* 1986). Vitamin C within the body is maintained in the reduced form by shuttling the dehydroascorbate across the erythrocyte membrane for reconversion to ascorbate (Orringer and Roe, 1979). Vitamin C exists as the enolate anion at physiological pH which spontaneously reduces superoxide, organic ($R^{\cdot}$) and vitamin E radicals, forming dehydroascorbyl radical ($AS^{\cdot}$).The $AS^{\cdot}$ undergoes a second reduction reaction to form dehydroascorbate which is recycled to ascorbate by dehydroascorbate reductase, a GSH-dependent enzyme present in all cells. This mechanism may not be sufficient to cope with oxidative stress hence the requirement of other antioxidant support. HSE pretreatment was reported to significantly prevent sodium arsenite-induced reduction of vitamin C levels in rats (Usoh *et al.*, 2005).

The results (table 3) showed a remarkable elevation in the serum activities of ALT, AST and ALP following CCl_4 intoxication in the rats, and pre-administration of 200 and 300mg/kg of the HSE prevented the elevated enzyme activities. The significant increase and decrease in serum activities of these enzymes in this experiment could be attributed to the damaged structural integrity of the liver by CCl_4 and to the hepatoprotective effectiveness of the HSE respectively. Epidemiologic studies have shown that those who take antioxidants such as vitamins E and C or β-carotene are not very susceptible to cardiovascular disease associated with increased oxidant lipids (Gaziano, 1990., Stampfer, 1991). In this study, the significant high levels of vitamins A, C, and β-carotene and a significant decrease in the concentrations of MDA, total cholesterol, LDL-C, TG, ALT, AST and ALP in the HSE-treated groups revealed that the extract possesses antioxidant and antihyperlipidemic and hepatoprotective properties capable of scavenging CCl_4- generated ROS and protecting LDL against oxidation in vivo. Since the data reported in this study were generated for short-term treatment with HSE, it is recommended that long-term animal studies be carried out to evaluate not only the effects of these extracts on biomarkers of oxidative stress but also the biochemical mechanism involving xenobiotic enzymes.

Table. 1: Effect of HSE on hepatic MDA, serum TC, HDL-C, LDL-C and TG concentrations in rats treated with CCl_4.

Treatment/Group	(MDA) µmol/mg	TC (mmol/l)	HDL-C mmol/l)	LDL-C mmol/l)	TG mmol/l)
A: control (only normal feed)	210.10±10.30*	1.97±0.14*	0.73± 0.06*	0.99±0.05*	1.25±0.04*
B:0.25ml/kg CCl_4 only	320.60± 46.26	3.49±1.00	0.58±0.05	2.56±0.10	1.74±0.05
C:200mg/kg HSE only	177.06±74.36*	1.83±0.08*	0.90±0.07*	0.71±0.08*	0.97±0.09*
D:200mg/kg HSE +0.25ml/kg CCl_4	111.10±24.20**	1.81±0.08*	0.85±0.09*	0.75±0.09*	1.04±0.08*
E:300mg/kg HSE+0.25ml/kg CCl_4	86.53±37.18**	1.79±0.10*	1.14±0.08**	0.45±0.04**	1.02±0.06*

Data are expressed as mean ± SD. *, ** are significantly different at $p < 0.05$ for each parameter. HSE - *Hibiscus sabdariffa* Extract; CCl_4 – Carbon tetrachloride; MDA - malondialdehyde ; TC - total cholesterol; LDL-C - low density lipoprotein cholesterol; HDL C – High densitry lipoprotein cholesterol and TG - triglyceride.

Table. 2: Effects of the Ethanolic Extract of HSE on Serum β-carotene, and Vitamins A and C in Rats.

Group	Vitamin A (µmol/L)	Vitamin C (mg/100ml)	ß-carotene (µg/100ml)
A: control (only normal feed)	1.60±0.10**	0.72±0.05**	19.10±0.50**
B:0.25ml/kg CCl_4 only	0.70±0.11*	0.43±0.01*	14.00±0.10*
C:200mg/kg HSE only	1.90±0.20**	0.95±0.08**	18.30±0.20**
D:200mg/kg HSE +0.25ml/kg CCl_4	1.00±0. 09**	0.80±0.02**	21.00±0.50**
E:300mg/kg HSE+0.25ml/kg CCl_4	1.30±0.10**	0.90±0.06**	22.00±1.22**

Data are expressed as mean ± SD. *, ** are significantly different at $p < 0.05$ for each parameter. HSE - *Hibiscus sabdariffa* Extract; CCl_4 – Carbon tetrachloride

Table 3: Effect of the ethanolic extract of HSE on serum activities of ALT, AST and ALP.

Group	ALT (U/I)	AST (U/I)	ALP (U/I)
A: control (only normal feed)	43.40 ± 5.0*	40.60 ± 3.9*	120.4 ± 12.2*
B: 0.25ml/kg CCl_4 only	101.30 ± 20.9**	120.30±36.5**	244.0 ± 19.2**
C:200mg/kg HSE only	40.50 ± 3.00*	45.20 ± 2.9*	99.5 ± 9.0*
D: 200mg/kg HSE+0.25ml/kg CCl_4	42.30 ± 4.0*	59.20 ± 6.2*	100.0±8.5*
E: 300mg/kg HSE +0.25ml/kg CCl_4	31.50 ± 4.2*	40.00 ± 2.0*	99.3 ± 7.2*

Data are expressed as mean ± SD. *,** are significantly different at $p < 0.05$ for each parameter. HSE - *Hibiscus sabdariffa* Extract; CCl_4 – Carbon tetrachloride; ALT – Alanine aminotransferases; AST – Aspartate aminotransferases; ALP – Alkaline phosphatase.

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