ORIGINAL ARTICLE

Antioxidant and hepatoprotective effect of the roots of *Hibiscus esculentus* Linn.

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The ethanol extract of *Hibiscus esculentus* Linn roots inhibited the formation of oxygen derived free radicals invitro with IC_{50} of 620 µg/ml, 2300 µg/ml, 870 µg/ml and 610 µg/ml. [Fe^{2+}/ascobate system], 730 µg/ml, [Fe^{3+}/ADP/ascorbate system], for superoxide radical production, hydroxyl radical generation, nitric oxide radical formation and lipid peroxide formation respectively. The oral administration of the extract (250 and 500 mg/kg body weight), significantly reduce CCl_4 induced hepatotoxicity in rats, as judged from the serum and tissue activity of marker enzymes [Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP)]. These results were comparable with standard drug silymarin [20 mg/kg, P.O.)

Key words: *Hibiscus esculentus* Linn, ethanolic extract, antioxidant activity, hepatoprotective effect

INTRODUCTION

Hepatitis is one of the major health problems, throughout the world more than 350 million peoples were affected with chronic hepatitis infection.[1] It is well known that free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation with subsequent tissue injury.[2] Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals.[3] Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the human diet. Plant phenolics comprise a great diversity of compounds, such asflavonoids (anthocyanins, flavanols, flavonols, flavones, among others) and several classes of non-flavonoids (phenolic acids, lignins, stilbenes).[4]

Several works have shown that phenolic compounds play a role as antioxidants through different mechanisms of action, such as: scavenging of free radicals, quenching of reactive oxygen species, inhibiting of oxidative enzymes chelating of transition metals or through interaction with biomembranes. These properties make those types of compounds good candidates as potential protectors against food oxidation and biological aging of tissues. Thus, phenolic compounds could be considered as natural antioxidants with potential applications in protecting the liver.[5]

Hibiscus esculentus L (Malvaceae) is an annual herb of 0.2 to 2 meters height is grown in many parts of India.[6] It is having different ethanomedical properties. Roots are used as stomachic,[7] to treat diabetes, ulcer,[8] used as laxative and treatment of jaundice. The phytochemical study shows the presence of carbohydrate, fixed oils, mucilage and flavanoid glycosides.[9] In the present study we aim to evaluate the antioxidant and hepatoprotective activity of ethanolic extract of *H. esculentus* roots.

Silymarin is a polyphenolic flavonoid derived from milk thistle (Silybum marianum), which has anti-inflammatory, cytoprotective, and anticarcinogenic effects, that suppress the TNF-induced production of ROS and lipid peroxidation[10,11] and improve the antioxidant status in blood and liver.[12]

MATERIALS AND METHODS

Plant Material

The plant materials used in this study, roots of *H. esculentus* were collected from the Botanical garden of Sankaralingam Bhuvaneswari College of Pharmacy, India in July 2005. The plant material was identified and authenticated by Dr.Sobhanam George, Scott Christian College, Nagarcoil, India. A voucher specimen of the collected sample was deposited in our institutional herbarium for the reference.

Preparation of Extract

The shade dried roots were powdered and passed through 10 mesh sieve. The coarse powder was soxhalated with 70% ethanol for nine hours [yield 5.7%]. Then the extracts were filtered and concentrated on rotary vacuum evaporator.[13] Preliminary qualitative phytochemical screening of roots gives the positive

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test for carbohydrates, fixed oils, flavanoids, glycosides and mucilage.[4] The extract was resuspended in water and used for antioxidant activity, for hepatoprotective studies the same extract at different doses (250 and 500 mg/kg) were received by the animals through oral gavage.

Animals

Male Wistar rats (150-200 gm) supplied from the Animal house of Sankaralingam Bhuvaneswari College of pharmacy, Sivakasi, India were used for this study. The rats were provided with standard rodent pellet diet (Lipton, India Ltd) and water *ad libitum* (The animals were maintained under standardized environmental condition, 27±2° C). Rearing of animals in the experimental period and their up key during the entire experimental span, confirm through ethical guidelines laid down by our institutional animal ethical committee.

Inhibition of Superoxide Radical Production

The effect on the superoxide radical production was evaluated using the nitroblue tetrazolium (NBT) reduction of McCord and Fridovic 1969.[14] The reaction mixture was: EDTA (6 μM; with 3 μg NaCN), riboflavin (2 μM), NBT (50 μM), *H.esculentus* extract (from 10 to 1000 μg/ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 530 nm before and after illumination.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system.[15] The reaction mixture was: deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), and the extract (from 10 to 3000 μg/ml) in a final volume of 1 ml. After incubation for one hour at 37° C, the deoxyribose degradation was measured as TBARS by the method of Ohkawa *et al*., 1979.[16]

Nitric Oxide Radical Inhibition Activity

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which were measured by Griess reaction.[17,18] The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the extract (from 10 to 1000 μg/ml) was incubated at 25° C for 150 min. After incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Griess reagent (1% sulfanilamide, 2%H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was evaluated at 546 nm.

Inhibition of Lipid Peroxide Formation

Induction by Fe^{2+}/ascorbate system

The reaction mixture containing rat liver homogenate (0.1 ml,25% w/v) in Tris- HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and different concentrations of the extract (from 10 to 1000 μg/ml) in a final volume of 0.5 ml was incubated for one hour at 37° C[19] and the resulting thiobarbituric reacting substance (TBARS) was measured by method of Ohkawa *et al*., 1979.[16] A 0.4 ml aliquot of the reaction mixture was treated with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%), and acetic acid (1.5 ml, 20% pH 3.5), made to a total volume of 4 ml by adding distilled water, and kept in a water bath at 95° C for one hour. After cooling, distilled water (1 ml) and 5 ml of *n*-BuOH 15:1 (v/v) were added. After shaking and centrifugation, the organic layer was separated and the absorbance measured at 532 nm.

Induction by Fe^{3+} / ADP / Ascorbate System

The reaction mixture containing rat liver homogenate (0.5 ml, 10% w/v), ferric chloride (100 μM), ADP (1.7 mM), ascorbic acid (500 μM), the extract (from 10 to 1000 μg/ml) and sufficient KCl (0.15 M) for a final volume of 1.5 ml, was incubated for 20 min at 37° C. Lipid peroxidation was then determined by the thiobarbituric acid reaction according to Ohkawa *et al*., 1979.[16] All these *in vitro* antioxidant activities were repeated with Silymarin as positive control at different concentration.

Hepatoprotective Effect Against Acute Dose of CCL_4 in Rats

Animals were randomized into five groups of five rats each. Group 1 served as normal, received only the vehicle (2% gum acacia, p.o), group 2 intoxicated by CCl_4, group 3 was treated with silymarin (20 mg/kg, p.o for 6 days) as standard, groups 4 and 5 received the extract (250 and 500 mg/kg p.o., respectively, for six days). On the day four, 2 h after treatment, groups 2-5 rats received CCl_4 (0.25 ml/kg, i.p.) in liquid paraffin (1:1).[20] The animals were killed, 48h after the acute dose of CCl_4. The blood was collected by heart puncture and serum was separated by centrifugation (3000 rpm at 4° C for 10 min). The liver was immediately removed. Tissue and serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were measured according to Kind and King,[21] alkaline phosphatase (ALP) activity according to Reitman and Frankel., 1957.[22]

Statistical Analysis

All the data were presented as mean ± S.E.M. and analyzed by student's t-test. Differences below the 0.05 level (P<0.05) were considered as statistically significant.[23]

Table 1: Effect of ethanolic extract *H.esculentus* roots on oxygen derived free radical generation *in vitro*[a]

Tested material	IC_{50} (µg/ml)[b]				
	Superoxide	Hydroxyl	Lipid peroxide		Nitric oxide
			A	B	
H.esculentus	620 ± 16*	2300 ± 41*	610 ± 23*	730 ± 61*	870 ± 52*
Silymarin	13.6 ± 2.3	54 ± 5.3	9.2 ± 1.5	13.4 ± 7	20.8 ± 9.3

[a]A, lipid peroxidation in Fe^{2+} / ascorbate; B, lipid peroxidation in Fe^{3+} / ADP / ascorbate. [b]Values are Mean± S.E.M. (n = 3). *P < 0.001*vs* Silymarin, student's t-test

Table 2: Effect of ethanolic extract of *H. esculentus* roots (p.o for 6 days) on tissue enzymatic changes in CCl_4-induced hepatotoxicity in rats[a]

Treatment	Dose (mg/kg)	GOT (u/l)	GPT (u/l)	ALP (u/l)
Normal (vehicle)	-	123±12.4*	92±7.8*	198±16.3*
CCl_4	0.25	638±28	709±29.1	993±48.4
Silymarin	20	109±8.3*	133.6±9.5*	271.2±15.3*
H.esculentus	250	451±21.3*	241.5±30.1*	391.3±18.2*
	500	388.7±18.3*	191.3±21.5*	318.9 ± 21.3*

[a]Values are mean ± S.D. (n = 5); **P*< 0.001 vs CCl_4, student's t-test. CCl_4 (0.25 ml/kg. i.p.) was administered to all groups except normal group on day 4. GPT - Glutamic pyruvic transaminase, GOT - Glutamic oxaloacetic transaminase, ALP - Alkalin phosphatase

Table 3: Effect of the ethanolic extract of *H.esculentus* (p.o. for 6 days) on serum enzymatic changes in CCl_4-induced hepatotoxicity in rats[a]

Treatment	Dose (mg/kg)	SGOT (u/l)	SGPT (u/l)	ALP (u/l)
Normal (vehicle)	-	60.3±3.3*	58.8±5.7*	141.6±12.2*
CCl_4	0.25	191.4±25	270.3±29.6	251.1±19.1
Silymarin	20	73.5±9.1*	49.3±3.5*	103.1±5.4*
H.esculentus	250	101.2±7.2*	201.5±22.3*	199.7±15.3*
	500	85.1±3.3*	71.3±6.4*	130±18.1*

[a]Values are mean ± S.D. (n = 5); **P*< 0.001 *vs* control, student's t-test. CCl_4 (0.25 ml/kg. i.p) was administered to all groups except normal group on day 4

RESULTS AND DISCUSSION

The ethanol extract of *Hibiscus esculentus* roots were found to have excellent scavenging effect on free radicals which was well comparable with standard drug Silymarin. It was found to scavenging the superoxides generated by riboflavin photoreduction method with an IC_{50} of 620 µg/ml while Silymarin showed IC_{50} of 13.6 µg/ml. [Table 1].

A remarkable elevation was observed in serum and tissue ALP, GPT and GOT activities following CCl_4 intoxication in the rat. In the groups orally treated with 250 and 500 mg/kg of the extract, the above enzyme activities were found to decrease when compared to CCl_4 treated control group [Tables 2 and 3].

Results from the study showed that *H.esculentus* root ethanol extract posses *in vitro* free radical scavenging activity (P < 0.001) which was well comparable with Silymarin. This could exert a beneficial action against liver damage induced by CCl_4. The preliminary phytochemical analysis of the extract showed the presence of flavonoids. Since the anti-oxidant and hepatoprotective activities of certain flavonoids from plant origin have already been established,[24] we can speculate that these constituents may be responsible for the observed protective effects. Phenolics are capable of scavenging peroxyl radicals;[25] we presume that these protective effects of the extracts are dependent on their phenolic composition in terms of lipophilicity/hydrophilicity partition, and molecular structures that define their reactivity against free radicals, as well as on the phenolic concentrations. Possibly, synergistic, additive or antagonistic actions are also present. Finally, the present study suggests that phenolic compounds of the roots *H esculentus* provide a good source of antioxidants that could offer potential protective effects against lipid oxidation and which could be exploited to make a hepato protective formulation.

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