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Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. leaves and calyces extracts in rats

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Antioxidant and antihyperlipidemic activities of the extracts of leaves and calyces of *Hibiscus sabdariffa* were investigated by studying their *in vitro* inhibitory activity on lipid peroxidation and *in vivo* effects on cholesterol induced hyperlipidemia. Highest antioxidant activity was exhibited by ethanolic extract of calyces followed by ethanolic extract of leaves followed by aqueous extract of leaves of *H. sabdariffa.* In cholesterol induced hyperlipidemic model, groups of rats treated with extracts of calyces and leaves of *H. sabdariffa* showed a significant decrease in the serum TC, LDL-C, VLDL-C, TAG values alongwith an increase in serum HDL-C levels. The treated groups also showed significant decrease in the atherogenic index, LDL-C: HDL-C risk ratios, and in the levels of SGOT, SGPT and ALP activities compared to cholesterol induced hyperlipidemic control group. Significant antihyperlipidemic activity was shown by ethanolic extract of calyces, followed by ethanolic extract of leaves. It was observed from the histopathological findings that rats fed with *H. sabdariffa* extracts showed decrease in granular degeneration caused by cholesterol feedings. Results suggest that the ethanolic extracts of calyces and leaves of *H. sabdariffa* containing polyphenols and flavanols possess significant antioxidant and antihyperlipidemic activities.

Keywords: Antihyperlipidemic, Antioxidant, *Hibiscus sabdariffa*

Recent studies have demonstrated that increased formation of free radicals/reactive oxygen species (ROS) contribute to cardiovascular disease (CVD) progression[1,2]. Reactive oxygen species induce cardiac dysfunction and cardiac apoptosis and/or necrosis in heart failure[3]. Reactive oxygen species are formed intracellularly and are controlled by antioxidant defense. The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification, and DNA breaks[4]. Reactive oxygen species induced depletion of antioxidants is a key factor for the initiation of atherosclerosis and the development of CVD[1].

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases[5]. World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year[5]. Hyperlipidemia is a metabolic disorder, specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), and Triglycerides (TAG) with a concominant decrease in the concentrations of High Density Lipoprotein Cholesterol (HDL-C) in the blood circulation[6].

Currently, the use of complimentary/alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use[1]. *Hibiscus sabdariffa* Linn. (Family: Malvaceae) is an annual dicotyledonous herbaceous shrub popularly known as '*Gongura*' in Hindi and Roselle or Sorrel in English[7,8]. The plant is well known in Asia and Africa and is commonly used to make jellies, jams and beverages[9]. In Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments like hypertension, pyrexia, liver disorders, and as antidotes to poisoning chemicals (acids, alkali, pesticides) and venomous mushrooms[10,11]. A number of active principles from this plant have been identified which include anthocyanins, flavonols and protocatechuic

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acid (PCA)[12-14]. The objective of the present research work was to investigate the antioxidant and antihyperlipidemic activity of extracts of leaves and calyces of *H. sabdariffa* by studying *in vitro* inhibitory activity on lipid peroxidation and *in vivo* effects on cholesterol induced hyperlipidemia respectively.

Materials and Methods

Plant material and extracts — Dried leaves of *H. sabdariffa* were obtained from Abirami Botanical Corporation, Tuticorin. Fresh calyces of *H. sabdariffa* were collected from suburbs of Mumbai and were dried at room temperature. Leaves and calyces of *H. sabdariffa* were authenticated at the Department of Pharmacognosy and Phytochemistry, Prin. K. M. Kundnani College of Pharmacy, Mumbai. Dried leaves of *H. sabdariffa* were powdered and subjected to soxhlet extraction at 60°C using 80% ethanol as solvent and also at 100°C using water as solvent for 12 hrs. Also the fresh calyces of *H. sabdariffa* were powdered and were subjected to soxhlet extraction at 60°C using 80% ethanol as solvent for the same period. After evaluation the main components of *H. sabdariffa* were found to be anthocyanins, polyphenols, flavones, glycosides, organic acids, proteins and carbohydrates.

Reagents — For antioxidant activity, the reagents used were, potassium chloride (0.15 *M* KCl); TRIS Buffer; ferrous sulphate (10 μ*M* $FeSO_4$); ascorbic acid (100 μ*M*); TBA reagent; saline (0.9% NaCl). Standard curcumin was purchased from Merck, Mumbai. For antihyperlipidemic activity, the drug and chemicals used were, Lovastatin obtained from USV Pharmaceuticals Ltd., Mumbai, India; Cholesterol (extra pure) obtained from Qualigens fine chemicals, Mumbai, India; Crude coconut oil procured from the local market; and sodium Carboxy Methyl Cellulose (CMC). All the Chemicals used in the study were of Laboratory Grade.

Experimental animals — For antioxidant activity, Swiss Albino mice (25-30 g) of either sex were obtained from Haffkiene Biopharmaceutical Corporation Ltd., Mumbai. For antihyperlipidemic activity, Albino rats of Wistar strain (150-200 g) of either sex were obtained from Nicholas Piramal Pvt. Ltd., Mumbai.

Rats were maintained under good hygienic conditions in the departmental animal house of Prin. K. M. Kundnani College of Pharmacy. Animals were maintained under standard environmental conditions (22-28°C, 60-70% relative humidity, 12 hr L:D cycle) and fed with standard feed and water *ad libitum.* The experimental protocol was approved by the Institutional Animals Ethics Committee constituted as per CPCSEA guidelines. Animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days period prior to experiments.

Evaluation of antioxidant activity by TBARs method — Mice of either sex weighing 25-30 g were sacrificed using anaesthetic ether. The liver was quickly removed and chilled in ice cold saline and was homogenized in 0.15 *M* KCl solution to get a 10% v/v liver homogenate. Fresh liver homogenate (0.2 ml) was mixed with 0.15 *M* KCl (0.1 ml) and TRIS-HCl buffer (0.4 ml, *p*H 3.5). Test extracts were then added in various concentrations (0.05 ml). Curcumin was used as a standard. *In vitro* lipid peroxidation was initiated by addition of 10 μ*M* ferrous sulphate (0.1 ml) and 100 μ*M* ascorbic acid (0.1 ml). After incubation for 1 hr at 37°C, the reaction was terminated by addition of thiobarbituric acid reagent (2 ml) and then boiled at 95°C for 15 min for development of coloured complex. On cooling, the tubes were centrifuged at 4000 rpm for 10 min.

Absorbance of supernatant was determined colorimetrically at 532 nm as Thio Barbituric Acid Reacting substances (TBARs)[15-17]. Percentage inhibition of TBARs formation was calculated with respect to control in which no test sample was added. The inhibition of lipid peroxidation was determined by calculating percentage decrease in the formation of TBARs. The IC_{50} values were calculated for all the test extracts by subjecting the results to linear regression.

Evaluation of antihyperlipidemic activity — Albino rats (Wistar strain) of either sex weighing 150-200 g were divided into following five groups with each group containing 6 rats.

Group I — Control group in which the rats were daily administered vehicle i.e., 1% w/v sodium CMC suspension.

Group II — Hyperlipidemic group in which the rats were daily administered cholesterol (25 mg/kg/day) in oil[6].

Group III — Positive control group in which the rats were daily administered Lovastatin (10 mg/kg/day) along with cholesterol in oil[18].

Group IV — Test group in which the rats were daily administered ethanolic extract of calyces of *H. sabdariffa* (500 mg/kg/day) along with cholesterol in oil[19].

Group V — Test group in which the rats were daily administered ethanolic extract of leaves of *H. sabdariffa* (500 mg/kg/day) along with cholesterol in oil.

A homogeneous suspension of the extracts and standard drug Lovastatin was freshly prepared individually using 1% w/v sodium carboxy methyl cellulose. Rats were fed daily with standard diet supplied by Pranav Agro Industries, Sangli. cholesterol in oil was given by oral route at 10 am and *H. sabdariffa* extracts or Lovastatin was given by oral route at 3 pm daily, to respective groups, for a period of 30 days. The normal control group was treated with vehicle instead of drugs. Initial and final body weights and food intake of rats were monitored.

At the end of the experimental study, animals were fasted for 12 hr and then sacrificed and the blood was collected by cardiac puncture under light ether anaesthesia. The liver sections were isolated and preserved in 10% formalin[20].

The main parameters assessed in hyperlipidemic model were as follows:

Biochemical lipid constituents/parameters[5] — The main biochemical parameters recommended by the National Cholesterol Education Program (NCEP) guidelines (2002) for lipid screening ie. Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), High Density Lipoprotein Cholesterol (HDL-C) and Triglycerides (TAG) were evaluated from the serum.

Cardiac risk indicators — The cardiac risk ratios recommended by NCEP guidelines (2002) were estimated by calculating the TC: HDL-C ratio (Atherogenic Index)[21] and LDL-C: HDL-C ratio.

Biological Parameters — Biological Parameters like Body weights were determined just before the animals were sacrificed.

Histopathological analysis — The liver sections were evaluated for histopathology to assess any architectural changes.

Marker enzymes viz; SGOT, SGPT and ALP — Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) activities were also measured.

Antihyperlipidemic activity evaluation — Antihyperlipidemic activities of the extracts of *H. sabdariffa* and of the standard drug Lovastatin evaluated by determining the percentage reduction in serum TC, LDL-C, VLDL-C, TAG, and percent increase in serum HDL-C levels, were calculated as,

$$\% \text{ reduction or increase} = [A\text{-}B/A] \times 100$$

where,

A: serum values of respective control ie. hyperlipidemia induced groups.

B: serum values of treated groups.

Statistical analysis — Results were expressed as mean $\pm$ S.E.M. Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests against the control (with cholesterol in oil) in multiple test groups. The P values < 0.05 were considered as statistically significant.

Results

Antioxidant activity — Highest lipid peroxidation inhibitory activity by *in vitro* studies was exhibited by the ethanolic extract of calyces of *H. sabdariffa* followed by the ethanolic extract of leaves of *H. sabdariffa* and then the aqueous extract of leaves of *H. sabdariffa* (Table 1).

Antihyperlipidemic activity — In cholesterol induced hyperlipidemic model, the groups treated with the extracts of *H. sabdariffa* and Lovastatin demonstrated a significant decrease in the serum TC, LDL-C, VLDL-C, TAG, besides an increase in serum HDL-C levels when compared to cholesterol induced hyperlipidemic control group (Table 2). The groups treated with the extracts of *H. sabdariffa* and Lovastatin demonstrated significant decrease in the Atherogenic Index and LDL-C: HDL-C risk ratios, and also a remarkable decrease in the levels of SGOT, SGPT and ALP activities when compared to

Table 1 — Inhibitory activity of *H. sabdariffa* extracts on lipid peroxidation

Test material	IC_{50} (μg/ml)	Inhibition (%)
Ethanolic extract of calyces of *H. sabdariffa*	32.77	71.3
Ethanolic extract of leaves of *H. sabdariffa*	34.64	69.41
Aqueous extract of leaves of *H. sabdariffa*	41.11	63.21
Curcumin (Standard control)	57.77	46.69

cholesterol induced hyperlipidemic control group (Tables 3 and 4). The groups treated with the extracts of *H. sabdariffa* also showed decrease in body weights when compared to cholesterol induced hyperlipidemic control group. In cholesterol induced hyperlipidemic model, the histopathological studies were conducted in the liver sections of rats and the histopathological changes were observed (Fig. 1). These figures illustrate the protective action of the extracts of *H. sabdariffa* against fatty infiltration and granular degeneration due to hyperlipidemia closely comparable to that with Lovastatin. The extracts of *H. sabdariffa* showed a significant antihyperlipidemic activity in the animal model and the best activity was shown by ethanolic extract of calyces, followed by ethanolic extract of leaves (Table 2).

Discussion

Administration of *H. sabdariffa* extracts significantly decreased levels of TBARs which may be due to the free radical scavenging property of *H. sabdariffa* extract[22]. In addition to this the alcoholic extract of calyces of *H. sabdariffa* showed maximum antioxidant property. The observed activity of antioxidants may be due to the presence of phenolic phytochemicals and bioflavonoids (like anthocyanins, glycosides, protocatechuic acid, hydroxycitric acid, etc.) in *H. sabdariffa* extract[23,24] which offer possible role in reducing the oxidative stress by inducing cellular antioxidant enzymes. Flavonoids and polyphenolic compounds are potent free radical scavengers and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules[25]. Most phenolic phytochemicals that have positive effects on health are believed to function by countering the effects of reactive oxygen species generated during cellular metabolism. Phenolic phytochemicals due to their

Table 3 — Effect of *H. sabdariffa* extracts on various biological parameters like Atherogenic Index and LDL-C/HDL-C ratio in hyperlipidemic rats

[Values are mean ± SE of 6 animals]

Groups	Atherogenic index	LDL - C/HDL - C
Normal control	2.79	1.25
Hyperlipidemic control	4.32	2.04
Lovastatin standard control	2.96	1.33
Calyces extract	2.31	1.07
Leaves extract	2.43	1.14

Table 2 — Effect of *H. sabdariffa* extracts on serum lipid profile in hyperlipidemic rats

[Values are mean ± SE of 6 animals]

Groups:	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL – C (mg/dl)
Normal control	64.5±1.19***	65.75±1.702***	23.31±1.111$^{n.s}$	28.04±1.5***	13.15±0.34***
Hyperlipidemic control	84.5±1.041	125±1.08	19.79±1.251	39.71±1.451	25±0.216
Lovastatin standard control	60.25±1.25*** (28.70%) ↓	64.25±0.8539*** (48.60%) ↓	20.47±0.97$^{n.s}$ (3.43%) ↑	26.93±0.3271*** (32.18%) ↓	12.85±0.171*** (48.60%) ↓
Calyces extract	62.25±1.931*** (26.33%) ↓	32.25±0.8539*** (74.20%) ↓	26.95±0.882*** (36.17%) ↑	28.85±1.175*** (27.35%) ↓	6.45±0.171*** (74.20%) ↓
Leaves extract	63.5±.0646*** (24.85%) ↓	37.75±1.031*** (69.80%) ↓	26.27±0.86** (32.74%) ↑	29.69±0.6*** (25.23%) ↓	7.55±0.206*** (69.80%) ↓

P values: *<0.05, **<0.01, ***<0.001, n.s. = not significant. Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↓ denotes decrease, ↑ denotes increase in respective serum levels.

Table 4 — Effect of *H. sabdariffa* extracts on SGOT, SGPT and alkaline phosphatase (IU/L) in hyperlipidemic rats

[Values are mean ± SE of 6 animals]

Groups	SGOT	SGPT	Akaline phosphatase
Normal control	18.75±1.109***	20.25±1.25***	89.75±1.548***
Hyperlipidemic control	42.75±0.854	50.75±1.887	319±6.621
Lovastatin Standard control	20.5±1.041***	36.75±1.25***	180.5±3.524***
Calyces extract	21.75±1.25***	33.25±1.25***	149.8±3.119***
Leaves extract	24±0.707***	35.25±1.931***	201±4.882***

P values: *<0.05, **<0.01, ***<0.001.

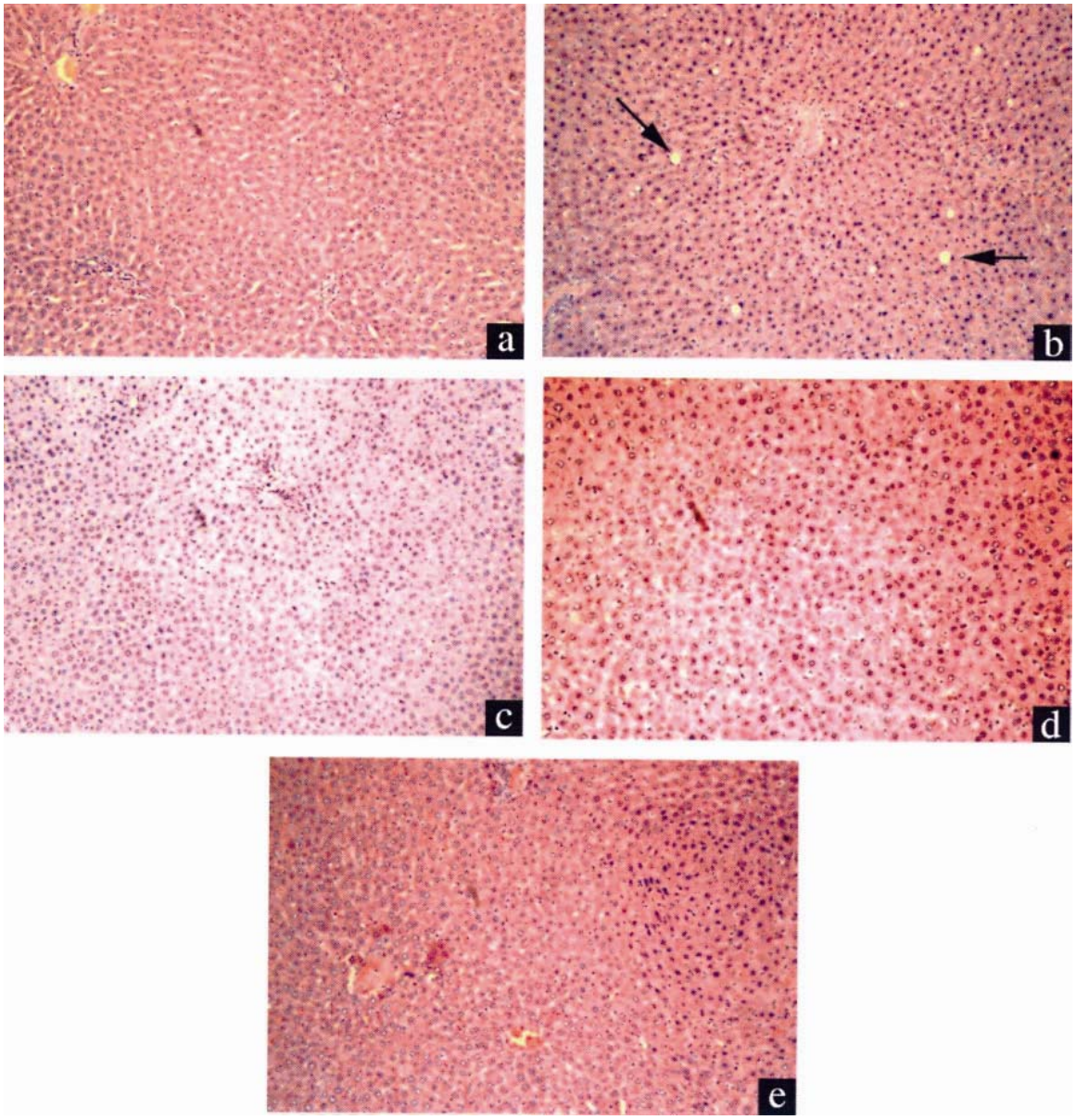

Fig. 1 — Hepatocytes of rats stained with hematoxylin and eosin (100 × magnification) — (a) control group showing normal architecture; (b) hyperlipidemic group showing fatty infiltration (→) and granular degeneration; (c) Lovastatin group showing negligible cytoplasmic fatty infiltration and granular degeneration; (d) group treated with calyces extract showing mild cytoplasmic fatty infiltration and mild granular degeneration; (e) group treated with leaves extract showing mild cytoplasmic fatty infiltration and mild to moderate granular degeneration.

phenolic ring and hydroxyl substituents similar to that found in Vitamin E can function as effective antioxidants due to their ability to neutralise hydroxy and related free radicals. It is, therefore, believed that dietary phenolic antioxidants can scavenge harmful free radicals and thus inhibit their oxidative reactions with vital biological molecules[26] and prevent the development of physiological conditions, which can manifest into many harmful diseases[27].

Results of the study has showed that ethanolic extract of calyces and leaves and aqueous extract of leaves of *H. sabdariffa* decreased lipid peroxidation confirming *H. sabdariffa* as a good source of antioxidants.

In the present study, the extracts of calyces and leaves of *H. sabdariffa* showed a significant antihyperlipidemic activity in cholesterol induced hyperlipidemic model of rats, which was almost

comparable to that of the standard Lovastatin drug used in treatment. Their actions may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptors and their protection etc. leading to an increased removal of LDL-C from the blood and its increased degradation and catabolism of cholesterol from the body. All these events either individually or in combination lead to decreased serum LDL-C levels which may have also reduced serum total cholesterol (TC) levels during the treatment with test extracts[28-30].

In the hyperlipidemic model, the purpose of inclusion of cholesterol and coconut oil might be attributed to the well established findings that addition of dietary cholesterol along with saturated fats results in accumulation of intracellular cholesterol and its esters in the body tissues as coconut oil contains approximately 92% of saturated fats i.e., saturated fatty acids (FA): of short chain 15%, medium chain 64.2% and long chain 12.2%[31]. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms given above and by others[28-32].

The extracts of *H. sabdariffa* induced an increase in serum HDL-C levels in the hyperlipidemic models. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as "reverse cholesterol transport" hence increased serum HDL-C levels may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process.

Rats treated with extracts of *H. sabdariffa* and Lovastatin caused significant decrease in the levels of SGOT, SGPT and ALP activities at the doses of 500 mg/kg/day and 10 mg/kg/day respectively. The histopathological findings in the liver of rats fed with the extracts of *H. sabdariffa* showed decrease in granular degeneration caused by cholesterol. All these beneficial effects of the extracts may be due to their antioxidant and antihyperlipidemic effects carried out by polyphenols and related compounds present in them.

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