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HYPOLIPIDEMIC EFFECT OF ETHANOLIC EXTRACT FROM THE LEAVES OF *HIBISCUS SABDARIFFA* L. IN HYPERLIPIDEMIC RATS

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Abstract: The present study is designed to investigate the hypolipidemic effect of ethanolic extract from the leaves of *Hibiscus sabdariffa* L. (HSEE) in hyperlipidemic rats. In the present work, HSEE was evaluated at three doses (i.e. 100, 200 and 300 mg/kg, orally) in cholesterol-induced (2 g/kg, orally) hyperlipidemic Wistar rats. Atorvastatin (10 mg/kg, orally) was used as the standard drug.

Administration of HSEE (200 mg/kg and 300 mg/kg) together with continuous cholesterol feeding for four weeks showed significant reduction in serum cholesterol level by 18.5% and 22%, respectively ($p < 0.05$); serum triglyceride level by 15.6% and 20.6%, respectively ($p < 0.05$); serum LDL level by 24% and 30%, respectively ($p < 0.05$), and serum VLDL level by 15.5% and 20.5%, respectively ($p < 0.05$), as compared to cholesterol group. However, no significant change in HDL level was observed. HSEE 300 mg/kg was more effective than HSEE 200 mg/kg dose but less effective than the standard drug, atorvastatin. HSEE 100 mg/kg did not show any significant reduction in lipid levels.

These results indicate that HSEE exhibit the hypolipidemic effect and among all HSEE groups investigated, HSEE 300 mg/kg has the best hypolipidemic effect.

Keywords: *Hibiscus sabdariffa* L. leaves, ethanolic extract, hyperlipidemic rats

Lipids and lipoproteins abnormalities are preceding risk factor for cardiovascular diseases and prevalence of this in general population has increased considerably in last few decades. Hyperlipidemia contributes significantly in the prevalence and severity of atherosclerosis and coronary heart diseases (1). Cardiovascular diseases are the primary cause of mortality and morbidity worldwide (2). Numerous factors, such as diet rich in saturated fats and cholesterol, age, family history, hypertension and life style play an important role in the development of high cholesterol and LDL levels, which are primarily responsible for the onset of atherosclerosis and coronary heart diseases (2, 3). Lowering of lipids levels, by a drug or diet management could reduce the risk of cardiovascular diseases. Current awareness of medicinal plants in the management of cardiovascular diseases has encouraged the researchers for exploring novel lipid lowering pharmaceuticals (4).

Among these sources, *Hibiscus sabdariffa* L. (Malvaceae), commonly known as Roselle, Jamaican Sorrel, Natal Sorrel, Red Sorrel; is an erect annual herb with a reddish cylindrical stem nearly glabrous with simple leaves and having petiole, 3-5 lobed or parted blade along with toothed or serrated lobes, which has been shown to possess cardiac activity in folk remedies (5). This drug is native to the West Indies and it is cultivated in Uttar Pradesh, Andhra Pradesh, West Bengal, Bihar, Punjab, Assam and Tamil Nadu states of India (6).

Tea made from the flowers and leaves of *Hibiscus sabdariffa* L. is a very common beverage in tropical regions. In Guinea, leaves are used as an emollient, antipyretic, sedative and as a soothing cough remedy, whereas in Central Africa and South

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India, leaves are poultice on the abscesses (7). Traditionally, flowers and leaves have been used orally for heart and nerve diseases, as a laxative, to reduce weight, as a diuretic, to activate and neutralize hepatic secretion, to activate gastric section as a digestive, for arteriosclerosis, as a diaphoretic, to give an euphoric impression and as an intestinal antiseptic, to relieve dry coughs, treat skin afflictions, cancer, and lower cholesterol levels and blood pressure (8-11).

Hibiscus sabdariffa L. has been reported to possess biological activities like antihypertensive, antimutagenic, chemopreventive, antioxidant, anticonvulsant, anxiogenic, CNS-depressant, serotoninergic activities, reducing oxidative liver damage, anti-inflammatory activities and hypoglycemic activity (7, 12-16) but there is hardly any scientific reports on the hypolipidemic effect of leaves of *Hibiscus sabdariffa* L. Therefore, the objective of the present study is to investigate hypolipidemic effect of *Hibiscus sabdariffa* L. leaves in cholesterol-induced hyperlipidemic rats.

MATERIALS AND METHODS

Plant material and preparation of extract

The leaves of *Hibiscus sabdariffa* L. were collected from the field of Leva, District-Jhansi in Uttar Pradesh state and were authenticated by Department of Botany, Bundelkhand University, Jhansi, India and voucher specimen was deposited at that Department under the reference no. BU/Botany/06/1653.

After authentication, the leaves of *Hibiscus sabdariffa* L. were separated, cleaned and dried under shade. After shade drying, the leaves were coarsely powdered and stored in well closed container for further use.

The coarse powder of *Hibiscus sabdariffa* L. leaves (600 g) was packed in Soxhlet apparatus and continuously extracted with 95% ethanol for 72 h. The solvent was removed from the extract by distillation, the last traces of the solvent being removed under reduced pressure by vacuum distillation, and dried in a desiccator. The percent yield of HSEE was 14.1% (w/w).

Preliminary phytochemical screening

Freshly prepared HSEE was subjected to preliminary phytochemical screening for the detection of various plant constituents like alkaloids, carbohydrates, proteins, amino acids, glycosides, flavanoids, tannins, phenolic compounds and triterpenoids by using standard procedures.

Acute oral toxicity study

The rats were given HSEE at doses of 1000, 2000 and 5000 mg/kg daily for a period of 4 weeks (five rats were taken for each dose). The animals were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 4 weeks for any physical signs of toxicity such as writhing, gasping, palpitation and decreased respiratory rate or mortality. The acute oral toxicity study for HSEE extract was conducted using preliminary limit dose test of the Up and Down Procedure statistical program – AOT425 guidelines.

Drug and chemicals

All chemicals and solvents used were of the analytical grade and were obtained from E. Merck, India. Cholesterol, triglyceride and HDL kits were purchased from Sigma Aldrich, USA. For inducing the hyperlipidemia, cholesterol puriss. from Sigma Aldrich, USA was used. Corn oil was obtained from Maxwell Inc., India and normal saline was purchased from Marck Biosciences Ltd., India. Atorvastatin is the cholesterol lowering synthetic drug available as Lipitor® (Parke Davis, U.S.A.) was purchased from respective vendor.

Experimental animals

Forty-two male, healthy, adult, albino rats of Wistar strain weighing about 120 g to 150 g were obtained from the animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animal house was well ventilated and the animals had 12 ± 1 h day/night schedules with a spacious hygienic cages during the course of the experimental period. The animals were fed with water and pellet feed *ad libitum*. The protocol for present experimental work was approved by the Institutional Animal Ethics Committee (716/02/a/CPCSEA).

Experimental design

All the animals were weighed and divided into non-cholesterol-fed group (control group) and cholesterol-fed group. The control group was subdivided into two groups. One group was continuously administered distilled water (control-I group). The other group was continuously administered corn oil (control-II group). Each group was composed of six rats.

Rats in cholesterol-fed group were induced to be hyperlipidemic by daily intragastric administration of cholesterol (2 g/kg). After induction of hyperlipidemia, which was confirmed by serum estimation of lipid levels, the rats were further randomly subdivided into five groups. Each group con-

tained six rats. Rats in groups 3-7 were treated with cholesterol (2 g/kg), atorvastatin (10 mg/kg) and HSEE at the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg, respectively. All the doses were intragastrically administered to rats as described, once a day and rats were continuously fed with cholesterol in corn oil for four consecutive weeks; while rats in non-cholesterol-fed groups (i.e. control groups) were continuously administered with distilled water and corn oil in control-I and control-II groups, respectively.

At the end of the study, rats were starved for 14 h before anesthetizing with diethyl ether. The blood was collected by tail vein into glass tubes with Na_2EDTA at the concentration of 1 mg/mL for biological serum estimation.

Biochemical analysis

Serum cholesterol, triglycerides and HDL levels were estimated by enzymatic methods using kits. Samples were analyzed on Lipid Auto analyzer 911 (Hitachi, Japan).

Statistical analysis

All experimental values were assessed as the mean ± standard error of the mean (SEM). The data were subjected to one way analysis of variance (ANOVA) and comparison by Dunnett's *post hoc* test for multiple comparisons among the groups. A 'p' values less than 0.05 were considered to be statistically significant.

RESULTS

Preliminary phytochemical screening

HSEE was found to possess carbohydrates, proteins, amino acids, glycosides, flavanoids, tannins, phenolic compounds and triterpenoids.

Acute oral toxicity and effect on general behavior of HSEE

The rats treated with 1000, 2000 and 5000 mg/kg doses of HSEE did not show any drug-induced physical signs of toxicity during the whole experimental period and no mortality was registered. Only behavioral changes observed included weight loss and diarrhea. These early symptoms subsequently disappeared.

Hypolipidemic study

During the induction of hyperlipidemia, the increase in body weight and amount of food intake were not significantly different among all groups (control and cholestrol-fed) of the study.

At the baseline, corn oil administration in the control-II group slightly increased the serum lipid level compared to control-I group but without significant difference at corresponding times. Rats in the cholesterol-fed groups exhibited a significant increase in serum lipid levels, as compared to control groups. The baseline level of serum lipids before the experiment was not significantly different within the subgroups of the cholesterol-fed group.

After four weeks of treatment, as shown in Table 1, the levels of cholesterol, triglycerides, HDL, LDL, VLDL in the serum of the rats of all the groups were examined. Among the control groups, control-II group have shown a significant increase in serum lipid levels whereas control-I group did not show any significant increase. Serum lipid level in the non-treated cholesterol group was maintained at the high level throughout four weeks of the treatment period.

Administration of atorvastatin (10 mg/kg), used as a positive control, in hyperlipidemic rats significantly decreased the levels of cholesterol, triglycerides, LDL and VLDL. HSEE (100 mg/kg) did not exhibit significant hypolipidemic effect at any time of treatment. However, administration of HSEE (200 mg/kg and 300 mg/kg) in hyperlipidemic rats had significantly decreased serum cholesterol by 18.5% and 22%, respectively ($p < 0.05$); serum triglyceride level by 15.6% and 20.6%, respectively ($p < 0.05$); serum LDL level by 24% and 30%, respectively ($p < 0.05$) and serum VLDL level by 15.5% and 20.5%, respectively ($p < 0.05$), as compared to cholesterol group after four weeks of treatment. Moreover, these lipid levels were significantly lower than in control groups.

Serum HDL levels were not significantly different among all the groups at corresponding times.

DISCUSSION AND CONCLUSION

In the present study, HSEE was shown to possess hypolipidemic and hypocholesterolemic activity. The treatment with 200 mg/kg and 300 mg/kg of HSEE showed significant reduction in lipid parameters over 4 weeks of study and even as per acute toxicity study, 300 mg/kg dose was found to be safe and most effective during the study. In the present study, atorvastatin was used as a positive control and hypolipidemic effect of atorvastatin was the highest.

Various studies reported the following results: the dried calyx at a conc. of 5% and 10% of the diet of albino rats had significantly reduced the total lipid level by 49% and 53%, respectively (10); aqueous extract from the flowers of *Hibiscus sab-*

Table 1. Levels of cholesterol, triglycerides, HDL, LDL and VLDL in serum of rats in various groups during 4 weeks of treatment.

	Control-I	Control-II (corn oil-fed group)	Cholesterol group	Atorvastatin 10 mg/kg	HSEE 100 mg/kg	HSEE 200 mg/kg	HSEE 300 mg/kg
Cholesterol level							
Baseline	94.3 ± 2.2	102.4 ± 6.2	159.6 ± 7.4[a]	154.3 ± 2.6	161.7 ± 2.9	158.7 ± 3.4	162.4 ± 4.2
4 weeks	100.4 ± 3.2	138.6 ± 3.7	163.8 ± 3.2[a]	110.0 ± 3.9[b,c,d]	157.4 ± 3.6	129.4 ± 6.3[b,c]	126.3 ± 5.4[b,c]
Triglycerides level							
Baseline	118.3 ± 2.7	112.4 ± 3.7	148.9 ± 4.2[a]	152.4 ± 6.4	156.7 ± 4.6	148.8 ± 4.2	153.4 ± 3.8
4 weeks	128.4 ± 6.2	139.4 ± 5.2	159.4 ± 3.8[a]	115.3 ± 7.3[b,c]	149.8 ± 2.6	125.5 ± 2.8[b,c]	121.8 ± 2.4[b,c]
HDL leve							
Baseline	21.7 ± 1.2	23.9 ± 1.2	23.4 ± 1.8	19.7 ± 3.8	20.3 ± 1.8	19.4 ± 3.8	20.4 ± 4.4
4 weeks	24.3 ± 2.4	25.4 ± 3.2	20.8 ± 0.9	23.5 ± 1.6	21.7 ± 1.6	21.3 ± 1.8	23.7 ± 4.8
LDL level							
Baseline	49.0 ± 2.8	56.0 ± 5.2	106.4 ± 3.9[a]	104.1 ± 4.2	110.1 ± 5.8	109.6 ± 4.4	111.3 ± 7.4
4 weeks	50.4 ± 5.8	85.5 ± 4.7[e]	111.1 ± 5.7[a]	63.4 ± 5.8[b,c,d]	105.7 ± 6.2	83.0 ± 2.9[b,c]	78.2 ± 5.4[b,c,d]
VLDL level							
Baseline	23.6 ± 1.8	22.5 ± 4.2	29.8 ± 4.7	30.5 ± 5.2	31.3 ± 3.4	29.7 ± 5.2	30.7 ± 2.8
4 weeks	25.7 ± 3.2	27.9 ± 3.7	31.9 ± 2.9	23.1 ± 4.3[b,c]	30.0 ± 2.6	25.1 ± 1.7	24.4 ± 4.6[b,c]

Data are the mean ± SEM obtained from six rats per group; [a] $p < 0.05$ as compared with corn oil fed rat (control-II group) at corresponding times; [b] $p < 0.05$ as compared with baseline level of serum cholesterol group; [c] $p < 0.05$ as compared with cholesterol group at corresponding times; [d] $p < 0.01$ as compared with baseline level of serum cholesterol group; [e] $p < 0.01$ as compared with control-I group

dariffa L. at 0.5% and 1.0% of the diet of New Zealand white rabbits inhibited the serum lipid levels and also possessed antiatherosclerotic activity (17, 18); another study reported that administration of 5% and 10% ethanolic extract from flowers of *Hibiscus sabdariffa* L. to cholesterol rich basal diet showed better results in the reduction of serum lipid levels (19) and administration of aqueous extracts of calyx of *Hibiscus sabdariffa* L. at 500 mg/kg and 1000 mg/kg reduced the cholesterol by 22% and 26%, respectively; triglycerides level by 33% and 28%, respectively and LDL level by 22% and 32%, respectively (20), suggesting that till now hypolipidemic effect has been studied only in flowers and calyces of *Hibiscus sabdariffa* L.

Chemical constituents reported in the leaves of *Hibiscus sabdariffa* L. are organic acids (15-30%), which include hibiscus acid, oxalic acid, tartaric acid, malic acid and succinic acid; anthocyanins, which involve cyanidine-3-sambubioside, cyanidine-3-glucoside, delphinidine-3-sambubioside and delphinidine-3-glucoside as major constituents. Beside these, leaves of *Hibiscus sabdariffa* L. also contain seven new flavonoids, protocatechuic acid, vitamin C, mucilages, pectin, quercetin, kaempferol and sitosterol-β-D-galactoside. (6, 21-23).

Therefore, possible explanations for hypolipidemic effects of the leaves of *Hibiscus sabdariffa* L. included the presence of hibiscus acid, sitosterol-β-D-galactoside, pectin, quercetin, kaempferol and flavonoids. It had been reported that hibiscus acid has inhibitory action against pancreatic α-amylase and small intestine α-glucosidase enzymes. This indicated that hibiscus acid could inhibit carbohydrate absorption and metabolism (24). Therefore, it might be hypothesized that hibiscus acid could inhibit lipogenesis, which in turn helps in treating type-IV and type-V hyperlipidemia.

Another research work proposed the role of polygalacturonic acid in the pectin molecule as responsible for the cholesterol-lowering properties of pectin (25). A further research work states that the 5 g/100 g apple pectin diet had no effect on plasma lipids, but effectively lowered liver cholesterol and triglycerides because pectin could induce HMG-CoA reductase and decrease mitochondrial fatty acid oxidation and phosphatidate phosphohydrolase and augment liver cholesterogenesis and lipogenesis (26).

Also, sitosterol-β-D-galactoside has the property to reduce the content of blood cholesterol and β-lipoproteins as well as it normalizes the cholesterol/phospholipid ratio during hypercholesterolemia as it regulates the synthesis of phospholipids in experimental hypercholesterolemia (27). Several naturally occurring constituents like quercetin, kaempferol and flavanoids have received considerable attention because of their potential antioxidant activity. Many reviews have emphasized the potential of diet rich in these compounds in the management of hypertension, hyperglycemia and hyperlipidemia (28).

Consequently, above stated reasons could be attributed to the hypolipidemic effect of leaves of *Hibiscus sabdariffa* L.

The conclusion of the study is that the treatment with HSEE (200 mg/kg, 300 mg/kg) significantly reduced the serum lipid levels over the four weeks study, whereas 100 mg/kg group did not show significant reduction and the results are consistent with the earlier studies on calyces.

Since calyces are not available throughout the year, hence to cater the demand of it; leaves can be the better raw material for the pharmaceutical industry.

However, more models and extracts are required to justify the hypolipidemic effect of *Hibiscus sabdariffa* L. leaves.

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