Antihyperlipidemic Effect of Ethanolic Extract of *Hibiscus rosa sinensis* Flowers in Hyperlipidemic Rats

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**ABSTRACT**

The antihyperlipidemic activity of *Hibiscus rosa sinensis* flowers ethanolic extract was investigated in triton (400 mg/kg b.w.) induced and atherogenic diet-induced hyperlipidemic rats in comparison of a known antihyperlipidemic drug simvastatin (10 mg/kg body wt.). Dose selection was made on the basis of acute oral toxicity study (50 mg to 5000 mg/kg body weight) as per OECD guidelines. Oral administration of 500 mg/kg body wt. of the ethanolic extract of *Hibiscus rosa sinensis* flowers exhibited a significant reduction ($p<0.01$) in serum lipid parameters total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats in comparison with hyperlipidemic control in both models. The drug has the potential to act as antihyperlipidemic drug.

**KEYWORDS:** *Hibiscus rosa sinensis*, Hyperlipidemia, Triton, Simvastatin

**INTRODUCTION:**

Hyperlipidemia with increased concentration of cholesterol, triglycerides carrying lipoproteins is considered to be the cause of arteriosclerosis with its dual sequel of thrombosis and myocardial infarction. Lipoproteins are divided into six major classes: chylomicrons, chylomicron remnants, VLDL (very low density lipoprotein), IDL (intermediate density lipoprotein), low density lipoprotein (LDL) and HDL (high density lipoprotein). HDL promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore increased levels of HDL are desirable. On the contrary high levels of VLDL and LDL promote arteriosclerosis. LDL especially in the oxidized form is taken up by macrophages via scavenger mechanism therefore anti-atherosclerotic drugs should reduce VLDL and elevate HDL. Epidemiological studies have demonstrated the strong causal relations in the levels of lipid parameters and hyperlipidemia. The LDL transfers cholesterol towards extra hepatic organs as major carrier, and HDL transports cholesterol from the periphery tissues to the liver for catabolism. HDL plays a pivotal role in the reverse cholesterol transport (RCT).

Hyperlipidemia is deeply involved in the etiology of arteriosclerosis. Moreover, results of various studies have revealed that hyperlipidemia is an important risk factor of coronary disease hence much attention is being given to primary and secondary prevention of hyperlipidemia. As a result, antihyperlipidemic agents having various pharmacological actions are being tested clinically. Because hyperlipidemia in many cases is caused by over-ingestion of alcohol or foods, attention is also being paid to treatment of patients with hyperlipidemia using strict dietary management and appropriate exercise. Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis therefore two ways are feasible to reduce hyperlipidemia; to block endogenous synthesis or to decrease absorption. Both factors can be evaluated in normal animals without artificial diets.

*Hibiscus rosa sinensis* is an erect, much-branched, glabrous shrub, 1 to 4 m high. Leaves are ovate, acuminate, coarsely toothed, 7 to 12 cm long, alternate, stipulate. Flowers are pedicillate, actinomorphic, pentamerous and complete; Corolla consists of 5 petals, red in color, obovate, entire, rounded tip, imbricate and about 3 inches in diameter. Inflorescence is solitary, axillary and very large. Outermost series of bracteoles - 6, lanceolate, green, and 8 mm long or less. Calyx is green, 2 cm long, lobes ovate. Stamens are forming a long staminal tube enclosing the entire style of the pistil and protruding out of the corolla. Ovary 5-celled, styles 5, fused below.

Some of the chemical constituents isolated from this plant are cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. Flavonoids are also present. Its flowers contain apigenidin, citric acid, cyanidin diglucoside, cyanin, fructose, gentisic acid, glucose, pelargonidin, quercetin, sucrose and tartaric acid.

*Hibiscus rosa-sinensis* petal infusion is widely used in ayurvedic medicine in India as a demulcent refrigerant drink in fever and decoction is given in bronchial catarrh. Previous studies showed that the plant possesses anti-complementary, anti-diarrhetic, anti-phlogistic activity. It has been reported that...
the plant flower possesses anti-spermatogenic and androgenic, anti-tumour and anticonvulsant activities. It helps in inducing abortion, provide treatment for headache. Young leaves are sometimes used as a spinach substitute. It also showed anti implantation, anti-inflammatory, anti-pyretic, anti-spasmodic, anti-spermatogenic and anti-viral activities. The decoction of the roots is used for coughs and colds. The infusion of the petals of the flowers soothes and protects the alimentary tract, relieves inflammation and lowers body heat. In fevers, an infusion of the flowers helps to reduce body temperature. The application of crushed flowers soothes external wounds and sores. Flowers can also be made into a kind of pickle or used as a purple dye for coloring foods such as preserved fruits and cooked vegetables. The leaves make a gentle laxative and soften inflamed parts. Root is edible but very fibrous. It's also good for hair treatment.

To the best of our knowledge no scientific data regarding the antihyperlipidemic effect of *Hibiscus rosa sinensis* flowers are available except in the treatise of Ayurvedic medicine. Thus, the present study was undertaken to evaluate the antihyperlipidemic effect of *Hibiscus rosa sinensis* flowers.

Several studies showed that systemic administration of triton WR1339 (ionic surfactant) in fasted rats causes elevation in plasma lipid level. Initially, there is a sharp increase in lipid level reaching a peak two to three times the control value by 24 hours after the administration of triton injection phase I (synthetic phase), this hyperlipidemia falls within next 24 hr i.e. 48 hrs after triton administration, phase II (Excretion phase). This increase in plasma lipid by triton is thought to be due to increased hepatic synthesis of cholesterol or removal of very low density protein (VLDL) from the blood due to their physical alteration by triton. Antihyperlipidemic drugs interfering with cholesterol synthesis were shown to be active in phase I while drug interfering with cholesterol excretion and metabolism were active in phase II. Triton-induced hyperlipidemia is rather simple and rapid method for evaluation of test substance and can be considered as the useful method for preliminary screening of antihyperlipidemic drugs.

The search for new drug with the ability to reduce or regulate serum cholesterol and triglyceride concentrations has gained momentum over the years, resulting in a plethora of publications reporting significant activity of a variety of natural and synthetic agents. Molecular modification of naturally occurring compounds has also given rise to potent agents like pravastatin and simvastatin; the former prepared by replacement of the methyl group of naturally occurring lovastatin by a hydroxyl group and the latter a methylated derivative of compaction. In continuation of our search for plant derived antihypercholesterolemic and hypolipidemic agents, we direct our attention to some Indian medicinal plants of which antihyperlipidemic activity has not been scientifically validated.

**MATERIALS**

**Plant material**

Flowers of *Hibiscus rosa sinensis* were collected in and around local forest area of Ankola in Western Ghats, Karnataka and authenticated by the Botanist Prof. G. S. Naik, Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GGSAC/HRS/01 was also preserved in the same college. The collected flowers were dried and powdered to coarse consistency in cutter mill. The powder was passed through 40 # mesh particle size and stored in an airtight container at room temperature.

**Atherogenic diet and chemicals**

Experimental diet consists of well pulverized mixture of Cholesterol (2%), Cholic acid (1%), peanut oil (10%), sucrose (40%) and normal laboratory diet (47%). A suspension of Triton –WR 1339 (S D Fine chemicals) in 0.15 M NaCl was used for inducing hyperlipidemia in experimental rats. Simvastatin (Dr. Reddy’s Laboratories, Hyderabad), Diagnostic kits for estimation of were purchased from Merck Diagnostics India Ltd. Anesthetic Ether (Ozone International, Mumbai), Distilled Water and All other chemicals were of Analytical grade.

**Animals**

Adult Albino rats of wistar strain (150-200 g) of either sex were procured and housed in the animal house of K L E S College of Pharmacy, Ankola with 12 h light and 12 h dark cycles. Standard pellets obtained from Goldmohar rat feed, Mumbai India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. Ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA). All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India.

**METHODS**

**Extraction of plant material**

Powdered crude drug (2.5 kg of the fresh air-dried) of *Hibiscus rosa sinensis* flowers were extracted with 95% ethanol by adopting simple maceration procedure at room temperature.
for seven days in a conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites. Extract was preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out by different methods of phytochemical analysis. A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

**Preparation of dose for dried extract and standard drugs**

Ethanolic extract (500 mg/kg b.w) of the selected plant were formulated as suspension in distilled water using Tween-80 as suspending agent. The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

Simvastatin 10 mg/kg was used as the reference standard drug for evaluating the antihyperlipidemic activity which was made into suspension in distilled water using Tween-80 as a suspending agent.

**Acute oral toxicity studies**

The acute oral toxicity studies of extract were carried out as per the OECD guidelines from CPCSEA. Administration of the stepwise doses of ethanolic extract of *Hibiscus rosa sinensis* from 50 mg/kg b.w. up to the dose 5000 mg/kg b.w. caused no considerable signs of toxicity in the tested animals. One tenth of upper limit dose were selected as the levels for examination of antihyperlipidemic activity.

**Diet-induced hyperlipidemic model:**

The animals were selected, weighed then marked for individual identification. Rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days. The rats were then given plant extract suspended in 0.2% tween 80 at the dose of 500 mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days. During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters.

**Triton-induced hyperlipidemic model**

Animals kept for fasting for 24 h, were injected a saline solution of Triton at the dose of 400 mg/kg b.w. intraperitoneally. The plant extract, at the dose of 500 mg/kg b.w., were administered orally through gastric intubation. The first dose being given immediately after triton injection and second dose 20 h later. After 4 h of second dose the animals were used for various biochemical parameters.

**Experimental design**

Animals were divided into four different groups with six animals in each group. Group I served as normal control, Group II was positive control which was given standard antihyperlipidemic drug simvastatin (10 mg/kg/day p.o.). Group III was hyperlipidemic control and this group did not receive any treatment except standard pellet diet. Group IV received the active plant extract of *Hibiscus rosa sinensis* flowers (500 mg/kg/day, p.o.). Treatment periods for all these groups were 14 days in atherogenic diet-induced hyperlipidemia and 48 hours in case of triton-induced hyperlipidemia.

**Collection of blood**

Blood was collected by retro-orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged at 4000 rpm for 10 minutes.

**Biochemical and HPTLC analysis**

The serum was assayed for total cholesterol, triglycerides, phospholipids and high-density lipoprotein (HDL) using standard protocol method. By using Friedwald formula the concentration of very-low density lipoprotein (VLDL) and low density lipoprotein (LDL) in serum were calculated.

An HPTLC chromatogram of active extract was also done by using CAMAG TLC SCANNER IV, densitometric evaluation system with CAT software instrument was used for scanning of thin layer chromatogram objects in reflectance or transmission mode by absorbance or by fluorescence at 254 or 366 nm respectively.

**Statistical Analysis**

The results of the study were expressed as mean± S.E.M. Data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett’s t-test for multiple comparisons. Values with (P<0.05) were considered significant.

**RESULTS AND DISCUSSION**

The effect of *Hibiscus rosa sinensis* flowers various extract were studied on serum lipids and lipoproteins level of triton (400 mg/kg b.w.) induced hyperlipidemic rats and results are expressed as change in serum lipid and lipoprotein levels.

As expected, administration of triton WR1339 led to elevation of serum lipid and lipoprotein levels, which were maintained over a period of study in hyperlipidemic control group and these rats, were given treatment with ethanolic...
Extract of *Hibiscus rosa sinensis* flowers. The results were comparable with reference standard simvastatin. There was a significant elevation in serum lipids and lipoproteins in triton-induced hyperlipidemic control (p<0.01) rats when compared with normal control.

In triton-induced hyperlipidemic model, *Hibiscus rosa sinensis* flowers Ethanolic extract showed significant serum lipid lowering effects in hyperlipidemic rats which brought down total cholesterol level, triglycerides, phospholipids, LDL, VLDL and elevated HDL level in comparison of similar parameters of hyperlipidemic control at 48th hr of study after the treatment (Table 1-2).

In diet-induced hyperlipidemic model, results showed serum lipid lowering potential of ethanolic extract of selected plant for study. In figure, tested drug results are presented for 14 days study. These results are comparable to standard drug simvastatin. Ethanolic extract of *Hibiscus rosa sinensis* demonstrated significant serum lipid lowering effects (P<0.01) after giving two doses of 500 mg/ kg,b.w. for 14 days treatment. It reduced total cholesterol 73±2.608, triglycerides 69.66±1.542, phospholipids 78±3.173, LDL 49±2.683, VLDL 25.5±1.335 and increased level of HDL 28.16±2.167 in comparison of diet induced hyperlipidemic control total cholesterol 101.16±2.613, triglycerides 86±2.280, phospholipids 107.66±2.642, LDL 81±2.556, VLDL 35±1.141 and HDL 21.08±1.172 at 14th day which was closer to the standard drug total cholesterol 68±2.864, triglycerides 65.33±1.801, phospholipids 75±1.528, LDL 43.83±2.182, VLDL 24±1.461 and increased level of HDL 28±1.571 anti-hyperlipidemic results. Standard anti-hyperlipidemic agent Simvastatin 10 mg/kg body weight also able to reduce the elevated serum lipid level towards the normal (Fig. 1).

HPTLC profile of ethanolic extract also reveals the presence of polyphenolic compound which may be involved in its antihyperlipidemic activity (Fig. 2).

There is a close relationship between atherosclerosis and an increase or decrease of serum lipids, in particular very low-density lipoprotein and LDL may be risk factors and HDL may be a protective factor. There was marked increase in the level of serum total cholesterol, triglycerides, phospholipids, LDL, VLDL and decrease in the level of good cholesterol carrier HDL in the animals treated with triton and atherogenic diet. Elevated level of blood cholesterol especially LDL was the major risk factor for the coronary heart disease and HDL as cardio protective protein. The data presented in this report show that repeated administration of *Hibiscus rosa sinensis* flowers ethanolic extract (500 mg/kg b.w) for 14 days induced a significant reduction of serum cholesterol, triglycerides, phospholipids, VLDL and LDL as compared to hyperlipidemic control. Decrease in the triglyceride level may be due to the increase in activity of the endothelium bound lipoprotein lipase which hydrolyses the triglyceride into fatty acid or due to inhibition of lipolysis so that fatty acids do not get converted to triglyceride.

There was significant increase in the HDL as compared to control. This effect may be due to the increased activity of lecithin: cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL and transferred back to VLDL and intermediate density lipoprotein.

**CONCLUSION**

In the present study, ethanolic extract of *Hibiscus rosa sinensis* flower extract showed significant antihyperlipidemic activity. The anti-hyperlipidemic activity was evaluated by using atherogenic diet induced and triton induced hyperlipidemia model. It was found that ethanolic extract significantly reduced serum elevated lipid level as compared to hyperlipidemic control group and proved to be an anti-
Table 1: Effect of *Hibiscus rosa sinensis* ethanolic extract on serum total cholesterol, triglycerides and phospholipids level in triton-induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
<th>Values are expressed as mg/dl, Mean±SEM</th>
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<tr>
<td></td>
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<td>Cholesterol</td>
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<td></td>
<td></td>
<td>6 hr</td>
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<tr>
<td>I</td>
<td>Normal control (vehicle only)</td>
<td>60.83±1.327</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic control</td>
<td>106.5±4.089</td>
</tr>
<tr>
<td>III</td>
<td>Simvastatin 10mg/kg</td>
<td>83.67±1.838 **</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract 500mg (EEHRS)</td>
<td>87.33±3.333 **</td>
</tr>
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*mg/kg/day for 48 hrs. Values are means±SEM; N=6. Values are statistically significant at *P*<0.05 and more significant at **P**<0.01. ns= not significant, *P*<0.01 vs Hyperlipidemic control. (ANOVA)

Table 2: Effect of *Hibiscus rosa sinensis* ethanolic extract on LDL, VLDL and HDL level in triton-induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
<th>Values are expressed as mg/dl, Mean±SEM</th>
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<td></td>
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<td>LDL</td>
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<td></td>
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<td>6 hr</td>
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<tr>
<td>I</td>
<td>Normal control (vehicle only)</td>
<td>55.5±1.232</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic control</td>
<td>105.66±2.319</td>
</tr>
<tr>
<td>III</td>
<td>Simvastatin 10mg/kg</td>
<td>64.83±2.272</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract 500mg (EEHRS)</td>
<td>77±2.260 *</td>
</tr>
</tbody>
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*mg/kg/day for 48 hrs. Values are means±SEM; N=6. Values are statistically significant at *P*<0.05 and more significant at **P**<0.01. ns= not significant, *P*<0.01 vs Hyperlipidemic control. (ANOVA)
hyperlipidemic agent in above mentioned hyperlipidemic models. In comparison to standard drug simvastatin effect of ethanolic extract of *Hibiscus rosa sinensis* flower extract was less but comparable notably. Present studies reveal that ethanolic extract of *Hibiscus rosa sinensis* flowers can be used as effective antihyperlipidemic agent and can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. Further experiments are required to prove the mechanism and advantage of this drug over other drugs.

REFERENCES


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