

Hypolipidemic activity of *Hibiscus rosa sinensis* root in rats

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Received 12 August 2009; revised 20 November 2009

The hypolipidemic activity of *Hibiscus rosa sinensis* (family Malvaceae) root extract was studied on triton and cholesterol-rich high fat diet (HFD) induced models of hyperlipidemia in rats. In triton WR-1339-induced hyperlipidemia, feeding with root extract (500 mg/kg body wt/day p.o.) exerted lipid-lowering effect, as assessed by reversal of plasma levels of total cholesterol (TC), phospholipids (PL) and triglycerides (TG) and reactivation of post-heparin lipolytic activity (PHLA) of plasma. The other model was fed with cholesterol-rich HFD and root extract (500 mg/kg body wt/ day p.o.) simultaneously for 30 days. This also caused lowering of lipid levels in plasma and liver homogenate and reactivation of plasma PHLA and hepatic total lipoprotein lipase activity. The hypolipidemic activity of *Hibiscus rosa sinensis* root was compared with a standard drug guggulipid (200 mg/kg body wt/day p.o.), a known lipid- lowering agent in both models. Histopathological findings in rat liver supported the protective role of *H. rosa sinensis* root extract in preventing cholesterol-rich HFD-induced hepatic steatosis.

Keywords: *Hibiscus rosa sinensis*, Triton, Cholesterol hyperlipidemia, Hypolipidemic agent, Post-heparin lipolytic activity, Hepatic lipoprotein lipase activity, Hepatic steatosis.

Hibiscus rosa sinensis (family Malvaceae; Hindi name Gudhal, Japa in Sanskrit and Shoe flower in English) is an Ayurvedic remedy that has been mentioned in many ancient Indian medical literatures and is reported to possess antipyretic¹, anti-complementary and anti-diarrheic activities². The flowers of the plant possess anti-spermatogenic, androgenic³ and anticonvulsant activities⁴. The leaves are useful in healing of ulcers and exhibit hair growth promoting activity⁵. Recent reports have also shown anti-tumor, antioxidant, anti-ammonemic, post-coital anti-fertility, cardio protective and wound healing activities⁶⁻¹⁴.

Cardiovascular diseases are leading cause of death in both industrialized and developing nations. Disorders of lipid metabolism, following oxidative stress are the prime risk factors for initiation and progression of these diseases^{15,16}. The known lipid lowering drugs, such as fibrates, statins and bile acid

sequestrants have many side effects in patients¹⁷. Thus, there is a considerable interest on development of lipid-lowering drugs from natural products in the recent years. In the present study, we have investigated hypolipidemic activity of *Hibiscus rosa sinensis* root in triton-WR-1339 and high fat diet-induced hyperlipidemia in rats.

Material and Methods

Preparation of root extract

H. rosa sinensis roots (primary, secondary and tertiary) were collected from local area of Lucknow and identified taxonomically by Department of Pharmacology, Era's Lucknow Medical College, Lucknow. A voucher specimen (HRS-001/06) was also submitted to Pharmacology Department. Roots were dried under shade, powdered and (500 g) extracted with 95% ethanol¹⁸ in a soxhlet extractor for 72 h, and the extract was concentrated to dryness under reduced pressure and controlled temperature (50-60°C), yielding 25 g of reddish brown semi-solid extract. This was stored in refrigerator and used to investigate hypolipidemic activity in rats. Guggulipid, a potent lipid-lowering agent from *Commiphora mukul* developed in Central Drug Research Institute, Lucknow was used as a standard drug.

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Abbreviations: HFD, high fat diet; LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; PL, phospholipids; TC, total cholesterol; TG, triglycerides.

Preparation of Cholesterol-rich high fat diet

Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow diet supplied by Ashirvad Industries, Chandigarh, India. Ingredient and nutrient composition of the normal rat diet was: casein 210; corn starch 440; sucrose 100; maltose dextrin 100; cellulose 50; soya bean oil 50; vitamins mix 10; and minerals 35 g/kg. Other ingredients included choline bitartrate (2 g/kg) and t-butyl hydroquinone (0.008 g/kg). Proximate analysis of diet showed it contained crude protein 21; crude fat 5; crude fiber 4; and ash 8%. Simultaneously, cholesterol (5 g) was dissolved in 300 g warm coconut oil and this oil solution was added slowly into powdered mixture of normal rat chow diet to obtain homogeneous soft cake. This cholesterol-rich (HFD) preparation was molded in the shape of pellets of about 3 g each¹⁶.

Animals

In vivo experiments were conducted as per the guidelines provided by Animal Ethics Committee of Central Drug Research Institute, Lucknow, India. Male adult rats of Charles Foster strain (200-225 g) bred in animal house of the Institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26°C, relative humidity 50-70% and 12/12 h light/dark cycle (light from 8:00 a.m. to 8:00 p.m.) and provided with standard rat pellet diet and water *ad libitum*.

Triton and cholesterol-rich HFD-induced hyperlipidemia

The rats were divided into four groups containing six animals in each group: control, hyperlipidemic, hyperlipidemic treated with *H. rosa sinensis* or guggulipid (standard drug). In the acute experiment to induce hyperlipidemia, triton WR-1339 (Sigma Chemical CO, St. Louis, MO, USA) was administered (400 mg/kg body wt/day p.o.) by intraperitoneal injection. *H. rosa sinensis* root extract and guggulipid were macerated with aqueous gum accacia (0.2% w/v) suspension and fed orally at the doses of 500 and 200 mg/kg body wt/day p.o.) respectively, simultaneously with triton and blood was collected after 18 h¹⁶. In the chronic experiment, hyperlipidemia was produced by feeding with cholesterol rich-HFD for 30 days. Drugs were administered orally at the same doses as above simultaneously with cholesterol-rich HFD in the drug-treated groups. Control animals received the same amount of vehicle. At the end of experiment, rats were fasted overnight, anaesthetized with thiopentone solution (50 mg/kg body wt/day i.p.) prepared in normal saline. Blood was withdrawn from retro-orbital plexus using glass capillary in EDTA coated tubes (3 mg/ml blood).

Thereafter, animals were sacrificed and liver was excised immediately, washed with cold 0.15 M KCl and kept it at -40°C till analyses. Blood was centrifuged and plasma was taken¹⁶.

Biochemical analysis of plasma and liver

Plasma post-heparin lipolytic activity (PHLA) was assayed spectrophotometrically using intralipid as artificial substrate¹⁹. Plasma was diluted with normal saline in a ratio of 1:3 and used for the analysis of total cholesterol (TC)²⁰, phospholipids (PL)²¹ and triglycerides (TG)²² using standard enzymatic kits supplied by Merck India Ltd. Mumbai, India. Liver was homogenized (10% w/v) in cold 100 mM phosphate buffer, pH 7.2 and used for the assay of lipoprotein lipase (LPL) activity¹⁹. The lipid extract of each homogenate prepared in a mixture of CHCl₃:CH₃OH (2:1, v/v) was used for estimation of TC²⁰, PL²¹ and TG²². Protein content of plasma and tissue were also estimated²³.

Histopathological study

Histopathological study of rat liver of all the four groups was also performed. Tissue sections of 3 µm thickness were stained with Ehrlich's hematoxylin and eosin and examined by light microscopy.

Statistical analysis

One-way-analysis-of-variance (ANOVA-New man's student t-test) was performed by comparison of values for hyperlipidemic groups with control, hyperlipidemic and drug-treated groups with hyperlipidemic. All hypothesis testing were two-tailed. $P < 0.05$ was considered statistically significant and results were expressed as mean \pm SD of six rats. The statistical analysis was done using the graph pad INSTAT 3.0 software²⁴.

Results

Effect of *H. rosa sinensis* root extract in triton-induced hyperlipidemia

The data in Table 1 show that acute administration of triton WR-1339 caused marked increase in the plasma levels of TC (161%), PL (148%) and TG (166%), followed by inhibition of PHLA by 20%. Treatment with root extract exerted a decrease in these levels of TC (23%), PL (19%) and TG (22.0%), simultaneously with reactivation of PHLA by 11%. However, the hyperlipidemic activity of guggulipid (31-35%) was comparatively higher to that of *H. rosa sinensis* root extract.

In the cholesterol-rich HFD-induced model of hyperlipidemia (Table 1), feeding with cholesterol-rich HFD in rats caused marked increase in

Table 1—Effect of *H. rosa sinensis* root extract and guggulipid on plasma lipids in triton and cholesterol rich HFD induced hyperlipidemia[Values are mean \pm SD of 6 animals]*In triton-induced hyperlipidemia*

	Total cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglycerides (mg/dl)	PHLA (n mol free fatty acid released/h/L)
Control	92.46 \pm 4.46	99.66 \pm 9.69	84.52 \pm 5.21	15.50 \pm 0.85
Triton-treated	240.89*** \pm 12.09	247.57*** \pm 24.86	224.60*** \pm 12.09	12.26** \pm 1.34
Triton + root extract	188.49*** \pm 7.49	201.02** \pm 13.85	176.06*** \pm 6.16	13.76* \pm 0.72
Triton + guggulipid	167.14*** \pm 7.85	157.14*** \pm 8.23	150.13*** \pm 8.65	14.56* \pm 0.68

Triton-treated group was compared with control, triton and drug-treated with triton *P<0.05; **P<0.01; ***P<0.001.

In cholesterol rich HFD induced hyperlipidemia

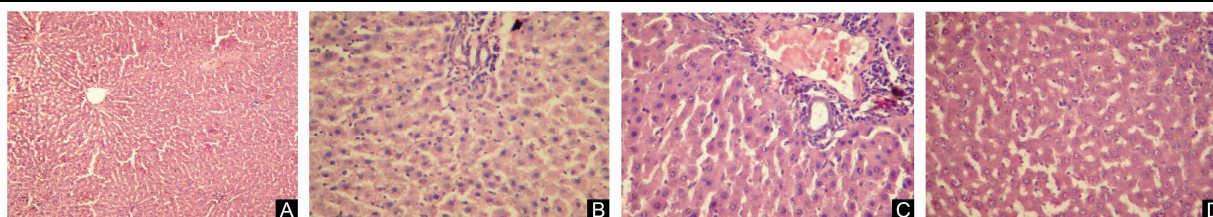
	Total cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglycerides (mg/dl)	PHLA (n mol free fatty acid released/h/L)
Control	91.29 \pm 7.06	78.72 \pm 5.00	114.90 \pm 11.20	18.71 \pm 1.20
Cholesterol rich HFD-treated	201.08*** \pm 20.10	159.27*** \pm 24.86	241.35*** \pm 24.59	12.10*** \pm 1.03
Cholesterol rich HFD + root extract	161.52** \pm 13.13	122.49*** \pm 7.47	192.0** \pm 7.46	14.33* \pm 1.62
Cholesterol rich HFD + guggulipid	143.39*** \pm 8.83	112.30*** \pm 8.77	169.92*** \pm 6.09	14.93* \pm 1.84

Cholesterol rich HFD-treated group was compared with control, cholesterol rich HFD and drug-treated groups with cholesterol rich HFD. *P<0.05; **P<0.01; ***P<0.001.

Table 2—Effect of *H. rosa sinensis* root extract and guggulipid on liver lipids in cholesterol rich HFD-induced hyperlipidemia[Values are mean \pm SD of 6 animals]

	Total cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	LPL activity (μ mol free fatty acid released/h/mg protein)
Control	5.85 \pm 0.30	21.63 \pm 2.71	4.84 \pm 0.60	82.55 \pm 3.82
Cholesterol rich HFD-treated	9.45*** \pm 0.62	37.58*** \pm 5.33	7.56*** \pm 1.02	53.26*** \pm 2.72
Cholesterol rich HFD + root extract	7.45*** \pm 0.72	29.30*** \pm 3.90	5.72*** \pm 0.74	64.33* \pm 3.75
Cholesterol rich HFD + guggulipid	6.86*** \pm 0.80	27.46*** \pm 4.04	4.80*** \pm 0.62	66.66** \pm 3.65

Cholesterol rich HFD treated groups were compared with control cholesterol rich HFD and drug-treated groups with cholesterol rich HFD. *P<0.05; **P<0.01; ***P<0.001.

Fig. 1—Photomicrographs of (A) normal rat liver (H & E X100); (B) HFD-treated rat liver (H & E X400); (C) HFD + *Hibiscus rosa sinensis* root extract treated rat liver (H & E X400); and (D) cholesterol rich HFD + guggulipid (standard drug) treated rat liver (H & E X400)

the plasma levels of TC (120%), PL (102%) and TG (110%), followed by inhibition of PHLA by 35%. Treatment with root extract for 30 days reversed the plasma levels of TC (20%), PL (23%) and TG (20%), simultaneously with reactivation of PHLA by 16.0%.

Feeding with cholesterol-rich HFD in rats also caused marked accumulation of TC (62%), PL (74%) and TG (56%), followed by decrease of LPL activity (36%) in their liver (Table 2). However, treatments with root extract caused decrease in the levels of TC (21%), PL (22%) and TG (24%), followed by reactivation of LPL activity (17%) in hyperlipidemic animals. Guggulipid showed higher hypolipidemic activity as compared with *H. rosa sinensis* root.

Effect of root extract on liver histopathology of cholesterol-rich HFD treated rats

As is evident from Fig. 1A, the histopathological alterations viz. steatosis, sinusoidal dilatation and congestion, Kupfer cell hyperplasia or portal triaditis were not present in normal rat liver. Histopathology of liver in rats fed with cholesterol-rich HFD showed microvesicular steatosis and occasional macrovesicular steatosis in zone 3 (perivenular area) of hepatic lobules along with sinusoidal dilatation and mild congestion. The portal triads revealed mild increase in inflammatory infiltrate of mononuclear cells and neutrophils. The overall hepatic architecture was maintained (Fig. 1B). Hyperlipidemic rats which

simultaneously received *H. rosa sinensis* root extract showed only mild feathery degenerative changes and mild vascular congestion, but no steatosis. Kupfer cells lining the sinusoids were prominent and increased in number. Their portal triads showed mixed inflammatory infiltrate rich in mononuclear cells admixed with polymorphs and fair number of eosinophils. The overall hepatic architecture was maintained (Fig. 1C). Liver histopathology of rats treated with cholesterol-rich HFD and guggulipid showed only mild sinusoidal congestion and Kupfer cell prominence. Portal tracts and the overall hepatic architecture were within normal limits (Fig. 1D).

Discussion

Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting into increased blood lipid concentration^{15,25}. The lipid-lowering effect of *H. rosa sinensis* root extract might be due to an early clearance of lipids from circulation in both hyperlipidemic models (triton and cholesterol-rich HFD-induced models) and it might be due to reactivation of lipolytic enzymes as evidenced by increased PHLA. Investigations with cholesterol rich HFD fed hyperlipidemic animals showed that root extract stimulated PHLA and hepatic LPL activity, both of which play a key role in lipid catabolism and their utilization in the body²⁸, leading to decrease in the level of plasma and liver lipids in above models. It is reported that hypolipidemic action of guggulsterone, the active principle of guggulipid is mediated through activation of PHLA, LPL and lecithin cholesterol acyl transferase (LCAT), inhibition of hepatic cholesterol biosyntheses and increased faecal bile acid excretion^{26,27,29}. The similar mechanism might also interplay in the hypolipidemic effect of *H. rosa sinensis* root extract.

Histopathological examination revealed hepatic steatosis induced by cholesterol rich HFD. These effects were not observed when the rats were given cholesterol rich HFD along with *H. rosa sinensis* root extract or the standard drug "guggulipid".

Acknowledgement

One of us (V K) is grateful to Director, CDRI, Lucknow for experimental support and Era's Lucknow Medical College, Lucknow for financial support.

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