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Research Article

PRELIMINARY DOSE DEPENDENT STUDY ON ANTI-HYPERLIPIDEMIC ACTIVITY OF *HIBISCUS ROSA SINENSIS* LINN LEAVES ON TRITON WR 1339 INDUCED HYPERLIPIDEMIC MICE MODEL

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ABSTRACT

The antihyperlipidemic activity of the methanol extract (ME) of the leaves of *Hibiscus rosa sinensis* Linn. against Triton induced hyperlipidemia was evaluated in mice. The ME was administered at a dose of 125, 250 and 500mg/kg to Triton induced hyperlipidemic mice. Simvastatin was used as a reference standard. Administration of a 500mg/kg (p.o) dose of ME resulted in a significant decrease in the levels of serum total cholesterol (71%), triglyceride (74%) and LDL (91%), in Triton induced hyperlipidemic mice model.

Keywords: *Hibiscus rosa sinensis,* Triton, anti-hyperlipidemic activity, cholesterol.

INTRODUCTION

In most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and death.[1,2] Elevated serum cholesterol levels leading to atherosclerosis can cause coronary heart disease (CHD). Reduction in serum cholesterol levels reduces the risk for CHD, substantially [3]. Even if statins remain the major hypolipidemic drug currently in use, an increasing number of patients that are treated with statins suffer from side effects such as hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function or they do not respond well to the therapy. [4] Thus, development of novel approaches to battle the world epidemic of hyperlipidemia remain relevant.

Hibiscus rosa-sinensis Linn [Malvaceae] is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. In medicine, however, the red flowered variety is preferred [5]. The principal constituents of *H. rosa. sinensis* Linn. are flavones; the other constituents being cyclopeptide alkaloid, cyanidin chloride, hentriacontane, riboflavin, ascorbic acid, thiamine, taraxeryl acetate, ß-sitosterol , cyclic acids, sterculic and malvalic acids. All parts of *H. rosa. sinensis* are used for their anti-tumor, antifertility, antiovulatory, anti-implantation, anti-inflammatory, analgesic, antiestrogenic, antipyretic, antispasmodic, antiviral, antifungal, antibacterial, hypoglycaemic, spasmolytic, CNS depressant, hypotensive and juvenoid activity.[6]

The present investigation is an attempt to determine whether the *Hibiscus* leaf extract has any potential for bringing about any changes in the different parameters of blood lipid profile, viz. total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL).

Several studies have shown that systematic administration of Triton WR1339 (non-ionic detergent) to fasted rats causes an elevation in the plasma lipid levels.[7] Initially, there is a twofold or three fold increase in the lipid levels over the control value, 24 hrs after administration of triton, (phase I - synthetic phase); this hyperlipidemia falls off within next 24 hrs i.e., 48hrs after triton administration, (phase II - excretion phase). The increase in the plasma lipids is thought to be either due to increased hepatic synthesis of cholesterol or a physical alteration in VLDL by triton resulting in their removal from the blood. Drugs interfering with cholesterol synthesis were shown to be active in phase one, while drugs interfering with cholesterol excretion and metabolism were found to be active in phase II.[8]

The method employed for triton-induced hyperlipidemia is rather simple and rapid for the evaluation of the test substance and can be considered as a useful method for preliminary screening of hyperlipidemic drugs.[9] It provides the supportive assessment in preliminary screening with advantage of simplicity and rapidity.

MATERIAL AND METHODS

Plant material

Leaves of *H. rosa sinensis* were collected from the local area of Nasik in the month of October and authenticated by a botanist. Leaves were washed thoroughly and dried under shade. Dried leaves were powdered and stored in airtight glass jars, until further use.

Preparation of extract

Fifty grams of dried and ground leaves were extracted for 36 hrs with methanol in a soxhlet apparatus. The extract was filtered and evaporated, at 30–40^{0}C, to obtain a semisolid material. The extractive value was found to be 17 %w/w on a dry weight basis. The dried extract was stored in a refrigerator for further pharmacological studies.

Preliminary Phytochemical analysis:

The Methanol extract of the leaves of *H. rosa sinensis* was subjected to preliminary phytochemical screening for the detection of various plant constituents such as alkaloids, glycosides, flavonoids, tannins, phenolic compounds and triterpenoids using standard procedures. [10, 11]

Chemicals

Triton WR 1339 was procured from Sigma Aldrich (USA) and diagnostic kits for Total cholesterol, triglycerides and HDL were purchased from SPAN diagnostic India Pvt. Ltd. All other reagents and chemicals used in the present study were of analytical grade (AR). Only double distilled water was employed for preparing the reagents.

Experimental Animals

Inbred albino Wistar mice (25-30g) were obtained from the Haffkine Institute, Parel, Mumbai. The animals were maintained in polypropylene cages, in a well ventilated room, at a temperature of 25±1°C with 12:12 hour light/dark cycle. Standard pellet feed (Lipton, India) and filtered tap water was provided *ad libitum* throughout the experimentation period. Animals were acclimated to laboratory conditions 10 days prior to initiation of experiments. The project proposal was approved by the Institutional Animal Ethical Committee (IAEC) and all the experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA).

Acute toxicity studies

Acute oral toxicity of the methanolic extract of *H. rosa sinensis* was determined using albino Wistar mice of either sex. The mice were fasted for 12 h prior to the experiment and were administered a single dose of 2000mg/kg leaf extract and observed for mortality up

to 48 h. Based on the short term toxicity study, the dose of the test animals was determined as per OECD guidelines 423.

Experimental design

The mice were randomly divided into 6 groups, of 6 mice in each group when experiments began. Triton WR-1339 was dissolved in 0.9 % normal saline to a final concentration of 5%. Plant extracts and simvastatin were dispersed in 0.5% CMC solution. All mice except the normal control group (group 1) were injected with triton WR-1339 (Sigma, USA) at a dose of 300 mg/kg body wt. to induce hyperlipidemia, while the normal control group was injected with normal saline of the same volume. Group 2 was given no treatment, and it served as a hyperlipidemic control group (triton control). Eight hours following triton WR-1339 administration, groups 3, 4 and 5 were orally administered the extract of *H. rosa. sinensis* leaf at a dose of 125, 250 and 500 mg/ kg body weight, respectively, by gastric intubation. Simultaneously, the positive control group (Group 6) was given simvastatin at a dose of 80mg/kg and 0.5% CMC was administered to the normal control group (group 1). Blood was collected from the retro-orbital plexus, 24 hours after triton WR-1339 administration; the serum samples were separated from the mouse blood samples and stored at -20^{0}C.

Determination of serum lipid levels

The amount of total cholesterol (TC) [12], triglycerides (TG) [13] and high density lipoprotein cholesterol (HDL-C) [14] in serum were assayed using standard diagnostic kits supplied by SPAN diagnostic, India[15, 16] ·LDL-cholesterol and VLDL-cholesterol were calculated by Friedwald formula.

Statistical analysis

The data are expressed as mean ± SD (n=6). Statistical analysis of data was performed using analysis of variance (ANOVA). P-values of 0.05 or less were considered significant.

RESULTS

Preliminary phytochemical screening

The Phytochemical tests with the methanol extract of *H. rosa sinensis* indicated the presence of flavonoids, glycosides, terpenes, saponins and mucilage.

Acute oral toxicity and effect on general behavior of Hibiscus methanolic extract

The methanol extract of *H. rosa sinensis* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals.

Antihyperlipidemic activity of *H. rosa sinensis* leaf extract

The data in table 1 show that administration of triton WR 1339 (300mg/kg body wt.) caused a marked increase in TC, TG and LDL approximately by 3, 13 and 7 folds respectively, accompanied by a decrease in HDL cholesterol.

Treatment with *Hibiscus* leaf extract at a dose of 125, 250 and 500 mg/kg body wt. resulted in a decrease in the levels of total cholesterol by 19%, 33% and 71% ($p<0.01$) respectively; serum triglycerides level by 42%, 50% and 74% respectively ($p < 0.001$); serum VLDL level by 42%, 50% and 75% respectively ($p<0.001$) while serum LDL level increased by 31% at a dose of 125 mg/kg. However, serum LDL levels were reduced by 2% and 91% in response to a dose of 250 & 500 mg/kg body weight respectively, when compared to the triton control group. These results indicate that methanolic extracts of *H. rosa sinensis* leaves exhibit a hypolipidemic effect. Among the three dose levels, a dose of 500 mg/kg proved to be the most effective against hyperlipidemia.

Table 1: Effect of Methanolic Extract of *H. ROSA. SINENSIS* (125 mg/kg, 250 mg/kg and 500 mg/kg) and Simvastatin (80mg/kg) on Serum Lipid Levels in Triton Induced Hyperlipidemic Mice.

	NORMAL	TRITON WR1339	*Hibiscus* EXTRACT (mg/kg)			SIMVASTATIN
			125 mg/kg	250mg/kg	500 mg/kg	
TC (mg/dl)	84.5± 11.56	248.98 #±18.19	201.19**± 20.55	165.89#± 25.26	71.63#± 16.92	181.78** ± 27.76
TG(mg/dl)	52.42± 19.42	695.35# ±82.81	400.97#± 49.76	346.77#± 73.34	175.54#± 38.13	448.54 **± 77.11
HDL(mg/dl)	62.97± 9.73	39.83 ±12.81.	28.6 ± 3.63	27.63 ± 4.04	30.26 ± 10.68	27.51 ± 3.79
LDL(mg/dl)	11.04± 4.33	70.08# ±26.37	92.40 ± 15.34	68.91 ± 32.49	6.27**± 21.28	64.57 ± 31.37
VLDL(mg/dl)	10.48± 3.89	139.07# ±16.56	80.19# ± 9.95	69.35#± 14.67	35.11# ± 7.63	89.71 **± 15.42

***n* =6; Values expressed as Mean ± S.D. Triton treated group was compared with control group and drug treated with triton treated group.**
*** *p*<0.05, ** *p*<0.01, # *p*<0.001**

DISCUSSION

Triton Wr-1339 has been widely used to block clearance of triglyceride-rich lipoproteins so as to induce acute hyperlipidemia in several animals. [17] While this model is widely used for a variety of purposes [18] the rat model particularly, has been used for screening natural or synthetic hypolipidemic drugs. [19] Interestingly, the results of the present study show that the leaf extract of *H. rosa sinensis* brings about a significant reduction in cholesterol levels and is also capable of reversing Triton induced hyperlipidemia in mice. Methanolic *H. rosa sinensis* extract at a dose of 250 and 500 mg/kg significantly lowered both plasma triglycerides and cholesterol levels. The large increase in plasma cholesterol and triglycerides due to Triton Wr-1339 administration possibly results from an increase of VLDL secretion by the liver accompanied by a noticeable decline in VLDL and LDL catabolism. [20] The lowering of total cholesterol level by the *H. rosa sinensis* extract in response to a dose of 500 mg/kg body wt. was associated with a decrease of its LDL fraction, which in fact is the target of several hypolipidemic drugs. Apparently, the cholesterol-lowering activity of the leaf extract is likely to be a result of rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids. [21]
It is well known that HDL-Cholesterol levels have a protective role in coronary artery disease. [22] Similarly increased levels of serum LDL-

cholesterol result in an increased risk for the development of atherosclerosis. [23] The decrease in cholesterol levels along with its LDL fraction as is evident from table 1 could be due to an increased cholesterol excretion and decreased cholesterol absorption through the gastro intestinal tract.

One aspect that needs consideration is that, the roots of *Hibiscus* are available in much smaller quantities as compared to leaves and further if roots are used the plants are destroyed completely. Another benefit which would accrue if leaf extract, serve the same purpose as root extracts is that it would provide ample raw material for use by the pharmaceutical industry.

CONCLUSION

The results of the present study thus demonstrate that the leaf extract of *H. rosa sinensis* serves as a potent lipid lowering agent which in turn possibly may contribute to its cardio protective and anti-atherosclerotic role. Further studies on drug metabolism as well as an assessment of the biological activity, of the leaf extract, of *Hibicus rosa sinensis* are in progress so as to elucidate its mode of action.

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