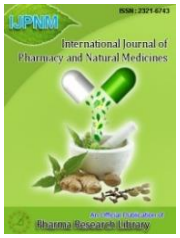

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Evaluation of Hypolipidemic Activity of *Hibiscus* in High-Fat Diet-Induced Hyperlipidemia in Wistar Rats

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A B S T R A C T

The global prevalence of obesity is increasing rapidly with high dietary fat intake as a major risk factor for the development of obesity. The present study was aimed at investigating hypolipidemic activity of *Hibiscus* by in vivo animal model. There is always a need for developing novel drugs with higher efficacy and fewer side effects. Though statins are generally well-tolerated drugs for hyperlipidemia with high efficacy they are not free from adverse effects. Herbal drugs are well known for their cost-effectiveness and minimal side effects. *Hibiscus* is one such plant with known hypolipidemic activity and wide availability in India. Hence this study is an attempt to verify and evaluate the extent of efficacy of *Hibiscus* as a hypolipidemic agent. The objective of the study is to compare the hypolipidemic activity of aqueous extract of *Hibiscus* with that of Rosuvastatin in cholesterol diet-induced hyperlipidemia in rats. Hyperlipidemia was induced in male albino rats of wistar strain in the first 30 days of feeding period and continued in the next 30 days of treatment period. Aqueous extract of *Hibiscus* (2.5 and 5g/kg, per oral) was administered as test drug in the treatment period. Rosuvastatin (10 mg/kg, per oral) was used as the standard drug. Serum lipid profile, atherogenic index and body weights were estimated for all rats on the day before the start of the feeding period and on day 0,15 and 30 of the treatment period. The results were analyzed statistically using student's unpaired and paired t-test wherever applicable. Serum lipid levels showed significant reduction ($p < 0.001$) in TC,TG, LDL-C and VLDL-C with significant elevation ($p < 0.001$) of HDL-C in both the Rosuvastatin and test groups, but the percentage reduction in lipid levels, percentage elevation of HDL-C and percentage protection from atherosclerosis was higher in Rosuvastatin group than in test groups. *Hibiscus* has a definite hypolipidemic potential. Although its effectiveness is lesser than Rosuvastatin its beneficial role as hypolipidemic agent may be tested in clinical studies.

Keywords: Cholesterol, Hyperlipidemia, Hypolipidemic, Rosuvastatin and Hibiscus.

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C o n t e n t s

1. Introduction

Hyperlipidemia or hypercholesterolemia is a metabolic condition and common form of dyslipidemia. It is characterized by elevated levels of blood lipids in the body. It is a broad category, but particularly refers to increase in cholesterol and triglyceride levels. Hyperlipidemia is defined as "elevation of fasting total cholesterol concentration which may or may not be associated with elevated triglyceride concentration". It is one of the forms

of disorder of lipid metabolism. Lipids are insoluble in plasma and hence transported by binding to protein particles known as lipoproteins. Hyperlipidemia is suggesting abnormalities of lipoprotein along with disorders of lipid metabolism. The condition is usually asymptomatic and rarely produces immediate serious complications. However, prolonged untreated hyperlipidemia accelerates atherosclerosis that leads to occlusion of blood vessels and obstruction to normal blood flow resulting in deadly CVDs. Both increase synthesis and decrease clearance of various lipids contributes to hyperlipidemia[1].

Plant Profile:

Botonical Name : ***Hibiscus***
Kingdom : Plantae
Order : Malvales
Family : Malvaceae
Genus : Hibiscus
Species : H. cannabinus

Fig.1: Leaves of Hibiscus

Hibiscus is a genus of flowering plants in the mallow family, Malvaceae. The genus is quite large, comprising several hundred species that are native to warm temperate, subtropical and tropical regions throughout the world. Member species are renowned for their large, showy flowers and those species are commonly known simply as "hibiscus", or less widely known as rose mallow. Other names include hardy hibiscus, rose of sharon, and tropical hibiscus. Several species are widely cultivated as or namental plants, notably Hibiscus syriacus and Hibiscus rosa-sinensis. A tea made from hibiscus flowers is known by many names around the world and is served both hot and cold. The beverage is known for its red colour, tart flavour, and vitamin C content.

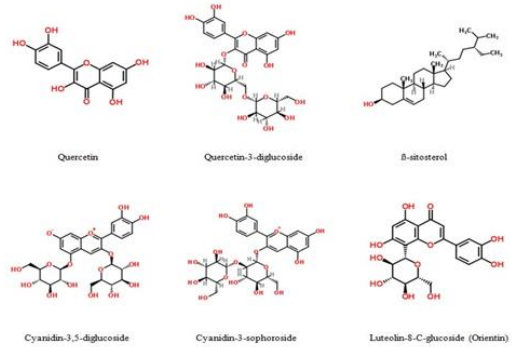

Fig.2: Structures of different bioactive compounds from H. rosa-sinensis: Quercetin, quercetin-3-diglucoside, ß-sitosterol,cyanidin-3,5-diglucoside, cyanidin-3-sophoroside, and luteolin-8-C-glucoside.

Reported pharmacological activities are:

- Comparative Study of Phytochemical Antibacterial Activity, Antifungal and Antioxidant Activity Hibiscus Cannabinus Using Various Solvents.
- Cytotoxic activity of lignans from Hibiscus cannabinus
- Haematinic activity of Hibiscus Cannabinus
- Comparative Study Of Anti-Inflammatory Activity Of Aqueous And Methanolic Extracts Of Hibiscus Cannabinus Leaf (Malvaceae)
- Antioxidative activity of Hibiscus cannabinus

2. Materials and Methods

The current study was conducted in the Central Animal House, department of pharmacology, Sura labs. The study was undertaken to evaluate hypolipidemic activity of aqueous extract of *Hibiscus* in cholesterol diet-induced hyperlipidemia in rats.

Preparation of the extract

The fresh leaves of *Hibiscus* were collected from Local market Hyderabad, Telangana, India. Identification and extraction of the plant was done at the Suralabs, India. The leaves of *Hibiscus* were isolated, chopped into small pieces and dried under shade at room temperature for seven days. The dried leaves were powdered and this powder was used for the preparation of aqueous extract by heat distillation process.

Animals

Animals were procured from central animal house of department of pharmacology. Healthy male albino rats of wistar strain (18-weeks-old), weighing 180-200 gm were used in the present study. They were inbred and grown under suitable laboratory conditions. They were housed two per cage in a room maintained at 12 h light-dark cycles and a constant temperature of 22±20 °C. The animals were provided with pellet chow and water ad libitum, except during experimentation. Animal experiments were conducted upon approval from the institutional animal ethics committee, AJIMS according to the CPCSEA guidelines of animal care.

Grouping

Thirty healthy male Albino rats with similar body weight were selected. They were randomized into treatment and control groups. All rats were allowed a one-week acclimatization period to become accustomed to the laboratory conditions. Rats were randomly divided into five groups, each comprising six rats.

Duration of the study

The duration of the study was for 2 months which included 30 days of feeding period and next 30 days of treatment period with continued feeding.

Feeding period: Group I served as normal control and was fed with standard rat chow throughout the study. Group II, III, IV and V were fed with high-fat diet for 30 days during the feeding period and then continued the same for the next 30 days of treatment period. Rats were supplied food and water ad libitum.

Treatment period

Group I served as normal control (N) and received normal saline (5ml/kg, per oral) daily for 30 days.

Group II served as hyperlipidemic control (H) and received normal saline (5ml/kg, per oral) daily for 30 days.
Group III served as standard drug control (R) and received Rosuvastatin (10 mg/kg, per oral) daily for 30 days.
Group IV served as test group (TA) and received *Hibiscus* (2.5 g/kg, per oral) daily for 30 days.
Group V served as test group (TB) and received *Hibiscus* (5.0 g/kg, per oral) daily for 30 days.

Induction of hyperlipidemia

High cholesterol diet (HCD) comprised the following ingredients: cholesterol 5 g (Himedia Pvt Ltd, Mumbai), deoxycholic acid 5 g (Sigma-Aldrich Pvt Ltd, Mumbai), coconut oil 300 ml (300 g), and standard rat chow 700 g. Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow diet. Simultaneously cholesterol (5g) was dissolved in 300 ml of warm coconut oil. This oil solution of cholesterol was added slowly into the powdered mixture and thoroughly mixed to obtain soft homogenous cakes. These cakes were daily supplied to rats in each cage in sufficient quantities.

Body weight

Body weights of all rats were checked on the day before the start of feeding period and on day 0, 15 and 30 of the treatment period.9 Total weight gain was calculated as:
Total weight gain on day 30=Final body weight–Initial body weight

Drug treatment

Daily single dosage of *Hibiscus* (dissolved in normal saline, 5 ml/kg) was given orally for 30 days in the treatment period to the test groups through oral gavage procedure. The control groups received normal saline alone. The standard group received Rosuvastatin 10 mg/kg/day orally dissolved in 5 ml/kg normal saline. Doses of *Hibiscus* and Rosuvastatin (Reddy labs Pvt Ltd, Hyderabad) were selected based on the reports in previous study which had hypolipidemic activity.7, 10, 11 All the doses were administered between 10-11 am.

Blood sampling

All blood samples were collected within a one-hour period between 8:00 am and 9:00 am. Twelve hours fasted blood samples were collected under light ether anaesthesia by retro orbital puncture. Blood samples were collected on the day before the start of feeding period and on day 0, 15 and 30 of the treatment period. These blood samples were used for serum lipid analysis.

Serum lipid analysis

Serum lipid profile were analyzed for all rats on the day before the start of feeding period and on day 0, 15 and 30 of the treatment period. Blood samples were allowed to clot for 30 minutes and serum was separated by centrifugation at 3,000 revolutions per minute (rpm) for 5 minutes in Remi centrifuge (INCO, Chennai) and transferred to sterile 1.5mL centrifuge tubes. Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL-C), low density lipoproteins (LDL-C), very low density lipoproteins (VLDL-C) were determined by endpoint colorimetric analysis using commercial kits and autoanalyser (Lablife Robochem, RFCL Ltd.) according to the manufacturer directions. Diagnostic reagent kit (RFCL Ltd, Dehradun) was used for estimation of triglycerides, total cholesterol and HDLC which used enzymatic glycerol-3-phosphate oxidase (GPO-ESPAS) and Cholesterol oxidase/peroxidase (CHOD-PAP) method. VLDL-C was calculated as onefifth the level of TG using empirical equation of Friedwald.13 The Friedewald method was used to calculate the LDL-C levels, which subtracts HDL-C and very low-density lipoprotein cholesterol (VLDL-C) from TC.

VLDL=TG/5 LDL=TC−(HDL+VLDL)

Percentage change from initial values (day 0 of treatment period) of serum lipid levels and body weights were calculated on day 15 and day 30 of treatment period using formula:

Percentage change=[(difference in lipid levels /day0 lipid levels) X 100]

Atherogenic index (AI) was calculated as AI=(total serum cholesterol/total serum HDL).

Percentage protection from atherosclerosis was calculated as Protection (%) =[(difference in AI between control and treated group/AI of control) X 100].

Statistical analysis

Results were expressed as mean+standard deviation (SD) of six values (n=6) for each group. Statistical differences between the controls and the treatment groups were evaluated by using student's unpaired and paired t-test wherever applicable using SPSS software package. Values were considered significant at $p < 0.001$.

3. Results and Discussion

Following were the effects seen in rats following administration of aqueous extract of *Hibiscus* (2.5/5 g/kg, p.o., once daily) and Rosuvastatin (10 mg/kg, per oral, once daily).

Effect on serum lipid levels

Comparison with normal group

On day 0 of treatment period the hyperlipidemic control group, Rosuvastatin and test groups showed significant increase ($p < 0.001$) in total cholesterol, triglyceride, LDLC, VLDL-C levels and significant decrease in HDL-C ($p < 0.05$) levels (Table1, Figure1).

Comparison with hyperlipidemic group

On day 30 of treatment period Rosuvastatin group, Test group A and Test group B showed a significant decrease ($p<0.001$) in total cholesterol, triglyceride, LDL-C, VLDL-C levels and a significant increase ($p<0.001$) in HDL-C levels. (Table 1, Figure 2).

Comparison with day 0 of treatment period (intragroup): A highly significant decrease in total cholesterol, triglyceride, LDL, VLDL levels and a significant increase in HDL levels was observed in Rosuvastatin group and both test groups on day15 ($p<0.001$) and day 30 ($p < 0.001$) in the same group (Table 1, Figure 1, Figure 2).

Percentage change in lipid levels

On day 30 when test groups were compared with rosuvastatin group they showed lesser percentage reduction in the serum total cholesterol, triglyceride, LDL-C and VLDL-C but test group B showed greater percentage elevation in the serum HDL-C than Rosuvastatin group (Table 2).

Effect on atherogenic index (AI)

On day 30 the hyperlipidemic group showed a significant increase ($p < 0.001$). The Rosuvastatin and the test groups both reduced AI significantly ($p < 0.001$). But the percentage protection against atherogenesis was greatest in the Rosuvastatin group than in the test groups (Table 3).

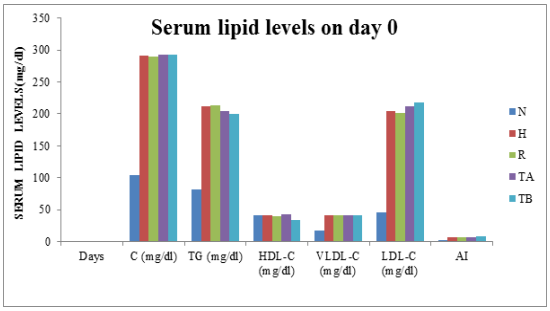

Fig.12: Serum lipid levels on day 0

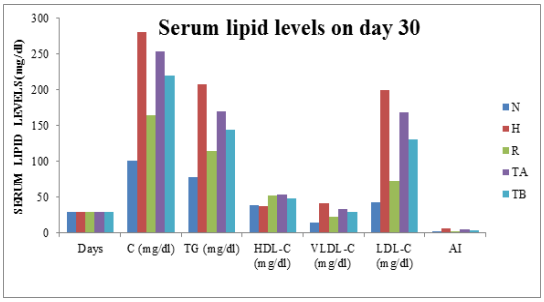

Figure 2: Serum lipid levels on day 30

Discussion

It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. This study has utilised deoxycholic acid, cholesterol, coconut oil and chow in the hyperlipidemic diet. A high-fat/high-cholesterol diet supplemented with cholic acid changes the lipoprotein profile to a more atherogenic one by increasing levels of LDL-C and lowering HDL-C. Many studies have shown that chow based diets supplemented with varying amounts of cholesterol and sodium cholate can induce hypercholesterolemia in mice and rats presumably by interfering with the hepatobiliary excretion of cholesterol. The mechanism of action of cholic acid is two-fold. It causes an increase in cholesterol absorption and a concomitant suppression of cholesterol 7α hydroxlyase activity that results in decreased cholesterol excretion. Cholic acid improves cholesterol absorption by its emulsifying property.

It is evident from the results that although both Rosuvastatin and *Hibiscus* showed significant lipid lowering and HDL-C increasing activity, the percentage change in lipid parameter except for HDL-C was lesser with *Hibiscus* than with Rosuvastatin. The explanation to this effect possibly lies in the difference in the mechanisms of hypolipidemic action of *Hibiscus* and the Rosuvastatin. Although multiple mechanisms have been suggested for *Hibiscus*, none of those is suggested to affect the de novo synthesis of cholesterol like that by statins. Statins act by blocking the HMG-CoA reductase enzyme, which catalyzes the rate-limiting step in de novo cholesterol synthesis. Rosuvastatin is the most effective statin to lower LDL-C, with reductions of up to 63% reported with a daily dose of 40 miligram.

The exact mechanism by which *Hibiscus* reduced the serum cholesterol is not clear. But the observation of increased HDL-C levels could be one of the possible mechanisms for decrease in serum lipid levels by *Hibiscus*. The increased HDL-C facilitates the transport of TG or cholesterol by a pathway termed ‘reverse cholesterol transport’ from serum to liver where it is catabolised and excreted out of the body. Increase of HDL-C is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of LCAT.

Studies have shown the presence of flavonoids, saponins, tannins, triterpenoids, steroids and polyphenolics in root extracts of *Hibiscus*. Flavonoids are reported to enhance the activity of lecithin cholesterol acyl transferase (LCAT) which plays a key role in the incorporation of free cholesterol into HDL causing increase in HDL-C. Saponins are highly active plant compounds reported to increase faecal cholesterol excretion and also increase the lipoportein lipase activity (LPL) which helps in faster removal of free fatty acid from circulation consequently decreasing total cholesterol. Tannins are reported to increase the activity of the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides. Plant sterols are also reported to decrease cholesterol absorption and increase its excretion.

The *Hibiscus* also has anti-oxidant property. These phyto constituents would have contributed to the lipid lowering effect of *Hibiscus* in this study. But the presence of these phyto constituents in the *Hibiscus* needs to be investigated by further studies (such as phytochemical screening, fecal bile acid excretion).

An ideal drug is one which raises HDL-C along with the lowering of LDL-C. Biochemical estimations showed that both the *Hibiscus* as well as Rosuvastatin increased the protective HDL-C level and decreased the atherogenic LDL-C and VLDL-C levels. Although *Hibiscus* has shown greater elevation of HDL-C when compared to Rosuvastatin,the percentage protection from atherosclerosis remains the similar. This is an important advantage in treatment of hypercholesterolemia particularly among Indians where low HDL-C is the most prevalent lipoprotein abnormality. High levels of total cholesterol and most importantly, LDL-C are the predictors of atherosclerosis. *Hibiscus* significantly reduced both the total cholesterol and LDL C. Recent studies show that triglycerides are directly or indirectly related to coronary heart diseases. In the present study, *Hibiscus* has markedly decreased the triglycerides level. These effects (although to a greater extent with Rosuvastatin) along with significant decline in atherogenic index without causing mortality or adverse effects in rats, points towards the efficacious role of

Hibiscus as hypolipidemic agent. The pleotropic actions and the distinct mechanism of hypolipidemic action definitely assign the statins as the clinically most efficacious hypolipidemic agents. But the significant decrease in lipid profile, elevation in the HDL, good safety margin in experimental models combined with traditional application as cardio protective agent definitely promote *Hibiscus* as an agent to be clinically tested for the treatment of hyperlipidemia and its associated cardiovascular disorders.

Table 1: Serum lipid levels and atherogenic index (AI) in various groups on Day 0, 15 and 30

Groups	Days	C (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	AI
N	0	103.45±25.10	82.14±2.25	41.45±1.52	17.52±2.16	46.24±12.10	2.42±1.63
	15	103.26±10.24	80.46±1.39	40.36±1.51	16.14±2.36	45.14±10.14	2.12±0.14
	30	101.14±1.25	78.14±2.68	39.15±2.41	14.25±1.78	43.59±06.24	2.24±0.39
H	0	291.81±12.14	211.51±11.90	41.02±1.01	41.60±2.13	204.42±10.10	6.71±0.41
	15	291.04±18.95	211.97±11.35	36.77±4.38	42.39±2.27	206.69±21.02	6.89±0.99
	30	280.23±15.15	207.81±9.52	38.01±4.43	41.56±1.90	199.16±18.36	6.83±0.72
R	0	290.08±03.15	213.51±11.12	40.01±1.97	41.21±2.06	201.88±12.88	6.99±0.83
	15	190.13±12.26	155.22±12.22	51.69±3.33	30.60±3.20	95.62±15.00	3.03±0.39
	30	165.03±16.95	115.01±16.68	52.85±5.10	22.45±2.22	72.70±15.13	2.26±0.25
T_A	0	292.14±16.41	204.15±12.52	43.14±7.17	40.83±2.50	211.70±19.65	7.02±1.24
	15	270.29±13.28	186.82±13.87	48.49±4.82	37.36±2.77	186.44±14.66	5.65±0.53
	30	254.35±12.13	169.70±15.34	53.30±2.60	33.94±3.07	169.26±14.40	4.82±0.30
T_B	0	292.95±14.06	200.41±10.21	34.12±4.31	40.48±2.66	218.19±14.19	7.69±0.96
	15	241.13±19.20	165.05±19.10	50.19±2.47	34.01±3.23	158.59±19.02	4.68±0.49
	30	220.15±19.58	144.46±21.21	48.07±3.08	29.30±2.26	131.31±19.54	3.60±0.44

Table 2: Mean percentage change (%) from day 0 values of serum lipid levels in different groups on day 15 and 30.

Groups	Day	Mean percentage change (%)				
		C	TG	HDL-C	VLDL-C	LDL-C
N	15	0.35	5.10	1.89	5.12	5.26
	30	4.12	6.98	0.10	9.61	2.02
H	15	0.63	7.05	9.61	0.52	0.43
	30	4.41	3.12	6.95	3.13	4.26
R	15	34.03	28.12	23.16	31.15	50.36
	30	43.21	47.16	35.12	47.26	68.12
T_A	15	8.12	9.41	14.14	9.05	10.41
	30	14.24	17.20	27.62	17.14	20.14
T_B	15	18.16	17.10	36.12	17.14	29.12
	30	27.12	30.45	52.12	25.14	36.19

Table 3: Atherogenic index (AI) in different groups on day 30

Groups	Atherogenic index	Protection (%)
N	4.36	-
H	9.20	-
R	5.12	61.12
T_A	6.14	37.50
T_B	5.14	53.19

Table 4: Weight gain in different groups on day 30 as compared to day 0

Groups	Weight gain (gm)
N	7.12±1.62
H	18.10±2.20*
R	9.1±3.40$^{\#}$
T_A	12.1±1.26$^{@}$
T_B	10.02±1.51$^{\$}$

4. Conclusion

From the observations made in the present study with reference to serum lipid profile and body weight gain percentage it can be concluded that, the combined suspension of *Hibiscus* extract with Rosuvastatin could have elicited a synergistic effect in lowering body weight and inhibition of cholesterol synthesis. The result shows that the *Hibiscus* has a definite hypolipidemic and hence cardioprotective and antiatherosclerotic potential. There is

also a valid scientific basis for consuming it for clinical benefits in the treatment of cardiovascular diseases in India. Hence, the present study helps to support the traditionally claimed cardioprotective activity of *Hibiscus*. However, further studies are necessary to support these findings. Also an extensive case-control study is required to document its therapeutic application in human beings.

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