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HYPOLIPIDEMIC PROPERTIES OF HIBISCUS ROSA SINENSIS FLOWER EXTRACTS IN ALBINO WISTAR RATS

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ABSTRACT

Background : The use of different parts of medicinal plant to cure specific ailments has been a traditional practice from ancient times. Herbal medicine was a traditional practice, is presently emerging as an alternative medicine since it makes health care affordable for all with scientifically proven properties of cure. Flowers of *Hibiscus rosa sinensis (HRS)* has been reported in the Indian medicinal literature with beneficial effects in several physiological disorders and were used for their medicinal properties. Hyperlipidemia is a World-wide adverse health problem and most of the antilipidemic drugs have side effects and hence herbal medications were scientifically tried as antilipidemic agents.

Objectives : To evaluate the possible lipid lowering properties of locally grown Hibiscus rosa sinensis flower extracts.

Methods : Male Wistar rats(180-230gm) were divided into seven groups of six each (n=6). The first group was the control. The second, third and fourth groups were given HRS flower extracts orally with the dose level of 80mg/kg, 160mg/kg and 240 mg/kg body weight once a day for 5 days (acute) and fifth, sixth and seventh groups were given same doses of HRS flower extracts for 30 days (chronic). At the end of treatment duration all animals were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and allowed to clot. Serum was separated for the estimation of Total cholesterol (TC), Triglyceride (TG), HDL, LDL, VLDL levels.

Results : Results of different groups were computed and analyzed for assessing the hypolipidemic effects of HRS extracts. There was a decrease in serum cholesterol and triglyceride levels and increase in high density lipoproteins level, in HRS treated groups.

Conclusion : The extract of Hibiscus rosa sinensis possess hypocholesterolemic/hypolipidemic effects.

Key Words:- Medicinal Plant, alternative medicine, hyperlipidemia, antilipidemic drugs.

INTRODUCTION

In recent years there has been an increased interest in areas related to newer approaches in the prevention of disease especially those involving natural compounds. *Hibiscus rosa sinensis* L. (Malvaceae) is an ornamental plant is a native of China and also distributed in India, Nepal, Bangladesh, Sri Lanka and Philippines. As a traditional medicine, the fresh juice of the flower is used to treat gonorrhea, the powdered root is used in the treatment of menorrhagia and the infusion of the petals is used as a refrigerant drink during fever[1]. Previous studies showed that the plant possesses anti-complementary, anti-diarrhetic, anti-phologistic activity[2]. A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn. was found to exhibit significant hypoglycemic activity in glucose induced hyperglycemic rats[3]. Similar results were obtained with *Hibiscus rosa sinensis(HRS)* leaf extract after repeated dosing[4]. It has been also shown to be beneficial in fever and bronchial catarrh[5]. Roots of HRS possess androgenic, antitumor, antiestrogenic[6], antiimplantation[7], wound healing[8], anticonvulsant and antispermatic properties.[9] Hyperlipidemia is a most common global health problem among the population. In allopathic approach of treatment statin group of drugs were the choice of antilipidemic agents, which were known to have long term side effects.[10] Scientific approaches towards alternative medicines involving herbal products were known to have positive results. There are little bit of information available in the literature towards the antilipidemic effect in different species of HRS flower extracts, on its antilipidemic property. Locally grown HRS flower may have greater antilipidemic effect which are easily available in and around of Sullia taluk of Karnataka

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State. Local people reveal that, they use petals of HRS as an hair vitaliser, common cold, fever, after heavy fat meal. Keeping these verbal information and literature we hypothesized that locally grown extracts of HRS flowers may exhibit antilipidemic properties. Hence the objective of the present study was to evaluate the lipid lowering properties of the extract of HRS flowers. The cholesterol lowering effect of HRS flower extracts at preclinical level need to be studied since there are less documented results.

MATERIALS AND METHODS

Plant material **-**Fresh and disease resistant flowers of *Hibiscus rosa sinensis* were collected from Kurunjibag locality, Sullia, D.K, Karnataka. A voucher specimen of the flower was identified and authenticated by the taxonomist at the Department of Botany, Nehru Memorial College, Sullia, Karnataka. A voucher herbarium specimen was also preserved in the same college and our laboratory for future reference. The flower petals were air dried completely under shade and powdered and stored in airtight container, until subjected to solvent extraction.

Preparation of extract:- The grounded powder was extracted in soxhlet apparatus using distilled water (1:10 ratio)[11]. The extract was filtered and concentrated in a rotatory evaporator, at 30–40 ?C under reduced pressure to obtain a thick dark brown extract. The extract yield was 14% w/w.

Phytochemical screening:- Preliminary screening of the flower extract for various phytochemical classes was carried out based on the reported methods.[12] The crude extract was screened for the presence of saponins, flavonoids, tannins, phenols, sterols, alkaloids, and anthocyanins.

Chemicals:- All reagents and chemicals used in the present study were of analytical grade (AR). Only double distilled water was employed for preparing the reagents.

Animals:- Wistar strain male albino mature(12-15 weeks old) rats of approximately same age group, having body weight 170–220 grams were used from animal house of K.V.G. Medical College, Sullia, D.K. All animals were housed in polypropylene cages in groups of 6 to provide sufficient space and allowed to acclimatize for 2 weeks before study at laboratory conditions. Rats were maintained as per the standard condition with pellet diet (Gold Mohur-Lipton India Ltd, Mumbai) and water ad libitum. All experimental procedures and animal maintenance were done under the guidelines of Institutional Ethics Committee for the use of animals in the experiment.

Experimental Groups Design and Treatment with HRS extract:-

After the acclimatization period 42 Male Wistar rats(180-230gm) were randomly divided into two groups. Experimental (36 rats) and control(6) groups. Experimental groups further divided into three acute groups and three chronic groups (n=6). The second group was the control(n=6). The experimental acute and chronic groups animals were given HRS flower extracts orally with the help of oral gavage. Each group received HRS flower extract at dose level 80mg, 160mg and 240mg /kg body weight daily once for 5 and 30 days as acute and chronic groups respectively. First treatment day with HRS was considered treatment day 1. The exact dosage for each rat was corrected every day for individual body weight by appropriate volume adjustment. Control group of animals received normal diet and water only. After 24 hrs of last HRS treatment, on 6th day (for acute groups) and on 31st day (for chronic groups), the animals were sacrificed by cervical dislocation. Thorax was opened and heart exposed and blood sample was collected by cardiac puncture and allowed to clot in a test tube and serum were collected. The serum was used to determine the total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) levels enzymatically, using commercially available kits E-Coline (Merck). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels were estimated indirectly by using the formula: VLDL=TG/5, LDL=TC-HDL-VLDL.

Statistical Analysis:

All the data was expressed as Mean ±SEM. Analyzed by using one - way ANOVA, followed by Bonferoni post – hoc test. The statistical test were obtained from statistical package of social sciences (SPSS) version 19, 2007. Values of $P<0.05$ were considered as statistically significant.

RESULTS

Phytochemical analysis of the crude extract of Hibiscus rosa sinensis (HRS) showed the presence of alkaloids, saponins, tannins, phenolics and flavonoids. All these test done at Bangalore Test House, Bangalore.

Lipid Profile of acute group animals

Acute group animals fed with HRS flower extracts showed decrease in total cholesterol level and LDL level($p<0.05$) as compare to control groups in 160mg and 240mg/kgBW HRS treated groups. Serum triglyceride level was also decreased significantly ($p<0.001$) with the similar HRS doses groups. The VLDL level was also decreased significantly with respect to 160mg/kg group and highly significant with 240mg/kg BW groups.($p<0.001$)(Fig-1)

Lipid Profile of chronic group animals

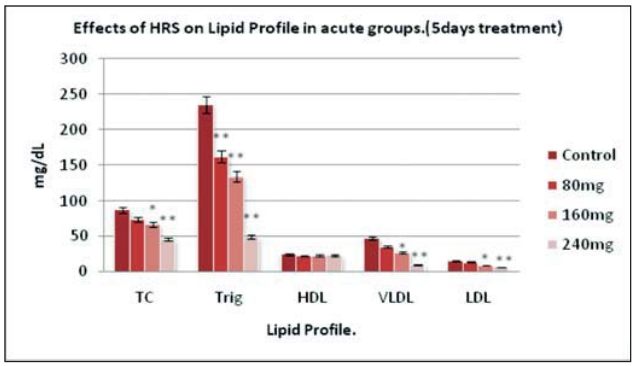
Chronic group animal fed with HRS flower extracts showed significant ($p<0.05$) decrease in total cholesterol level as compared to control groups in 80mg/kgBW treated groups and highly significant ($p<0.001$) with 160mg, 240mg/kgBW treated groups. Serum triglyceride level was decreased significantly ($p<0.001$) with all the dose groups. The VLDL level was also decreased significantly with respect to 160mg/kg group and highly significant with 240mg/kg BW groups. The LDL level was also decreased in both 160mg and 240mg/kg BW treated groups significantly ($p<0.05$). (Table no-1)

Table-1 : Effects on blood Lipid Profiles with Chronic Groups (30 Days Treatment)

	TC	TRIG	HDL	VLDL	LDL
Control	87.36 ±9.51	235.62 ±78.38	24.92 ±2.74	47.44 ±15.37	15.00 ±16.96
80 mg	76.47* ±13.8	186.83** ±42.95	23.65 ±6.57	37.36 ±8.59	14.57 ±3.69
160mg	54.40** ±18.4	57.92** ±15.37	31.07 ±8.87	24.65* ±8.77	8.96* ±2.19
240mg	38.80** ±9.32	44.35** ±21.42	31.17 ±7.65	8.86** ±4.29	4.63** ±1.20

* The mean difference is significant at the ($p< 0.05$)level,
** The mean difference is highly significant at the ($p< 0.001$) level.

Fig:- 1.



* The mean difference is significant at the ($p< 0.05$)level,
** The mean difference is highly significant at the ($p< 0.001$) level.

DISCUSSION

Hypercholesterolemia is extremely common in the general population, is regarded as a high risk factor for several health problem in this 21st century. This may be due to an increased trend towards the Western life style and high cholesterol rich diet intake. Drug based cholesterol control is a common norm in allopathic treatment, though there are number of side effects. In this study the lipid lowering activity of Hibiscus rosa sinensis flower extract may be attributed to the phytoconstituents present in it, such as saponins and phenolics ingredients.[13,14,15] These components are reported for other plant extracts such as saponins derived from *Medicago sativa* were reported to reduce blood cholesterol by competing with cholesterol at binding sites or interfering with cholesterol biosynthesis in the liver.[16] Several studies have showed that plant saponins also act as antihyperlipidemics by one of the following mechanisms as by binding with cholesterol in intestinal lumen, so that cholesterol is less readily absorbed or bile acids causing reduction in its extra hepatic circulation and increasing metabolism of cholesterol to sterol through their fecal excretion. Increase in bile acid excretions offset by enhanced synthesis from cholesterol in the liver consequently lowers the plasma cholesterol. Saponins are also reported to increase the lipoprotein lipase activity which is considered as helpful in faster removal of free fatty acid from circulation that causes in turn a decrease in total cholesterol.[17,18]

Phenolic active principles present in *Anethum graveolens* were observed to be responsible for lowering TC and LDL-C and elevating HDL-C in hypercholesterolaemic rats.[19]

The observed lipid lowering effects of HRS in this study is of high interest, may be possibly due to its content of plant sterols (such as campesterol, fucosterol, etc).[20] Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids that results in decrease of body lipids.[21] Secondary plant metabolites such as flavonoids, saponins and poly phenolics from polar extracts may be responsible for the anti

hyperlipidemic activity. Flavonoids leads to augmented the activity of lecithin acyl transferase(LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells.[22]

Plant proteins are considered as less hyperlipidemic than animal proteins.[23] The exact mechanism by which proteins act as antihyperlipidemics is not yet clear but the following mechanism may be considered. In plant proteins, especially the ratio between lysine: arginine (L:A) is less than 2, which is important for the control of the progression in hyperlipidemia and arteriosclerosis. L:A ratio of well documented plants viz soy proteins, garlic proteins and coconut proteins are 0.84, 0.7, 0.86 respectively.[24]

The findings of this study reveal that the crude extract of HRS flowers exhibited lipid lowering effect in rats. The chronic treatment appears to be has more pronounced effect on lipid lowering activity as compared to the acute treatment. It can effectively control the blood lipid levels by interfering with the biosynthesis of cholesterol and utilization of lipids and antioxidant activities. Thus this study provides a pharmacologic rationale to the medicinal use of Hibiscus rosa sinensis in dyslipidemia and may be a good candidate to be developed as antidyslipidemic medicine, with therapeutic potential in obesity and metabolic syndrome.

This study need to be explored further by finding the fractionation of extracts isolation, purification and characterization of active constituents responsible for the antilipidemic activity and their mechanisms. Further the extract need to be explored on mammalian model before authenticating with clinical trials to confirm its antilipidemic potential.

REFERENCES

1. Duke JA, CRC Handbook of medicinal herbs, Roca Raton.1985;228-229.
2. Shimizu N, Tomoda M, Suzuki T, Takada K, Plant mucilages. XLIII. A representative mucilage with biological activity from the leaves of *Hibiscus rosa sinensis*. Biological and Pharmceutical Bulletin.1993; 16, 735–739.
3. Ragunathan V, Sulochana N, A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn. and its hypoglycemic activity. Journal of Indian Chemical Society.1994;71, 705–706.
4. Sachdewa A, Nigam R, Khemani L.D, Hypoglycemic effect of *Hibiscus rosa sinensis* L. leaf extract in glucose and streptozotocin induced hyperglycemic rats. Indian Journal of Experimental Biology. 2001; 39, 284–286.
5. A. Sachdewa and L. D. Khemani, Effect of *Hibiscus rosa sinensis* Linn. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *Journal of Ethno pharmacology*. 2003; 89, 61–66.
6. S. D. Kholkute and K. N. Udupa, Antiestrogenic activity of *Hibiscus rosa sinensis* Linn. flowers, *Indian Journal of Experimental Biology*.1976; 14, 175–176.
7. N. Vasudeva and S. K. Sharma, Post-coital antifertility activity of *Hibiscus rosa-sinensis* Linn. roots, *Evidence-Based Complementary and Alternative Medicine*. 2008; 5, 91– 94.
8. B. Shivananda Nayak, S. Sivachandra Raju, F. A. Orette, and A. V. Chalapathi Rao, Effects of *Hibiscus rosa sinensis L (Malvaceae)* on wound healing activity: a preclinical study in a Sprague Dawley rat. *International Journal of Lower Extremity Wounds*. 2007; 6, 76–81.
9. V. S. Kasture, C. T. Chopde, and V. K. Deshmukh, Anticonvulsive activity of *Albizzia lebbeck, Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. *Journal of Ethnopharmacology*.2000; 71, 65–75.
10. Annie L. Culver, BPharm; Ira S. Ockene et al. Statin Use and Risk of Diabetes Mellitus in Postmenopausal Women in the Women's Health Initiative, Arch Intern Med. 2012;172:144-152.
11. Soxhlet, F. Die gewichtsanalytische Bestimmung des Milchfettes, Polytechnisches J. Dingler's. 1879; 232, 461.
12. Evans W C: Phytochemistry. Trease and Evans Pharmacognosy. 5th edition Delhi: Elsevier;2006,135-150.
13. Pengelly Andrew, Triterpenoids and saponins, The constituents of medicinal plants (CABI Publishing, USA), 2004,74.
14. Regisusan Daniel, Mathew Biju C, Devi K S and Augusti K T, Antioxidant effect of two flavonoids from the bark of Ficus bengalensis Linn in hyperlipidaemic rats, Indian J Exp Biol, 1998;36, 902.
15. Marudamuthu Amarnath Satheesh and Leelavinothan pari, Effect of pterostilbene on lipid and lipid profiles in streptozotocin –nicotinamide induced type 2 diabetes mellitus, J Appl Biomed.2008; 6, 31-33.
16. Lanksy P S, Plants that lower cholesterol, Acta Horticulture. 1993;332, 131.
17. Sidhu G S and Oakenful D G, A mechanism for hypocholesterolaemic activity of saponins, Br J Nutrition.1986; 55, 643.
18. Sidhu G S and Oakenful D G, Could saponins be a useful treatment for hypercholesterolaemia? Eur J Clin Nutr. 1990; 44, 79.
19. Yazdanparast R and Bahramika S, Evaluation of the effect of Anethum graveolens L crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats, DARU.2006; 6, 88.
20. Cleghorm CL, Skeaff C M, Mann J and Chisholm A, Plant-sterol enriched spread enhances the cholesterol lowering potential of fat-reduced diet, Eur J Clin Nutrition. 2003;57,70.
21. Guimaraes P R, Galavao A M P, Batista, C M, Azovedo G S, Oliveira R D, Lamounier R P, Frieire N, Barros AMD, Sakurai E, Olivera JP, Vieira E C and Aalvarez J I, Eggplant (Solanum melongena) infusion has modest and transitory effect on hypercholesteremic subjects, Braz J Med Biol Res. 2000; 33, 1027.
22. Ghule B V, M H Ghante, A N Saoji and P G Yeole, Hypolipidemic and antihyperlipidemic effects of Lagenaria siceraria (Mol.) fruit extracts, Indian J Exp Biol. 2006;44, 905-909.
23. Biju C, Daniel R S and Augusti K T, Hypolipidemic effects of garlic protein substituted for casein in diet of rats compared to those of garlic oil, Indian J Exp Biol.1996; 34, 337.
24. Kritchevsky D, Tepper S A, Czamake S K, Klureld D M and Story J A, Experimental atherosclerosis in rabbits for cholesterol free diets. Parts -9-Beef protein and textured vegetable protein, Atherosclerosis.1981; 39, 169.