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Research Article

ANTI-HYPERLIPIDEMIC ACTIVITY OF LEAVES OF *Sphaeranthus indicus* ON ATHEROGENIC DIET INDUCED HYPERLIPIDEMIC IN RATS

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ABSTRACT

Hyperlipidemia is a secondary metabolic imbalance associated with diabetes. Besides this, elevated serum level of triglycerides, cholesterol and LDL are major risk factors for the premature development of cardiovascular disease like arthrosclerosis, hypertension, and coronary heart disease. The leaves of *Sphaeranthus indicus* Lam., belongs to Asteraceae family, are used by the Indians in their herbal medicine as a hypolipidemic agent in obese patients. The aim of the present study is to evaluate the metholic extract of the leaves of *S. indiucus* for anti hyperlipdemic activity. Albino Wistar rats were fed with methanolic extract of *S. indicus* (100, 200mg/kg, *p.o.*) and Gemfibrozil (10 mg/kg, *p.o.*) along with hyperlipidemic diet for 30 days. *S. indicus*, Gemfibrozil were found to lower the serum cholesterol, triacylglyceride, VLDL, LDL ($p<0.001$), and atherogenic index,($p<0.05$) but were found to increase the HDL($p>0.001$) as compared to the corresponding high fed cholesterol diet group (control). The *S.indicus* methanolic *extract* was also investigated on liver total cholesterol levels in hyperlipidemic rats. This study demonstrates that *S. indicus* possesses a hypolipidemic effect.

Key words: Hyperlipidemia, *Sphaeranthus indicus*, Lipid profile, Total cholesterol.

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INTRODUCTION

Hyperlipidemia is a collective term used to describe human conditions when a plasma level of one or more classes of lipids, namely cholesterol, triacylglycerides, phospholipids and fatty acids increases above normal levels (Ghule *et al.*, 2009). Hyperlipidemia is one of the major causes of the development of cardiovascular disorder (Ansarullah *et al*., 2009). Although lot of efficacious lipid-lowering synthetic drugs available, none is effective for all lipoprotein disorders, and all such agents are associated with more side effects. Therefore, it is a need of the day to search other materials from natural sources that are less toxic, less expensive, which can provide better safety and efficacy on a long term usage. Natural products from plants are a rich source used for centuries to cure various ailments.

Sphaeranthus indicus Linn.is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments (Kirtikar *et al*., 1981). It is mostly used in *Ayurvedic* system of medicine to treat serious conditions of epilepsy, mental illness, jaundice, diabetes, leprosy, fever cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. There are reports providing scientific evidences for hypotensive, anxiolytic neuroleptic, immunomodulatory, antioxidant, anti-inflammatory, bronchodilator, antihyperglycemic and hepatoprotective activities of this plant. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude

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(Chatterjee A & Pakrashi SC, 2003). A much branched, strongly-scented annual with winged stem and the wings toothed. Leaves obovate-oblong, narrowed at the base, dentate and serrate. Flowers compound heads, globose avoid, Flowering time November to January in Indian conditions; glandular hairy. Achene staled.

MATERIALS AND METHODS

Plant material

The fresh leaf of *Sphaeranthus indicus* Linn were collected from Coimbatore district, Tamil Nadu, India, identified and by authenticated by Dr. P Jayraman, Director of plant Anatomy Research Centre Chennai. A voucher herbarium specimen number SCOPS/SI/01 was also preserved in the Sanjo College of pharmaceutical studies, Palakkad. The collected leaves were dried in shade and powdered to coarse consistency in cutter mill. The powder was passed through 60 # mesh particle size and stored in an airtight container at room temperature.

Preparation of Extract

The powdered leaf material was subjected to batch extraction in Soxhlet apparatus. The solvent used was 95% Methanol. The powdered leaf of *Sphaeranthus indicus* was evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an. electric heating mantle with thermostat control. Appearance of brown solvent in the siphon tube was taken as the termination of extraction. The filtrate was concentrated using a rotary evaporator at low temperature (40-45 $^{\circ}$C) and pressure and percentage yield was calculated (Mukherjee PK, 2002).

Test for Phytochemical Analysis

The conventional chemical tests were carried out for the Methanolic extract of *Sphaeranthus indicus* to identify the presence of various chemical constituents (Khandelwal KR, 2008).

Chemicals and Drugs

Gemfibrozil was purchased from Sigma Co. (Sigma St. Louis, MO). Analytical grade of Methanol was purchased from Merck (German). Other reagents were of analytical grade.

EXPERIMENTAL ANIMALS

Adult male albino rats (180-200g) were procured from the laboratory animal house, Sanjo College of pharmaceutical studies, Pallakad, Kerala, India and used in the study. The animals were kept under standard environmental conditions of room temperature (22 ±1ºC), relative humidity (50% ± 5%) and 12 hours light and dark cycle. The animals were housed in the colony cages (either three rats) and provided feed (Excel feed, Ilorin) and water ad libitum. All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee.

Experimental procedure

Acute oral toxicity study

Acute oral toxicity was conducted as per OECD guidelines (organization of economic cooperation and development) 423 (Acute toxic class method). The acute toxic class method is a step wise procedure of three animal of a single sex per step. Depending on the mortality and / or moribund status of animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses defined doses, (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which causes acute toxicity. The method previously described by Lorke[9] was adopted.

Atherogenic Diet (AD) induced hyperlipidemic model

Preparation of Feed

Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 1%, Cholic acid 0.5 %, casein 18%, and were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals. Thirty albino rats were randomly divided into five groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats are then given test plant extracts i.e., MESI (100 and 200 mg/kg, p.o) and Gemfibrozil (10 mg/kg, p.o) once daily in the morning orally for 14 consecutive days. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidemic control i.e., group II animals received the hyperlipidemic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle.

Group 1: Administered vehicle and served as normal control (Yokozawa T & Ishida A, 2003).

Group 2: Fed with atherogenic diet (AD) and served as hyperlipidemic control.

Group 3: Administered MESI (100mg/kg), p.o., and fed with AD.
Group 4: Administered MESI (200mg/kg), p.o., and fed with AD.
Group 5: Administered Gemfibrozil (10mg/kg), p.o.,and fed with AD.

On day 21, animals were anaesthetized with Diethyl ether and blood was collected by retro orbital puncture. The blood was subjected to centrifugation for 15 min at 2500 rpm to obtain serum. The collected serum was analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol (Parab S and Mengi SA, 2003; Sikarwar MS, Patil MB, 2012).

Liver total cholesterol:

The livers of all the rats were collected, dried on tissue paper, stored. The liver of each rat was homogenized, the total cholesterol was extracted with a mixture of chloroform and methanol (2:1v/v) and the amounts of total cholesterol ere determined using commercial kit.

Atherogenic index

Atherogenic index is a measure of the Atherogenic potential of an agent was calculated using the following formula

$$AI = \frac{\text{Total cholesterol-HDL cholesterol}}{\text{HDL cholesterol}}$$

Statistical analysis

The results were analyzed for statistical significance using Student t-test and p value<0.001was considered significant.

Fig 1. Diagrammatic representation Effects of methanolic extracts of *S. indicus* on hyperlipidemic rats.

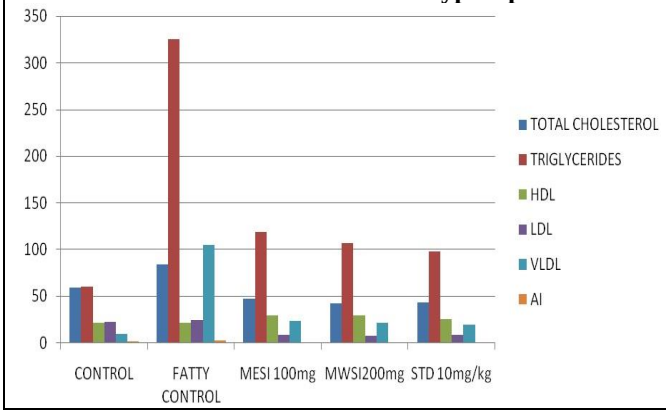

Fig 2. Diagrammatic representation of Effects of *S. indicus* extract on liver total cholesterol levels in Hyperlipidemic rats.

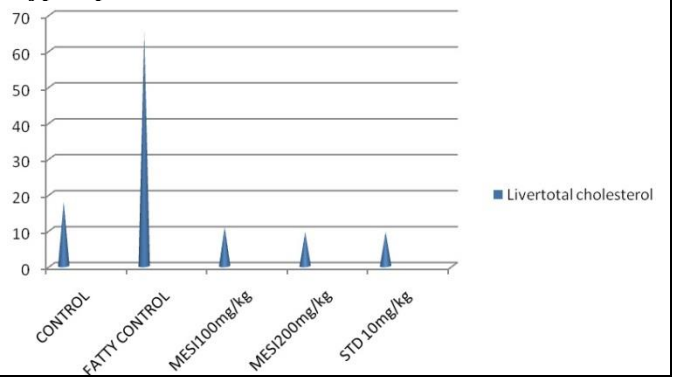

Table 1. Effects of methanolic extracts of *S. indicus* on hyperlipidemic rats

Groups	Treatment	Cholesterol mg/dl	Triglycerides	HDL cholesterol	LDL	VLDL	A.I.
I	Control	59.2 ± 0.6952	50.2 ± 0.6782	22.2 ± 0.6782	22.8 ± 0.730	10.00 ± 0.761	1.66 ± 0.304
II	Fatty Control	84.6 ± 0.574	285.6 ± 1.412	21.8 ± 0.6164	24.75 ± 0.567	105.1 ± 0.8483	2.88 ± 0.426
III	Methanolic extract 100mg/kg	47.9 ± 0.840	118.7 ± 0.6878	29.75 ± 0.4290	8.65 ± 0.4090	23.75 ± 0.6360	0.610 ± 0.16**a**
IV	Methanolic extract 200mg/kg	42.4 ± 0.790	107.4 ± 0.7119	29.90 ± 0.3615	8.42 ± 0.4188	21.65 ± 0.5945	0.418 ± 0.37**a**
V	Standard Gem fibrozil (10mg/kg)	43.4 ± 0.734	98.4 ± 0.836	25.40 ± 0.7118	8.8 ± 0.6271	19.7 ± 0.6683	0.70 ± 0.037**a**

Values are mean ± SEM Statistical significance (n=6) P<0.001 Vs control; P[a]< 0.05 Vs control; student 't' test [10] AI – Atherogenic index.

Table 2. Effects of *S. indicus* extract on liver total cholesterol levels in Hyperlipidemic rats

Group	Dose (mg/kg body wt/day)	Liver total cholesterol (mg/g tissue)
Normal rats	-	17. 9. ± 0.672
Hyperlipidemic rats (fatty control)	-	63.4 ± 0.9865
Methanolic extract	100mg/kg	10.8 ± 0.748
Methanolic extract	200mg/kg	9.4 ± 0.6733
Standard (Gemfibrozil)	10/mg/Kg	9.5 ± 0.673

Values are mean ± SEM Statistical significance P<0.01 Vs control; student 't' test.

RESULTS AND DISCUSSION

The dried and powdered leaf of *Sphaeranthus indicus* was subjected to soxhlet extraction with 95% methanol and yielded to 8%w/w extract. Phytochemical analysis of the leaf showed that the presence of chemical constituents sterols, terpenoids, carbohydrates, flavonoids (Isoflavone), tannins and volatile oil. Presence some chemical constituents like flavonoids, terpenoids have Hypolipidemic properties.

Serum total cholesterol, Low density lipoprotein (LDL), High density lipoprotein (HDL), Very low density lipoprotein (VLDL), Triglycerides and Atherogenic index, and Total cholesterol level in liver were determined. Rats fed with atherogenic diet revealed a statistically significant increase in serum level of total cholesterol, triglycerides, LDL, VLDL and decrease in the level of HDL cholesterol (P<0.001). The methanolic extract of *Sphaeranthus indicus* at 100 mg / kg was found to statistically lower the serum cholesterol, LDL, Triglycerides, VLDL and the HDL cholesterol levels were raised than that of the baseline values. The hypolipidemic activity was found to be pronounced with the methanolic extract of *Sphaeranthus indicus* at a dose of 200 mg/kg. Since total cholesterol and LDL brought down than that of the baseline values and HDL values were raised than that of the baseline. Although both Gemfibrozil and methanolic extract were failed to bring the triglycerides and VLDL levels back to baseline values. However both Gemfibrozil and methanolic extract were found be statistically superior in efficacy with respect to lowering of the serum cholesterol, LDL and raising the levels of HDL cholesterol as compared to control. The effects of extracts, standard drug is reflected in the atherogenic index. Where the lowest Atherogenic index is seen with methanolic extract at a dose of 200mg/kg, thus demonstrating statistically superior efficacy in comparison to the control and fatty control.

It has been well established that nutrition plays a most important role in the etiology of hyperlipidimia and atherosclerosis. Athergenic diet is used for induction of hyperlipidemia in chronic model.

Diet containing saturated fatty acids increases the activity of HMG-CoA reductase the rate determining enzyme in cholesterol biosynthesis this may due to higher availability of acetyl CoA which stimulate the cholesterogenesis rate. Besides this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet which could also explain the elevation of serum LDL-C levels either by changing hepatic LDL receptor activity or the LDL-C production.

LCAT enzyme is involved in the transesterfication of cholesterol, the maturation of HDL-C and flux of cholesterol from cell membranes into HDL. The activity of the enzyme lends to decrease in diet induced hypercholesterolemia.

The possible mechanism of methanolic extract of *Sphaeranthu sindicus* involve increase of HDL-C which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase.

The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed reverse cholesterol transport where it is catabolished and excreted out of the body (Kulkarni SK, 1999).

CONCLUSION

The results of the Antihyperlipidemic study clearly demonstrate the efficacy of methanolic extract of *Sphaeranthus indicus* at a dose of 200mg/kg in lowering serum cholesterol, LDL and atherogenic index and elevating serum HDL level. This property of *S.indicus* suggests it to be an imminently vascular protective drug in the overall management of coronary and cerebral artery diseases.

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CONFLICT OF INTEREST

No Interest

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