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A preclinical antihyperlipidemic evaluation of *Artemisia vulgaris* root in diet induced hyperlipidemic animal model

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Abstract

Hyperlipidemia is a major cause of atherosclerosis, coronary heart disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease. The main objective of this study is to evaluate the hypolipidemic activity of aqueous root extract of *Artemisia vulgaris* in cholesterol diet induced hyperlipidemic rats. Rats were randomly divided into five groups each comprising six rats. The study was conducted for two months which included 30 days of feeding period and next 30 days of treatment period. Group I served as normal control, group II, III, IV & V were fed with high-fat diet for 30 days during the feeding period and then the high-fat diet was replaced by standard diet for the next 30 days of treatment period. *Artemisia vulgaris* extract showed significant serum lipid lowering effects in hyperlipidemic rats which brought down total cholesterol level (C) till 180 ± 9.48, triglycerides (TG) 147.2±1.28, LDL 126.3±9.54, VLDL 28.2±2.26, increased level of HDL 68±5.19 and Atherogenic Index (AI) 2.63±1.82 in comparison of diet-induced hyperlipidemic control, total cholesterol 282.23 ±15.15, triglycerides 243.2 ±9.52, LDL 209.16 ±18.36, VLDL 47.56 ±1.90 , HDL 34.17±2.312 and Atherogenic Index (AI) 8.2 ± 0.72 at 30th day and hypolipidemic activity of *Artemisia vulgaris* was compared with rosuvostatin in diet induced hyperlipidemic rats.
Keywords: Hypolipidemic activity, *Artemisia vulgaris*, extraction & Atherogenic Index

1. Introduction

Hyperlipidemia is a major cause of atherosclerosis, coronary heart disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease. Lipoproteins play an essential role in the absorption of dietary cholesterol, long-chain fatty acids, and fat-soluble vitamins transport of triglycerides, cholesterol, and fat-soluble vitamins from the liver to peripheral tissues and the transport of cholesterol from peripheral tissues to the liver. The plasma lipoproteins are divided into five major classes based on their relative density they are as chylomicrons, very low density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) [1]. The two major clinical sequelae of hyperlipidemias are acute pancreatitis and atherosclerosis. Atherosclerosis is a systemic disease process in which fatty deposits cause inflammation of cells and scar the tissue build up within the walls of arteries, it is the underlying cause of the majority of clinical cardiovascular events. The metabolic disorders that involve elevations in lipoprotein are termed as hyperlipoproteinemias or hyperlipidemias. *Artemisia vulgaris* is a tall herbaceous perennial plant growing 1-2 m (rarely 2.5 m) tall with a woody root. The leaves are 5-20 cm long, dark green, pinnate, with dense white tomentose hairs on the underside. The erect stem often has a red-purplish tinge [2,3]. The aim of this study is to evaluate the hypolipidemic activity o f aqueous root extract of *Artemisia vulgaris* in cholesterol diet-induced hyperlipidemic rats and compare with rosuvastatin in cholesterol diet- induced hyperlipidemic rats.

2. Material and methods

2.1. Drugs and Chemicals

Cholesterol :(Himedia Pvt Ltd, Mumbai); Deoxycholic Acid:**(** Sigma-Aldrich Pvt Ltd, Mumbai); Rosuvastatin: (Reddy labs Pvt Ltd, Hyderabad); Serum Total cholesterol diagnostic kit:(RFCL Diagnova, Dehradun); Serum Triglyceride diagnostic kit :(RFCL Diagnova, Dehradun); Serum HDL cholesterol diagnostic kit: (RFCL Diagnova, Dehradun)
Other chemicals and reagents were of analytical grade.

2.2. Equipments

Oral feeding tube, Oral feeding needle, Microphage tubes (centrifuge tubes, 1.5 ml), Micro Pipettes (10µl, 100 µl,

1000 µl), Tuberculin syringe, Remi Centrifuge, UV spectroscopy (Single Monochromator UV-2600 company shimadzu).

2.3 Preparation of extract

The air-dried stem of *Artemisia vulgaris* were subjected to hydrodistillation for four hour using a clevenger-type apparatus [4,5].

2.4 Experimental Animals

Animals were procured from Sainath agencies, Hyderabad. Healthy albino rats of wistar strain weighing between 180-200g were used in this study. Thirty male albino rats were randomized into treatment, standard and control groups. All rats were allowed one-week acclimatization period to become accustomed to the laboratory conditions. The study protocol was approved by our Institutional Animal Ethical Committee (IAEC/SUCP/08/2012).

Rats were randomly divided into five groups, each comprising six rats. The study was conducted for two months which included 30 days of feeding period and next 30 days of treatment period.

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

Group I served as normal control (N) & received normal saline (5ml/kg, per oral by oral feeding needle with tuberculin syringe) daily for 30 days.

Group II served as hyperlipidemic control (H) & received normal saline (5ml/kg, per oral by oral feeding needle with tuberculin syringe) daily for 30 days.

Group III served as standard drug control (R) hyperlipidemic control (H) & received Rosuvastatin (10 mg/kg, per oral by oral feeding needle with tuberculin syringe) daily for 30 days.

Group IV served as test group - A test drug extract (25 mg/kg per oral by oral feeding needle with tuberculin syringe) daily for 30 days.

Group V served as test group - B (test drug extract (50 mg/kg per oral by oral feeding needle with tuberculin syringe) daily for 30 days.

2.5 Induction of hyperlipidemia

High cholesterol diet (HCD) comprised the following ingredients: cholesterol 5g, deoxycholic acid 5g, coconut oil 300 ml (300 g), and standard rat chow 700g. Deoxycholic acid was mixed thoroughly with of powdered rat chow diet, Simultaneously cholesterol 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 [4-6].

Body weights of all rats were checked on the day before the start of feeding period and on day 1, 15 & 30 o f the treatment period.

Calculation of weight gain: Total weight gain on day 30 = Final body weight – Initial body weight

Daily single dosage of *Artemisia vulgaris* extract (dissolved in normal saline 5 ml/kg) were given orally for 30 days in the treatment period to the test groups through oral by oral feeding needle with tuberculin syringe. 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 *Artemisia vulgaris* extract and rosuvastatin were selected based on the reports in previous study which had hypolipidemic activity. All doses were administered between 10-11am.

All blood samples were collected within one hour period between 8:00am 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 1, 15 & 30 of the treatment period. These blood samples were used for serum lipid analysis.

Serum lipid profile were analyzed for all rats on the day before the start of feeding period and on day 1, 15 & 30 of the treatment period. Blood samples were allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 revolutions per minute for 5 minutes in Remi- centrifuge and transferred to sterile 1.5mL centrifuge tubes. Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL), low density lipoproteins (LDL) ,very low density lipoproteins (VLDL) were determined by endpoint colorimetric analysis using commercial kits and UV Spectroscopy(Single Monochromator UV-2600 company shimadzu) using with biochemical diagnostic kit (RFCL Diagnova, Dehradun) according to the manufacturer directions[17-18].

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 paired t-test wherever applicable using prism-5 software package. Percentage change from initial values (day 1 of treatment period) of serum lipid levels were calculated on day 15 and day 30 of treatment period.

Calculation of Percentage Change in serum parameters

$$\text{Percentage Change (\%) on day 30} = \frac{\text{Day 1 serum levels} - \text{Day 30 serum levels}}{\text{Day 1serum levels}} \times 100$$

$$\text{Percentage Change (\%) on day 15} = \frac{\text{Day 1 serum levels} - \text{Day 15 serum levels}}{\text{Day 1 serum levels}} \times 100$$

Calculation of Atherogenic Index (AI)

$$\text{Atherogenic Index} = \frac{\text{Total serum cholesterol}}{\text{Total serum HDL-C}}$$

Calculation of Percentage Protection

$$\text{Protection (\%)} = \frac{\text{AI of Control} - \text{AI of Treated group}}{\text{AI of Control}} \times 100$$

3. Results and discussion

Artemisia vulgaris extract showed significant serum lipid lowering effects in hyperlipidemic rats which brought down total cholesterol level (C) till 180 ± 9.48, triglycerides (TG) 147.2 ± 1.28, LDL 126.3 ± 9.54, VLDL 28.2 ± 2.26, increased level of HDL 68 ± 5.19 and Atherogenic Index (AI) 2.63 ± 1.82 in comparison of diet-induced hyperlipidemic control, total cholesterol 282.23 ± 15.15, triglycerides 243.2 ± 9.52, LDL 209.16 ± 18.36, VLDL 47.56 ± 1.90 , HDL 34.17 ± 2.312 and Atherogenic Index (AI) 8.2 ± 0.72 at 30th day [Table 1].

Standard antihyperlipidemic agent Rosuvostatin 10 mg/kg body weight also able to reduce the elevated serum lipid level toward the normal. It brought down total cholesterol 183.3±5.74, triglycerides 139.2±1.08, LDL 102.36 ±5.13, VLDL 20.1±2.22, increased level of HDL 68.2±2.53 and Atherogenic Index (AI) 2.68 ±2.26 when compared to diet-induced hyperlipidemic control, total cholesterol 282.23 ±15.15, triglycerides 243.2 ±9.52, LDL 209.16 ±18.36, VLDL 47.56 ±1.90, HDL 34.17±2.312 and Atherogenic Index (AI) 8.2 ± 0.72 at 30th day.

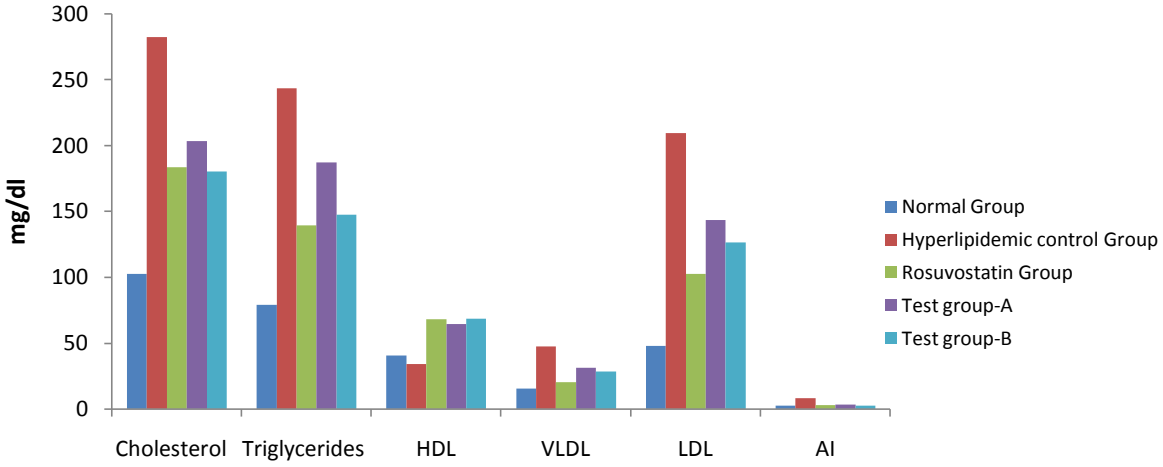
Hyperlipidemia is a well known risk factor for cardiovascular disease, especially atherosclerotic coronary artery disease (CAD) [7-9]. It is one of the major causes of premature death globally and is expected to be the most important cause of mortality in India by the year 2010.

From the results obtained it was observed that keeping the animal on High cholesterol diet (HCD) significantly increased the total cholesterol (TC), TG, LDL-C level in serum as compared to rats on normal diet. With administratio n o f *Artemisia vulgaris* root extracts, the elevated levels of TC, TG and LDL showed a considerable decline as compared to High cholesterol diet rats, thus indicating the efficacy of *Artemisia vulgaris* extract in decreasing levels of various components of lipid profile under experimental condition of diet-induced hyperlipidemia. Rosuvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor. Rats treated with rosuvastatin showed marked reduction in all serum lipoproteins and increase in HDL level as compared to High cholesterol diet (HCD) group. Though both rosuvastatin and *Artemisia vulgaris* showed significant TC, TG, LDL lowering activity and significant HDL increasing activity, the percentage reduction of TC, TG, LDL and percentage increase in HDL was lesser with *Artemisia vulgaris* than with rosuvastatin. Also 50mg/kg of *Artemisia vulgaris* showed greater response than 25mg/kg of *Artemisia vulgaris.*

An ideal drug is one which raises HDL along with the lowering of LDL. In the present study, *Artemisia vulgaris* has markedly decreased the triglycerides level. Studies have shown the presence of flavonoids, saponins, tannins, triterpenoids, steroids, and polyphenolics in root extracts of *Artemisia vulgaris*. Flavonoids are reported to increase HDL concentration and decrease LDL and VLDL levels in hypercholesteremic rats. Several studies show that plant saponins and steroids are known to possess both hypolipidemic and antihyperlipidemic activities [10-12].

The aqueous extract of *Artemisia vulgaris* root has anti-oxidant property[5,14]. This property could be another factor that contributes to hypolipidemic & anti-atherosclerotic effect. Further investigation is required to elucidate this mechanism. Atherogenic index (AI) is a marker of atherogenecity, showed significant decline with both *Artemisia vulgaris* and rosuvastatin. During the experimentation rats did not show any mortality or any other adverse effects. This indicates that the *Artemisia vulgaris* has a good margin of safety. The result shows that the *Artemisia vulgaris* has a definite hypolipidemic activity hence it may have cardioprotective and antiatherosclerotic activity[15-16]. There is also a valid scientific basis for consuming it in the treatment of coronary artery diseases in India. Hence, the present study helps to support the traditionally claimed cardioprotective and cardiotonic activity of *Artemisia vulgaris*. 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.

Figure 1: Serum lipid levels, Atherogenic Index (AI) of different groups on 30th day of treatment.



HDL-High-density lipoprotein; VLDL-Very-low-density lipoprotein; LDL-Low-density lipoprotein; AI-Atherogenic Index

Table 1: Serum lipid levels, Atherogenic Index (AI) of different groups on the 1st day, 15th day and 30th day.

Groups	C(mg/dl) Mean ± SD			TG(mg/dl) Mean ± SD			HDL(mg/dl) Mean ± SD			VLDL(mg/dl) Mean ± SD			LDL(mg/dl) Mean ± SD			AI Mean ± SD		
	1st day	15th day	30th day	1st day	15th day	30th day	1st day	15th day	30th day	1st day	15th day	30th day	1st day	15th day	30th day	1st day	15th day	30th day
N	104.93±14.18	103.0±3.59	102.3±8.94	80.32±8.13	79.00±8.71	78.75±6.63	41.83±2.91	41.0±2.1	40.3±4.48	16.6±1.6	15.89±1.74	15.3±1.21	49.43±16.1	48.00±1.65	47.6±6.55	2.60±0.53	2.50±1.64	2.53±0.42
H	283.80±18.89	272.0±18.9	282.23±15.15	263.1±15.9	251.9±11.3	243.2±9.52	40.77±8.23	36.7±2.1	34.17±2.31	49.6±3.1	46.39±2.27	47.56±1.90	216.4±19.1	210.6±21.0	209.16±18.3	6.96±0.95	7.39±0.99	8.2±0.72
R	292.28±13.88	196.5±15.67	183.3±5.74***	215.5±10.3	158.2±16.0	139.2±1.08***	42.29±4.98	60.2±5.4	68.2±2.53***	43.1±2.0	31.65±3.20	20.1±2.22***	206.8±12.8	109.6±15.0	102.36±5.13***	6.99±0.83***	3.26±0.39	2.68±2.26***
T-A	285.67±16.41	242.2±13.2	203.1±2.93	204.3±12.5	196.8±13.8	187.2±5.34	42.1±7.17	48.4±4.8	64.3±2.60	48.2±2.5	37.36±2.77	31.2±3.07	212.7±19.6	186.4±14.6	143.2±4.40	6.78±1.24	5.40±0.53	3.15±1.12
T-B	292.0±14.26	205.0±19.9	180.31±9.48***	206.4±13.2	170.0±16.1	147.2±1.28***	42.5±4.31	52.4±2.4	68.5±5.19***	47.2±2.6	34.01±3.23	28.2±2.26***	210.8±14.19	158.5±19.0	126.3±9.54***	6.87±0.96	4.68±0.49	2.63±1.82***

Data expressed as Mean + SD of (n=6) ; N=Normal, H= Hyperlipidemic control, R=Rosuvostatin group, T-A= Test group-A, T-B= Test group-B, Atherogenic Index (AI).; *** Significant at P<0.001; ** Significant at P< 0.01; *Significant at P<0.05 compared to diet-induced hyperlipidemic control.

Table 2: Weight Gain Different between 30th day to 1st day in various groups .

Groups	Weight Gain(gm) Mean ± SD
N	6.66 + 2.58
H	17.3 + 3.76
R	7.5 + 2.73
T-A	10.0 + 4.47
T-B	8.16 + 3.76

Data expressed as Mean + SD of (n=6); N=Normal, H= Hyperlipidemic control, R=Rosuvostatin group, T-A= Test group-A, T-B= Test group-B, Atherogenic Index (AI).

4. Conclusion

The aqueous extract of roots of *Artemisia vulgaris* has a definite hypolipidemic effect in cholesterol diet-induced hyper lipidemia in rats. The aqueous extract of root of *Artemisia vulgaris* has hypolipidemic activity same as rosuvastatin. These findings provide some biochemical basis for the use of aqueous root extract of *Artemisia vulgaris* as hypolipidemic agent in hyperlipidemia. Further studies are required to gain more insight into the possible mechanism of action.

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