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Hypolipidemic effect of *Achyranthes aspera* on High fat diet induced atherogenic rats

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ABSTRACT

Present investigation was undertaken to evaluate the hypolipidemic activity of aqueous extract of *Achyranthes aspera* in high fat diet induced atherogenic rats. High fat diet produced a significant increase in total cholesterol, VLDL, LDL, PL, Triglycerides, Free fatty acids and decrease in HDL. It also increased HMG CoA reductase activity. Reduction in the activity of Lipo protein lipase was observed. High fat diet also decreased the levels of SOD, CAT and reduced glutathione with associated increase in lipid peroxidation. Treatment with *Achyranthes aspera* (200mg./kg b.wt.,) altered the deranged metabolic profile and was effective in producing hypolipidemia.
Keywords: *Achyranthes aspera*, high fat diet, hypolipidemic agent, hyperlipidemia.

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INTRODUCTION

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease.

Atherosclerosis is a complex disease and usually presents with atheromatosis (intimal fat accumulation) and arteriosclerosis (media calcification and elastin damage) Heart disease is raising an epidemic scale in Indian men and women, vegetarians and nor-vegetarians whether they are living in India or abroad [1].

The World Health Organization report emphasizes that the cardio vascular diseases to be the largest cause of death and disability in India by 2020.The nature of the carbohydrates in the diet has been reported to influence the level of serum cholesterol in both man and experimental animals. The basic pathogenesis of atherosclerosis involves an insult to the endothelial and smooth muscle cells of the arterial wall by various harmful factors such as viral infection, mechanical damage .and dislipidemia, especially abnormal oxidized low-density lipoprotiens. A plant-based diet rich in fruits, vegetables, legumes and low in saturated fat along with regular exercise is the standard prescription for individuals with elevated risk of cardiovascular disease. Various anatomic, physiological and behavioral risk factors for atherosclerosis are known [2].These can be divided into various categories: congenital acquired, modifiable or not, classical or non-classical. Hyperlipidemia, hypertension and cigarette smoking together increase the risk seven times.

Herbs constitute a major part in all traditional systems of medicines. Because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. *Achyranthes aspera* (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The plant possesses anti parasitic, Hypoglycemic, Hepatoprotective, analgesic, anti inflammatory, antimicrobial, nephro protective and immuno modulatory activities [3-8]. In the present study, hypolipidemic effect of Achyaranthes aspera has been evaluated through various biochemical parameters.

MATERIALS AND METHODS

Collection and preparation of Herbal extract

Fresh leaves of *Achyranthes aspera* are collected fron Athiyur village, Thanjavur district, Tamil Nadu, India. Collected leaves are washed with distilled water, shade dried and powdered well . The leaf powder was soaked in water (50g in 300 ml water), soxhleted, filtered and the filtrate is stored at 4°C in an airtight container for future use.

Animals

Male wistar rats weighing about 150-200 g were procured and maintained in laboratory condition. The animals were fed with standard pellet diet (Kamathenu Agencies, Bangalore, India) and clean water ad libitum, and routinely housed in controlled conditions with temperature of 25–26°C, relative humidity of 60–80% and 12-h light/dark cycle. The animals were acclimatized for 2 weeks before experimentation.

Chemicals

Cholesterol and lecithin were purchased from Hi Media Laboratories Ltd, Mumbai, India. Triolein was purchased from Sisco Research Laboratory Pvt. Ltd, Mumbai, India. Bovine serum albumin, digitonin and dextran sulfate were purchased from Sd. Fine Chemicals Ltd, Mumbai, India. The reagent kit for high-density lipoprotein (HDL) and total cholesterol estimation was obtained from Span Diagnostics Ltd, Surat, India. All other chemicals and solvents were of analytical grade from standard companies, and the solvents were distilled before use.

Phytochemical Analysis

Phytochemical Analysis for major phytoconstituents of the plant extract was undertaken using standard methods as described by various authors. The plant extracts were screened for the presence of biologically active compounds like sugars, aminoacids, proteins, phenols, terpenoids, etc.,

Experimental Design

The animals were divided into 4 groups of 6 animals in each group. Control group animals were fed with standard pellet diet where as HFD group animals were fed with standard pellet mixed with 2% of cholesterol, 0.125% of cholic acid and 20% of coconut oil.

Group 1	:	Control rats received standard pellet diet
Group 2	:	Received high fat diet
Group 3	:	HFD + 200mg/kg body weight *Achyranthes aspera*
Group 4	:	*Achyranthes aspera* (200 mg/kg body weight)

The animals were maintained in their respective groups, monitored closely daily and weighed every week. Total duration of the experiment was 10 weeks, at the end of which some of the rats were fasted overnight to eliminate chylomicrons, anaesthetized with ketamine hydrochloride (30 mg/kg body weight i.p.), and then sacrificed by cervical decapitation. Blood was collected in tubes containing ethylenediaminetetraacetic acid (1 mg/mL), and plasma was obtained by centrifuging the blood (1500 g, 15 min, 4C). The liver, heart and aorta were excised and immediately chilled in normal saline. Tissues were homogenized (5% w/v) in appropriate buffer, centrifuged and the supernatant assayed for enzyme activity.

Statistical Analyses

All the grouped data were evaluated statistically and significance of changes was determined using one-way analysis of variance followed by Duncan's multiple range test (Duncan 1957) using SPSS 11.0 for windows. Results are presented as mean ± SD among values of 6 rats from each group. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Cardiovascular disease (CVD) has by far the highest prevalence of all diseases in affluent industrialized countries. The role of lipids as an important factor in the pathogenesis of CVD has now been firmly established. Different approaches were used to reduce the incidence, and to treat CVD. Therapeutic effects of plant foods and extracts in reducing CVD were reviewed and nonnutrient phytochemicals are increasingly being recognized as potential health promoters in reducing the risk of CVD and atherosclerosis [9].

The plasma, liver, heart and aorta FFA, PL and TG levels were significantly elevated in HFD-fed rats as compared to control rats. *Achyranthes aspera* supplementation to rats simultaneously along with HFD reduced plasma lipid concentrations to basal levels and thereby, *Achyranthes aspera* may play a role in maintaining membrane integrity and activities of membrane-bound enzymes.

VLDL and LDL are known to have a positive role in obesity and other related disorders [10]. These lipoproteins are chemically modified by oxidation or glycation in the initial stages of atheroma formation. In the present study, plasma VLDL and LDL were significantly higher in HFD-fed rats than those in control rats, whereas VLDL and LDL levels were significantly lowered on Achyranthus aspera administration (Table.3).

HDL is considered to be a beneficial lipoprotein [11] and has a negative effect on hyperlipidemia, obesity and atherogenesis. In the present study, plasma HDL was significantly lowered in HFD-fed rats than those in control rats, whereas HDL levels are significantly elevated on Achyranthus aspera administration (Table, 3).

LPL plays an important role in the metabolism of plasma lipoproteins and thus, the transport of lipids to peripheral tissues. A number of studies reported that plasma LPL, directly or indirectly, may promote or protect against atherosclerosis. [12] Reported that increased LPL activity is antiatherogenic, and other researchers showed that a decrease in LPL activity is atherogenic[13]. Significantly lowered LPL activity in HFD-fed rats can cause accumulation of cholesterol and VLDL. Achyranthus aspera administration resulted in the optimum activity of plasma LPL (Table, 4). Thus, comparatively low levels of VLDL and LDL found in the Achyranthus aspera treated animals may be correlated with the optimal activity of plasma LPL observed in these animals.

HMG CoA reductase, the enzyme that converts HMG CoA to mevalonate using reduced nicotinamide adenine dinucleotide phosphate as a reducing equivalent, is the rate-limiting step in cholesterol biosynthesis. In the present study, HMG CoA reductase activity was higher in HFD-fed rats. This may be because high dietary saturated fat is known to cause increased fatty acid oxidation in animal models [14], leading to increased availability of the substrate acetyl CoA required for HMG CoA *Achyranthes aspera* reductase activity in the tissues. Simultaneous supplementation of A.aspera along with the high-fat diet significantly reduced the activity of HMG CoA reductase indicating the potential cholesterol-lowering effect of Achyranthes aspera (Table,5).

Table 1 : Levels of Total Cholesterol, Triglycerides, Free fatty acid and Phospholipids in Plasma and liver of control and experimental animals

Groups	Treatment	TC mg/dL	TG mg/dL	FFA mg/dL	PL mg/dL
Group I	Control	92.38±8.88	92.38±8.88	19.64±1.89	2.14±0.20
Group II	HFD	186.07±17.91^{+}	176.89±1702^{+}	36.21±3.48^{+}	2.40±0.23^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	154.93±14.91*	137.76±13.26*	27.62±2.68*	0.27±0.02*
Group IV	*Achyranthes aspera(*200mg/kg.b.wt)	90.10±10.72*	90.32±8.88*	17.64±1.89*	2.00±0.20*

Values are mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Table:2 Levels of total Cholesterol , Triglycerides, Free fatty acids and phospholipids in liver of control and experimental animals

Groups	Treatment	TC mg/dL	TG mg/dL	FFA mg/dL	PL mg/dL
Group I	Control	0.73±0.07	49.25±4.74	22.86±2.20	0.92±0.08
Group II	HFD	1.81±0.17^{+}	75.93±7.30^{+}	35.64±3.43^{+}	2.94±0.28^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	0.78±0.07*	56.08±5.39*	29.47±2.83*	0.90±0.08*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	0.68±0.08*	43.25±4.74*	2086±2.20*	0.78±0.08*

Values are mean ± SD (N=6)
+P<0.05 Compared to normal Control
*P<0.05Compared HFD Induced Control

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Table: 3 Level of Total Cholesterol, Triglycerides, Free fatty acids and Phospholipids in heart of control and experimental animals

Groups	Treatment	TC mg/dL	TG mg/dL	FFA mg/dL	PL mg/dL
Group I	Control	0.48±0.04	7.85±0.75	10.47±1.00	0.59±0.05
Group II	HFD	1.57±0.15^{+}	13.31±1.28^{+}	17.47±1.68^{+}	2.06±0.19^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	0.44±0.04*	10/05±0.96*	12.34±1.18*	1.59±0.15*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	0.39±0.03*	7.27±0.70*	10.33±1.06*	0.585±0.05*

Values are mean ± SD (N=6)
+P<0.05-compared t with normal Control
*P<0.05compared with HFD Induced Control

Table: 4 Levels of Total Cholesterol, Triglycerides, Free fatty acids and phospholipids in aorta of control and experimental animals

Groups	Treatment	TC mg/gtissue	TG mg/ g tissue	FFA mg/g tissue	PL mg/ g tissue
Group I	Control	0.59±0.05	3.88±0.37	4.31±0.41	0.74±0.07
Group II	HFD	2.06±0.19^{+}	7.19±0.69^{+}	7.22±0.69^{+}	2.44±0.23^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	1.59±1.15*	5.45±0.52*	5.61±0.63*	0.73±0.07*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	0.52±0.06*	3.00±0.38*	4.19±0.44*	0.65±0.07*

Values are mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05compared with HFD Induced Control

Table : 5 Levels of VLDL-C, LDL-C and HDL –C in the plasma of experimental and control rats

Groups	Treatment	VLDL-C mg/dL	LDL –C mg/dL	HDL-C mg/dL
Group I	**Control**	14.13±1.36	38.65±3.72	42±3.88
Group II	HFD	27.94±2.69^{+}	114.62±11.03^{+}	22±2.48^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	21.87±2.10*	97.81±9.41*	38±3.65*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	13.53±1.30*	36.37±5.81*	41.±3.33*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

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Table:6 Levels of Lipo Protein lipase in plasma and tissues of control and HFD administered rats

Groups	Treatment	LPL (mmol of glycerol liberated/ h/mL plasma)
Group I	Control	79.5±7.6
Group II	**HFD**	58.10±5.59^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	76.39±7.35*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	83.53±8.04*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Table: 7 Levels of tissue HMG CoA reductase in plasma and tissues of control and HFD-administered rats

Groups	Treatment	Liver U/L	Heart U/L	Aorta U/L
Group I	Control	4.04±0.38	2.31±0.22	1.26±0.012
Group II	HFD	3.08±0.29^{+}	5.81±0.55^{+}	0.88±0.08^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	7.84±0.75*	1.97±0.19*	1.29±0.13*
Group IV	*Achyranthes aspera* (200mg/kg.b.wt)	9.64±0.92*	4.30±0.44*	1.27±0.12*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Table:8. Levels of TBARS in Plasma, Liver, Heart and Aorta of control and experimental animals

Groups	Treatment	Plasma mg/dL	Liver mg/dL	Heart mg/dL	Aorta mg/dL
Group I	Control	5.84±0.56	26.69±2.56	11.65±1.12	18.44±1.77
Group II	HFD	11.57±1.11^{+}	76.82±7.39^{+}	50.33±84.0^{+}	43.55±4.19^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	8.53±0.82*	43.58±4.19*	24.56±2.36*	30.80±2.96*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	4.34±0.70*	22.92±2.20*	11.08±1.06*	17.68±170*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

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Table:9 Levels of SOD in Plasma, Liver, Heart and Aorta of control and experimental animals

Groups	Treatment	Plasma U/L	Liver U/L	Heart U/L	Aorta U/L
Group I	Control	1.85±0.17	4.79±0.46	49.85±4.79	33.57±3.23
Group II	HFD	1.63±0.15^{+}	3.02±0.29^{+}	50.07±4.81^{+}	23.08±2.22^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	1.81±0.17*	3.41±0.32*	39.82±3.83*	24.88±2.39*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	2.06±0.19*	5.02±0.48*	51.15±4.92*	33.15±3.19*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Table:10 Levels of CAT in Plasma, Liver, Heart and Aorta of control and experimental animals

Groups	Treatment	Plasma U/L	Liver U/L	Heart U/L	Aorta U/L
Group I	**Control**	7.18±0.69	31.11±2.11	3.03±0.37	2.68±0.25
Group II	HFD	5.90±0.56^{+}	30.09±2.89^{+}	2.66±0.25^{+}	1.95±0.18^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	6.95±0.66*	26.31±2.53*	3.08±0.29*	2.19±0.21*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	7.16±0.68*	32.02±3.08*	4.02±0.38*	2.80±0.26*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Table:11 Levels of GSH in Plasma, Liver, Heart and Aorta of control and experimental animals

Groups	Treatment	Plasma mg/dl	Liver mg/dl	Heart mg/dl	Aorta mg/dl
Group I	Control	3.59±0.34	5.31±0.51	10.99±1.05	6.44±0.62
Group II	HFD	2.94±0.28^{+}	3.02±0.29^{+}	7.74±0.74^{+}	5.07±0.48^{+}
Group III	HFD+*Achyranthes aspera*(200mg/kg.b.wt)	3.33±0.32*	4.23±0.40*	8.13±0.78*	6.32±0.60*
Group IV	*Achyranthes aspera*(200mg/kg.b.wt)	3.59±0.34*	5.31±0.51*	0.99±1.05*	6.44±0.62*

Values are Mean ± SD (N=6)
+P<0.05 compared with normal Control
*P<0.05 compared with HFD Induced Control

Free radical mediated lipid peroxidation leads to accumulation of lipid peroxidation products such as malondialdehyde, hydrogen peroxide which in turn propagate lipid peroxidation process, and causes damage to the membrane, resulting in cell death [15].

SOD has been touted as the first line of the erythrocyte antioxidant against oxygen free radicals CAT have a major role in preventing LPO by neutralizing hydrogen peroxide and lipid peroxide, there by maintaining the structure and function of biological membrane. In the presence of HFD , the antioxidant system fails to neutralize the high amount of ROS formed and the resultant lipid peroxidative damage is greater than their ability to counter them. This lowered activities of these antioxidant enzyme may be due to their increased utilization to combat the excessive erythrocyte oxidative stress in hypercholesterolemic rats.

Simultaneous supplementation of *Achyranthes aspera* with HFD resulted in increase in the activities of the antioxidant enzymes (SOD, CAT) may be because *Achyranthes aspera* is highly lipophilic in nature, helps in inhibiting the lipid peroxidation initiated by free radicals , thus preventing or delaying the damage to cells.

GSH is a multifunctional intracellular non-enzymic antioxidant which scavenge hydroxyl radical and singlet oxygen directly, and also detoxifies hydrogen peroxide and other lipid peroxide radicals by the catalytic action. In the present study A.aspera Supplementation produced increased in the level of reduced glutathione.

Elevated TBARS concentrations the final products of LPO found in the tissue of HFD and / or treated experimental animals is a clear manifestation of excessive formation of free radicals and activation of LPO system. In our study TBARS significantly elevated in the tissue of rats fed with HFD suggesting that hypercholesterolemia could enhance the process of LPO and increase oxidative stress, evoking a series of events which dysregulate the cellular function [16].

Significant decline in the TBARS concentration and increase in the enzymic and non enzymic antioxidant in plasma and tissue on *Achyranthes aspera* treatment have shown that *Achyranthes aspera* significantly inhibited oxidative stress.

Hence it is evident from the present study that Achyanthes aspera have immense potential to lower serum lipid levels.

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