HYPOLIPIDEMIC ACTIVITY OF *ACHYRANTHES RUBROFUSCA* LINN. WHOLE PLANT EXTRACTS IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS

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Summary

High fat diet fed rats showed significant increased levels of plasma and tissue total cholesterol, triglycerides, free fatty acids, phospholipids, plasma LDL cholesterol and decreased level of plasma HDL cholesterol. Ethanol and aqueous extract of *Achyranthes rubrofusca* Linn. administration to high fat diet fed rats showed near to normal levels of the above lipids in plasma and tissues. Ethanol extract showed comparable results with standard drug atorvastatin. It is concluded that the ethanol extract at the dose 300mg/kg body weight of A. rubrofusca possesses significant hypolipidemic activity in high fat fed rats.

Key words: Achyranthes rubrofusca, Hypolipidemic effect, Cholesterol, Triglyceride.

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Introduction

Achyranthes rubrofusca Linn. (*A. rubrofusca* Linn.). is a tribal medicine belonging to the family Amaranthaceae used for reproduction related disorders and common ailments such as diarrhea, bone fracture cuts and boils. Previous study showed that the main constituents of this genus were steroids, Flavanoids, triterpenes and phenols[1-3]. It is a small herb found all over India. Hyperlipidemia is recognized as one of the greatest risk factors for coronary artery disease[4]. Several experimental animal & interventional studies indicated morbidity & mortality in coronary heart diseases with reduction of serum total cholesterol and / or improvement in HDL cholesterol[5]. Currently available Hypolipidemic drugs have associated with number of side effects[6]. The current scenario sees the emergence of a number of medicinal plants being evaluated for various disease and disorders. Although a number of medicinal plants like Commiphora mukal, *Gymnema sylvestre, Ptercarpus narsupium, Trigonella foemgraceum, Azadirachta indica, Terminalia arjuna and Boerwellia serrata* have been evaluated of their Hypolipidemic activity, only Commiphora mukal has been commercially well established.

Materials and methods

Whole plants of *Achyranthes rubrofusca* Linn. were collected from Sankarankoil, Tirunelveli district of Tamilnadu, India. Taxonomic identification was done from Botanical Survey of Medicinal Plant Unit, Siddha Government of India, Palaymkottai, Tamilnadu. Four month old whole plants were dried under shade, segregated and pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered materials were successively extracted by hot continuous percolation method in Soxhlet apparatus[8]. The extracts were suspended in 0.5% Carboxy Methyl Cellulose. Wistar male rats of 16-19 week of age, weighing 150-175g were procured from P.S.G Institute of Medical Science and Research, Coimbatore. The animals were kept in plastic bags, 3 per cage, with 12:12 hour light and dark cycle at $25^0 \pm 2^0$C. The animals were maintained in their respective diets and water *ad libitum*. Animals were divided into following 5 groups of 6 animals each.

Group I : Control diet.
Group II : High fat diet.
Group III : High fat diet + aqueous extract of Achyranthes rubrofusca 300mg/kg/day.
Group IV: High fat diet + 300mg/kg body weight of ethanol extract of A. rubrofusca.
Group V : High fat diet + standard drug atorvastatin (1.2mg/kg/day)

The compositions of the two diets were as follows
Control diet : Wheat flour 22.5%, roasted Bengal gram powder 60%, Skimmed milk powder 5%, Casein 4%, Refined oil 4%, Salt mixture with starch 4% and vitamin & choline mixture 0.5%.

High fat diet : Wheat flour 20.5%, roasted Bengal gram 52.6%, Skimmed milk powder 5%, Casein 4%, Refined oil 4%, Coconut oil 9%, Salt mixture with starch 4% and Vitamin and Choline mixture 0.5%, Cholesterol 0.4%.

Rats of groups III and IV were orally fed with ethanol and aqueous extracts respectively and rats of group V were fed with standard drug atorvastatin. Both the *A. rubrofusca* Linn. extracts and atorvastatin were suspended in 1% carboxy methyl cellulose and fed to their respective rats in addition to their respective diets. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after over night fasting. Animals were given enough care as per the animal ethical committee's recommendations.

Plasma total cholesterol, triglycerides, phospholipids, free fatty acids, LDL Cholesterol and HDL Cholesterol were estimated using Boehringer Mannherrm Kits by Erba Smart Lab Analyzer, USA. Ester cholesterol and free cholesterol[8] were analyzed using digitonin. Portion of liver, heart and aorta tissues were blotted, weighed and homogenized with methanol (3 volumes) and lipid extract was obtained by the method Eblan et al. and were used for the estimation of ester cholesterol, free cholesterol, Triglycerides and phospholipids.

Results and discussion

Results are presented in Table 1-3. The average food in take per rat per day was found to be 19.2 $\pm$1.0g. Results clearly show that feeding high fat diet increased plasma and tissue lipids and lipoprotein levels. Earlier studies have also shown a significant elevation in the plasma and tissue lipid parameters in response to high fat diet[9-11].

Both plasma and tissue cholesterol as well as phospholipids were reduced remarkably on treating the high fat diet rats with ethanol and aqueous extract of A. rubrofusca. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increased excretion of fecal sterols. Like many species *A. rubrofusca* Linn. may stimulate hepatic microsomal cytochrome P450 dependant aryl hydrolase activity which is believed to be involved in the hydroxylation of endogenous steroids such as cholesterol[12], and thereby increases the catabolic conversion of cholesterol to bile acids in liver[13].

Concentration of phospholipids decreased on treatment with A. rubrofusca extract in the high fat diet fed rats. Marked reduction in cholesterol and phospholipids levels may be attributed significantly to ethanol extract of A. rubrofusca which is comparable to the standard drug atorvastatin.

Increased levels of plasma free fatty acids were observed in high fat diet fed rats compared to the controlled rats. The significant increase of free fatty acids may be due to the breakdown of membrane phospholipids by the action of oxygen derived free radicals induced during hyper lipidemic or may be due to the increased activity of Phospholipase[14]. Treatment with *A. rubrofusca* Linn. extract decreased the free fatty acid concentration.

Table no. 1
Effect of extracts of Achyranthes rubrofusca on plasma lipid profile in HFD rats.
(Values are mean ± SE of 6 rats)

Group	Total cholesterol	Free cholesterol	Ester cholesterol	Free fatty acids	Phospho lipid	Arhero-genic index	Total Glyceride	HDL cholesterol	LDL cholesterol	VLDL cholesterol
Group I Control	107.88 ± 1.38	21.88 ± 1.02	86.01 ± 1.02	40.21 ± 1.08	88.14 ± 0.78	1.78 ± 0.048	55.18 ± 0.88	60.26 ± 1.14	37.09 ± 0.08	10.12 ± 0.88
Group II High fed	174.84 ± 1.86^{a}	40.78 ± 1.2^{a}	134.06 ± 1.04^{a}	57.88 ± 1.12^{a}	105.84 ± 1.18^{a}	3.84 ± 0.058^{a}	70.07 ± 0.88^{a}	47.32 ± 0.88^{a}	111.98 ± 1.08^{a}	12.14 ± 0.8^{NS}
Group III Aqueous extract	123.28 ± 1.48^{a}	28.76 ± 1.18^{a}	94.52 ± 1.20^{a}	44.24 ± 1.08^{c}	93.18 ± 1.48^{b}	2.14 ± 0.08^{a}	63.28 ± 0.82^{a}	50.14 ± 0.78^{a}	59.85 ± 0.88^{a}	12.14 ± 0.88^{NS}
Group IV Ethanolic extract	99.4 ± 1.52^{b}	22.9 ± 0.8^{NS}	76.5 ± 1.46^{b}	37.85 ± 1.18^{NS}	80.16 ± 0.66^{a}	1.68 ± 0.04^{a}	50.88 ± 1.18^{a}	57.35 ± 1.08^{NS}	30.14 ± 0.252^{a}	10.2 ± 0.88^{NS}
Group V Standard drug 1.2 g atrovastatin	96.96 ± 1.08^{a}	21.27 ± 0.90^{NS}	75.69 ± 0.96^{c}	49.25 ± 0.96^{b}	82.88 ± 0.80^{b}	1.68 ± 0.08^{a}	54.74 ± 0.88^{NS}	56.98 ± 0.98^{NS}	28.88 ± 0.70^{a}	10.15 ± 0.40^{NS}

P values : P ^{a}<0.001, P ^{b}<0.01, P ^{c}<0.05
NS : Non significant

Table No. 2
Effect of extracts of Achyranthes rubrofusca on tissue cholesterol level in HFD rats.
(Values are mean ± SE of 6 rats)

Group	Free cholesterol mg/g tissue			Ester cholesterol mg/g tissue		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I Control	0.783 ± 0.02	0.668 ± 0.12	0.48 ± 0.012	2.58 ± 0.08	1.88 ± 0.03	1.78 ± 0.014
Group II HFD	1.10 ± 0.01[a]	0.938 ± 0.02[a]	2.57 ± 0.012[a]	6.52 ± 0.02[a]	3.42 ± 0.02[a]	7.28 ± 0.17[a]
Group III Aqueous extract	0.978 ± 0.01[a]	0.88 ± 0.96[a]	1.76 ± 0.01[a]	4.18 ± 0.02[a]	2.62 ± 0.02[a]	5.76 ± 0.01[a]
Group IV Ethanol extract	0.80 ± 0.08[b]	0.66 ± 0.01[NS]	0.66 ± 0.01[a]	2.92 ± 0.12[a]	1.97 ± 0.02[c]	2.66 ± 0.01[a]
Group V Standard drug	0.88 ± 0.01[a]	0.72 ± 0.01	0.82 ± 0.01[a]	3.04 ± 0.02[a]	2.18 ± 0.01[a]	2.98 ± 0.02[a]

P values : P [a]<0.001, P [b]<0.01, P [c]<0.05
NS : Non significant

Table No. 3
Effect of extracts of Achyranthes rubrofusca on tissue lipid content in HFD rats.
(Values are mean ± SE of 6 rats)

Group	Phospholipid mg/g tissue			Triglyceride mg/g tissue		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I Control	17.56 ± 0.38	23.48 ± 0.48	8.41 ± 0.14	7.18 ± 0.18	10.52 ± 0.48	9.18 ± 0.44[a]
Group II HFD	27.48 ± 0.37[a]	36.22 ± 0.48[a]	15.06 ± 0.24[a]	28.96 ± 0.68[a]	48.18 ±1.08[a]	21.42 ± 0.55[a]
Group III Aqueous extract	23.68 ± 0.48[a]	36.40 ± 0.64[a]	12.48 ± 0.32[a]	21.07 ± 0.42[a]	36.91 ± 0.58[a]	16.84 ± 0.35[a]
Group IV Ethanol extract	18.98 ± 0.54[c]	26.51 ± 0.48[b]	10.4 ± 0.40[a]	10.52 ± 0.32[a]	18.12 ± 0.4[a]	12.00 ± 0.98[a]
Group V Standard drug	19.98 ± 0.48[a]	28.28 ± 0.88[b]	11.08 ± 0.45[a]	12.48 ± 0.62[a]	21.10 ± 0.44[a]	12.82 ± 0.32[a]

P values : P [a]<0.001, P [b]<0.01, P [c]<0.05
NS : Non significant

Supplementation of A. rubrofusca extracts lowered the concentration of triglyceride level significantly in high fat diet fed rats. High fat diet fed rats showed decreased activity of lipoprotein lipase in adipose tissue. Stimulation of the activities of skeletal muscle lipoprotein lipase and adipose tissue hormone sensitive lipase may be responsible for the increased uptake of triglycerides from plasma by skeletal muscle and adipose tissue[15].

Increased concentration of VLDL and LDL were observed in the plasma of high fat diet fed rats when compared to the controlled. Treatment with A. rubrofusca extract reduced VLDL and LDL levels significantly. Ethanol extract of the plant was found to have the effect to the extent when compared to standard drug atorvastatin. VLDL is highly rich in triglycerides and is involved in the transport of triglycerides from liver to extra hepatic tissues whereas LDL is mainly formed from VLDL in presence of heparin releasable lipoprotein lipase, an enzyme present in the endothelial cells of the blood vessels. Studies show that both LDL and VLDL have a positive role in atherogenesis[16]. Ethanol extract of A. rubrofusca was found to be more effective than aqueous extract in reducing the concentration of LDL in plasma. Reduced levels of LDL in A. rubrofusca extracts on HFD fed rats may be possible due to increase in catabolism of LDL.

HDL is synthesized mainly in intestine and liver. It has a high phospholipid content and is involved in reverse cholesterol transport. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. HDL concentration on plasma was significantly increased on ethanol extract treatment and the present investigation shows that ethanol extract of A. rubrofusca has a preventive role in the development of atherogenesis.

Atherogenic index is used as marker to assess the susceptibility of atherogenesis. It was markedly increased on high fat diet fed rats. It was significantly decreased on feeding the experimental plant, ethanol extract of A. rubrofusca, thus emphasizing the protective role of A. rubrofusca against atherogenesis. Further investigation is needed to explore the exact mechanism of action of the plant extract.

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