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Research Article

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Hypolipidemic effect of Methanolic extract of *Mussaenda frondosa linn*. Leaves in high fat diet fed rats

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ABSTRACT

High fat diet fed rats showed significant Increased levels of plasma and total cholesterol, triglycerides, free fatty acids, phospholipids plasma LDL cholesterol and decreased level of plasma HDL cholesterol. Methanolic extract of *M.frondosa* administration to high fat diet fed rats showed near normal levels of the above lipids in plasma and tissues. When higher dose of the extract (400-450 mg/kg body weight) showed prominent results with the standard drug atorvastatin (1.2mg/kg). Results obtained from this study suggest that the hypolipidemic effect of Methanolic extract of *Mussaenda frondosa* may be due to the inhibition of hepatic cholesterogenesis and may have slight antioxidant activity. It is therefore concluded that the methanolic extract of M.frondosa possesses hypolipidemic activity in high fat diet fed rats.

Key words: *Mussaenda frondosa*, Hypolipidemic effect, Rubiaceae, Mussaein.

INTRODUCTION

Medicinal plants have been used in various traditional systems, as they have immune potential against numerous diseases. Cardio vascular diseases remain by far the number one cause of death for both men and women. Although many causative factors of these diseases are recognized (smoking, high blood pressure, genetic back ground, diabetes mellitus and obesity) high serum LDL – C and elevated total cholesterol levels are the most prevalent indicators for susceptibility to atherosclerotic heart diseases[1]. Atherosclerosis is a disorder of the arterial wall characterized by accumulation of cholesterol ester in cells derived from the monocyte – macrophage line, smooth muscle cell proliferation and results in narrowing the blood vessels [2]. An association of dietary cholesterol with cardiac and cerebral vascular diseases is based on several lines of evidence, including studies in animal models and epidemiological data in humans[3]. There are many classes of lipid lowering agents available, these drugs have different mechanisms of action and variable efficacy depending on the lipid profile of an individual. Inspite of their lipid lowering effect, these drugs have many side effects. Thus research is still pursuing to find out novel agents that are more effective and safe. M.frondosa linn, microfiche number IDC 86.11 belongs to family Rubiaceae is distributed in central Nepal, India and Srilanka. The juice of the root is used to treat blemishes on the tongue and the sepals are diuretic[4]. Its some what smaller, 6 to 9 ft tall with an equal spread. The foliage is lighter green, on the terminal flower clusters have orange to yellow with a single white enlarged calyx[5]. The phytochemical constituents are Iridoids, flavanoids, triterpenes[6] and mussaein. The leaves of M.frondosa yield astragaline, isoquercitin, Drutinoside[7] (WHO 1998).

It has been used in Chinese folk medicine as a diuretic, anitphlogistic and antipyretic. It is used to detoxify mushroom poisons and terminate early pregnancy. M.frodosa has been found to posses anti bacterial effect. But its hypolipidemic activity have been not yet reported so far. In short the M.frondosa treatment demonstrated a promising hypolipidemic activity in rats and therefore, needs further clinical evaluation in humans. The present work aims at investigating the hypolipidemic activities of M.frondosa leaves in high fat diet fed rats. Atorvastatin has adopted in this study as a reference hypolipidemic drug for data comparison.

MATERIALS AND METHODS:

The leaves of M.frondosa were collected between june – September from southern parts of India. Taxonomic identification was made from our pharmacognosy institute. The leaves were collected in a one year plant, dried under shade, segregated and pulverized and passed through a 40 mesh sieve. The leaves were kept free from possible fungus infection. The powdered materials were successively extracted by hot continuous percolation method in soxhlet apparatus[8]. The leaf extracts were suspended in 2% tween 80[9]. Young wistar male rats weighing 160 – 180g were used for this study. The animals were procured from K.M.College of Pharmacy, Madurai – 625 107. The animals were kept in plastic cages, with 12:12 hr light and dark cycle at $25^0 \pm 2^0$C. The animals were maintained on their respective diets and water *ad libitum*.Animals were divided into following 5 groups of 6 animals each:

Group I (Control): Standard diet

Group II : High fat diet

Group III : High fat diet + methanolic extract of *M.frondosa* (dose 200mg/kg body wt)

Group IV : High fat diet + methanolic extract of *M.frondosa* (dose 400mg/kg body wt)

Group V : High fat diet + standard drug atorvastatin (1.2mg kg body wt)

The compositions of 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 0.9%.

Rats of groups III and IV were orally fed with methanolic extracts of M.frondosa dose 200mg and 400mg/kg body wt respectively and rats of group V were fed with standard drug atorvastatin. Both the M.frondosa extracts and atorvastatin were suspended in 2% tween 80 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 committees recommendations and the work were carried out after obtaining proper approval from institutional animal ethics committee.

Plasma total cholesterol, triglyceride, phospholipids, free fatty acids, LDL Cholesterol and HDL Cholesterol were estimated using Boehringer Mannheim kits by Erba smart lab analyzer, USA. Ester cholesterol[10] and free cholesterol[10] were analysed using digitonin. Portions of liver, heart and aorta tissues were blotted, weighed and homogenized with methanol and lipid extract was obtained by the method of folch et al[11] and were used for the estimation of ester cholesterol, free cholesterol, triglyceride[11], phospholipids[12].

Results were expressed as mean ± SE of 6 rats in each group. Students 't' test was used to determine the statistical significance between each control group and its test groups. Significance level was fixed at 0.05.

RESULTS AND DISCUSSION:

Results are presented in Tables 1 – 3.

Results clearly show that feeding HFD increased plasma and tissue lipids and lipoprotein levels [13,14] when compared with earlier studies. Both plasma and tissue cholesterol as well as phospholipids were reduced remarkably on treating the HFD with methanolic extract of M.frondosa. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis and catabolic conversion of cholesterol to bile acids in liver[15,16]. Marked reduction in cholesterol and phospholipids levels may be attributed to higher doses of drug of M.frondosa which is comparable to the standard drug atorvastatin.

Increased levels of plasma free fatty acids were observed in HFD rats as compared to the control rats. This significant increase of free fatty acids may be due to the breakdown of membrane phospholipids by the action of oxygen derived free radicals. M.frondosa extract lowered the concentration of triglyceride level in HFD fed rats. HFD fed rats showed decrease activity of lipoprotein lipace in adipose tissue[17].

Increased concentration of VLDL and LDL were observed in the plasma of HFD treated rats when compared with the control. Treatment with M.frondosa extract reduced VLDL and LDL levels significantly. Studies show that both LDL and VLDL have a positive role in atherogenesis [18].

HDL is synthesized mainly in intestine and liver. It has a

Table1- Effect of methanolic extract of M.Frondosa on plasma lipid profile in HFD rats

[values are mean + SE of 6 rats]

Group	Total Cholesterol	Free Cholesterol	Ester Cholesterol	Free fatty acid	Phospho lipid	Atherogenic index	Triglyceride	HDLCholesterol	LDLCholesterol	VLDL Cholesterol
Group I	109.54±1.21	22.42 ± 1.21	86.02 ± 1.21	41.71 ± 1.02	87.21 ± 0.96	1.72 ± 0.04	52.14 ± 0.75	58.24 ± 0.74	36.04 ± 0.04	11.26 ± 0.96
Group II	173.32 ± 1.94^{a}	45.26 ± 1.26^{a}	126.21 ± 1.31^{a}	56.07± 1.12^{a}	108.32 ± 1.17^{a}	3.24 ± 0.074^{a}	70.02 ± 0.84^{a}	49.34 ± 0.62^{a}	114.92 ± 1.04^{a}	12.22 ± 0.76^{NS}
Group III	122.21 ± 1.62	28.16 ± 1.01^{a}	92.22 ± 1.21^{a}	42.24 ± 1.16	92.24 ± 1.05^{b}	2.24 ± 0.04^{a}	64.26 ± 0.94^{a}	46.29 ± 0.72^{a}	58.50 ± 0.51^{a}	12.17 ± 0.24^{NS}
Group IV	100.47 ± 1.61^{b}	24.71 ± 0.98^{NS}	74.29 ± 1.32^{b}	36.04 ± 1.08^{NS}	82.56 ± 0.96^{a}	1.74 ± 0.07^{a}	52.22 ± 1.21^{c}	54.35 ± 1.06^{NS}	32.72 ± 0.025^{a}	10.21 ± 0.94^{NS}
Group V	96.32 ± 1.84^{a}	20.94 ± 0.91^{NS}	82.06 ± 0.94	49.17 ± 1.14^{b}	82.21 ± 0.74^{b}	1.62 ± 0.09^{a}	59.72 ± 0.96^{NS}	59.17 ± 0.94^{NS}	30.24 ± 0.80^{a}	10.74 ± 0.84^{NS}

P Values : ^{a}<0.001 , ^{b}<0.01, ^{c}<0.02,NS: Non – Significant

Group I : Control;Group II: High fat diet (HFD);Group III: HFD + Methanolic extract of *M.Frondosa* (200mg/kg body wt/day);Group IV: HFD + Methanolic extract of *M.Frondosa* (400mg/kg body wt/day);Group V: HFD + atorvastatin (1.2mg/kg body wt / day.

Table 2 – Effect of Methanolic extract of *M.Frondosa* on tissue cholesterol level in HFD rats
[value 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	0.723 ± 0.01	0.729 ± 0.01	0.48 ± 0.014	2.68 ± 0.04	1.84 ± 0.04	1.80 ± 0.014
Group II	1.16 ± 0.02[a]	0.96 ± 0.01[a]	2.64 ± 0.012[a]	6.24 ± 0.04[a]	3.24 ± 0.04[a]	7.26 ± 0.014[a]
Group III	1.16 ± 0.04[a]	0.89 ± 0.01[a]	1.72 ± 0.012[a]	4.72 ± 0.04[a]	2.72 ± 0.06[a]	4.72 ± 0.06[a]
Group IV	0.84 ± 0.07[b]	0.64 ± 0.01[NS]	0.69 ± 0.021[a]	2.72 ± 0.04[a]	1.72 ± 0.01[b]	2.67 ± 0.01[a]
Group V	0.86 ± 0.01[a]	0.71 ± 0.02	0.84 ± 0.02[a]	3.09 ± 0.05[a]	2.21 ± 0.01[a]	2.99 ± 0.01[a]

P Values : [a]<0.001, [b]<0.01, [c]<0.02NS: Non Significant

Table -3 Effect of methanolic extract of *M.Frondosa* 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	16.21 ± 0.34	22.94 ± 0.61	9.21 ± 0.14	6.21 ± 0.24	11.21 ± 0.42	9.21 ± 0.34[a]
Group II	27.21 ± 0.39[a]	34.22 ± 0.58[a]	14.04 ± 0.32[a]	28.04 ± 0.69[a]	46.54 ± 1.04[a]	21.21 ± 0.48[a]
Group III	24.21 ± 0.24[a]	39.21 ± 0.72[a]	11.21 ± 0.22[a]	21.24 ± 0.44[a]	36.32 ± 0.61[a]	19.52 ± 0.34[a]
Group IV	19.21 ± 0.21[c]	24.21 ± 0.42[b]	9.21 ± 0.76[a]	11.21 ± 0.44[a]	16.21 ± 0.42[a]	12.92 ± 0.72[a]
Group V	20.21 ± 0.21[a]	24.26 ± 0.59[b]	11.21 ± 0.34[a]	12.92 ± 0.21[a]	20.04 ± 0.24[a]	12.94 ± 0.46[a]

P Value : [a] < 0.001, [b]< 0.01, [c]<0.05,NS: Non-Significant

high phospholipids 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 in plasma was significantly increased on M.frondosa treatment in this present investigation. Atherogenic index is used as a marker to assess the susceptibility of atherogenesis. It was increased during feeding with HFD to rats, and significantly decreased on plant extract, thus emphasizing a protective role against atherogenesis. Further investigation is needed to explore the exact mechanism of action of the plant extract.

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