HYPOLIPIDEMIC ACTIVITY OF AQUEOUS EXTRACT OF *MELOTHRIA MADERASPATANA*

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Summary

The present study investigates the hypolipidemic effect of aqueous extract of whole plant of *Melothria maderaspatana* (AEMM) in high fat diet fed rats. In this study three doses (0.5, 1 and 2 gm kg^{-1}) of AEMM were used. Treatment with AEMM (2 gm kg^{-1}, p.o.) had shown significant reduction in the lipid level in high fat diet fed animals. The AEMM exhibited significantly hypolipidemic activity comparable with the navaka guggulu (400mg kg^{-1}) in high fat diet induced rats. In histological evaluation of the hepatic tissue, marked degenerative and fatty changes in liver of rats (high fat diet fed) were observed. Whereas, on treatment with the aqueous extract of *M. maderaspatana* and Navaka guggulu illustrate, striking micro vesicular fatty changes with control and reference drug navaka guggulu in rat's liver.

Key Words: Hypolipidemic activity, High fat diet induced rats, *Melothria maderaspatana.*

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 men and women [1]. Hyperlipidemia is the primary risk factor of coronary heart disease and atherosclerotic heart disease [2]. It is characterized by elevated level of triglyceride, cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and decreased high-density lipoprotein (HDL) level in the blood [3]. Ischemic heart disease (IHD) is one of the leading cause of morbidity and mortality in both developing and developed countries.

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An underlying cause of IHD involves retention and deposit of serum lipids in coronary arteries. Many drugs (conventional and herbal) were used to lower levels of serum cholesterol to prevent IHD. The Ayurvedic medicine pharmacopoeia identified few herbs that might contribute to decrease in cholesterol and therefore reduce the risk of IHD.

In modern society, herbal medicine continues to flourish and play a pivotal and indispensable role in public health care. Medicinal plants used in traditional folk medicine would be a good source for this area of research. In India, different medicinal systems make use of a number of plants in the treatment of hyperlipidemia. Some drugs of plant origin like *Commiphora mukkul* [4]*, Terminalia chebula* [5]*, Emblica officinalis* [6]*, Annona muricata* [7]*, Anthocephalus indicus* [8]*, Sphaeranthus indicus* [*9],* and *Carica papaya [10]* have shown varying degree of anti hyperlipidemic activity.

There are many classes of lipid lowering agents available, in the market with different mechanisms of action and variable efficacy depending on the lipid profile of an individual. In spite of their lipid lowering effect, these drugs have many side effects [11]. Thus research is still pursuing to find out novel agents that are effective and with minimum side effects on long term uses.

Melothria maderaspatana Linn. (family: Cucuribitacea) is an annual monoecious tendril climber, popularly known as Agumaki in Hindi and Musimusikkayi in Tamil. Plant is found throughout India ascending up to 1800m in the hills [12]. *M. maderaspatana* reduces the fever, anxiety and improved appetite and did not produce adverse effects such as nausea or vomiting. The seed of *M. maderaspatana* in decoction are sudorific, inflatulence [13], seeds in crushed used an aching bodies especially as sprained backs and when masticated relieves tooth ache, the tendrils shoots and tender leaves are used as a gentle aperlent and present bed in vertigo and biliousness [14]. The roots are recommended as laxative and diuretic in constipation [15]. The phytochemical constituents present in *M. maderaspatana* are sugar, amino acid and flavanoids. The leaf of *M.maderaspatana* contains several phytochemicals, including spinasterol, 22, 23-dihydrospinasterol, *β*-sitosterol, decosaenoic acid, triterpenes, phenolic compounds, and multiple glycosides (22,23-dihydrospinasterol-3-*O*-*β*-d-glucoside) [16, 17]. Chemical constituents, such as, columbin from roots, linolenic, lenoleic and arachidic acids from seeds have been reported in literature. *M. maderaspatana* leaf tea consumption gradually decreases the blood pressure in hypertensive patients [18] and it has been reported to have anti-inflammatory [19], anti-arthritic and antipyretics in animals [15]. *M. maderaspatana* has been found to posses anti bacterial effect. But hypolipidemic activity of *M. maderaspatana* has not been yet reported so far. This has been established that *M. maderaspatana* (leaf tea form) had shown promising hypolipidemic effect in humans, so it is worthwhile to study the mechanism of action of *M. maderaspatana* as hypolipidemic agent in HFD rats. In this research investigation, we evaluated the hypolipidemic activities of *M.maderaspatana* in high fat diet fed rats, with reference to Navaka Guggulu as hypolipidemic drug for data comparison.

Methods

Preparation of the Extracts:

Dried aerial parts of plant *M. maderaspatana* were purchased from local herbal market of Chennai, India and were authenticated by Mr. D. Narayanappa (Retd. chief Botanist, TAMPCOL Chennai). The voucher specimen (SH/MM/02) has been kept in the department of pharmaceutics Banaras Hindu University, Varanasi for future references. Whereas, aqueous extracts of *M. maderaspatana* was prepared separately by boiling the powdered material with distilled water, concentrated and dried. The dried extract was formulated as suspension in distilled water by using 2% Tween 80.

Preparation of high fat diet:
For the preparation of high fat diet wheat flour (52.6%), milk powder (23.2%) and cholesterol (2.5%) were mixed well. Thereafter, yeast powder (3.5%), sodium chloride (1.2%) and water were added to the above mixture. From this dough, the pellets of high fat diet were prepared using a manually operated pelletizing machine. These pellets were baked at 100^0C for three hours and stored in air tight container [20].

Animals:
Pharmacological experiments were carried out on albino rats of either sex (Charles Foster strain) were bought from the Central Animal House (Reg.No. 542/02/ab/CPCSEA), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The experiments were conducted according to the norms of committee for the purpose of control the supervision of the experiments in Animals (CPCSEA) New Delhi India, and Institutional Animal Ethical Committee (IAEC).The body weight of animals ranged between 150-180 g. The rats were housed in polypropylene cages at an ambient temperature of 25 ± 2^0C and 55-60% relative humidity. The animals were given commercially available rat feed (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum.* The animals were divided in to following six groups with each group containing 6 rats. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of six animals). Each animal was used only once. The experiments were conducted between 9:00 am and 4:00 pm.

Group I (control) **–** normal diet
Group II – High fat diet
Group III – High fat diet + navaka guggulu (400 mg kg^{-1};po)
Group IV – High fat diet + *M. maderaspatana* (500mg kg^{-1};po)
Group V – High fat diet + *M. maderaspatana* (1g kg^{-1};po)
Group VI – High fat diet + *M. maderaspatana* (2g kg^{-1};po)

High fat diet model
The diet was started with 9 g/rat/day and experiment was started when the rats have consumed 15 g/rat/day of diet and the schedule was followed till the completion of the experiment. In experimental evaluation one group of rats received a normal diet and served as a control. Whereas pellets of high fat diet were given for a period of 7 weeks to the rats groups II, III, IV, V and VI. Navaka guggulu churna (400 mg kg^{-1}; po) and aqueous extract of *M. maderaspatana* in three different doses (500 mg, 1g, 2g kg^{-1}; po) were administered to rats of group III, IV, V, and VI, respectively [4]. Body weight of animals were measured after the duration of 3, 5 and 7^{th} weeks during the experiment.
After 7 weeks, the blood was collected from all of the rats through retro orbital venous plexus under light anesthesia. The plasma was separated and processed for the estimation of plasma lipids. Finally animals were sacrificed; the livers were dissected out and used for histopathological studies. The plasma lipids *i.e.* total cholesterol, HDL, LDL, VLDL, triglycerides (TG) and Atherogenic index (AI) were determined by using commercially available diagnostic kits [21-22]. The lipid peroxidation level in the liver was also estimated [23].

Statistical Analysis:
Results were expressed as mean ± SEM by using two- way ANOVA followed by Dunnett□s multiple comparisons test *versus* HFD, $P < 0.001$ implies more significant.

Results and discussion

Feeding the high fat diet (HFD) to the animals for 7 weeks was found to increase the body weight (Table 1), plasma cholesterol, triglycerides, lipoproteins (HDL and LDL) and atherogenic index (Table 2) levels in rats. Hepatic LPO was also increased in HFD rats (Table 2). Administration of aqueous extract of *M. maderaspatana* (2g kg^{-1};po) along with the high fat diet to rats showed a significant decrease in body weight, plasma cholesterol, triglycerides and lipoproteins. However no significant changes were observed in the atherogenic index.

Table 1: Changes in the body weight of normal, hyperlipidemic, and treated group of rats.

Groups	% Increase in the body weight		
	After 3 weeks	After 5 weeks	After 7 weeks
Normal rats	7%	16%	18%
HFD	14%	20%	32%
Navaka Guggulu	11%	17%	23%
Aq. ext. *M. maderaspatana* (500 mg kg^{-1}; po)	13%	21%	28%
Aq. ext. *M. maderaspatana* (1 gm kg^{-1}; po)	15%	19%	25%
Aq.ext. *M. maderaspatana* (2 gm kg^{-1}; po)	20%	24%	29%

After this study the decrease in hepatic LPO level was observed as compare to HFD treated rats. This lipid lowering effect of this Aq. extract may either be due to the inhibition of hepatic cholesterogenesis or catabolic conversion of cholesterol to bile acids in liver. The presence of *β*-sitosterol and few sterol compounds in the leaves of *M. maderaspatana* has been already reported [18]. The structure of *β*-sitosterol is similar to the cholesterol except for the substitution of an ethyl groups at C_{24} of its side chain and it is a cholesterol lowering agent [24]. *β*-sitosterol reduced absorption of cholesterol by 42% in a meal containing 500 mg of cholesterol [25]. Therefore, *β*-sitosterol may be a bioactive phyto-constituent in the leaves of *M. maderaspatana* which may decrease the plasma cholesterol by increasing the LDL receptor activity. The level of HDL-cholesterol increased after the administration of Aq. Extract of *M. maderaspatana* (2g kg^{-1}; po), might be due to the increase in the activity of lecithin acyl transferase, which may contribute to the regulation of blood lipids [26].

Table 2: Effect of aqueous extract of *M. maderaspatana* on high fat diet hyperlipidemic rats

S. NO.	Plasma cholesterol (mg dl^{-1})	HDL (mg dl^{-1})	Triglyceride (mg dl^{-1})	LDL (mg dl^{-1})	VLDL (mg dl^{-1})	AI %	Liver LPO (n moles gm^{-1})
Normal	141.60 ± 0.622	53.81 ± 1.22	52.46 ± 1.91	77.30 ± 0.565	10.47 ± 0.850	2.63 ± 0.822	370.01 ± 3.25
HFD	312.54 ± 2.09	111.50 ± 0.889	202.05 ± 0.747	160.63 ± 5.07	40.40 ± 0.748	2.80 ± 0.137	1138.44 ± 2.30
Navaka guggulu (400mg kg^{-1})	189.25* ± 3.46	82.01* ± 1.22	100.08* ± 0.521	87.23* ± 3.63	20.01* ± 0.520	2.30 ± 0.26	688.74 ± 3.37
M. maderaspatana (Aq. Extract) (500mg kg^{-1})	258.65• ± 0.708	61.02* ± 1.78	166.28 ± 3.76	164.37 ± 0.636	33.25* ± 1.68	4.23 ± 0.577	983.01 ± 3.59
M. maderaspatana (Aq. Extract) (1 gm kg^{-1})	213.82* ± 9.27	64.77* ± 2.03	141.34* ± 2.83	120.78Δ ± 7.17	28.26* ± 1.26	3.30 ± 0.648	870.98 ± 2.48
M. maderaspatana (Aq. Extract) (2 gm kg^{-1})	197.31* ± 4.27	73.84* ± 0.664	117.71* ± 4.42	99.92* ± 4.40	23.54* ± 1.97	2.67 ± 0.703	771.58 ± 1.96

*p< 0.001 compared with HFD

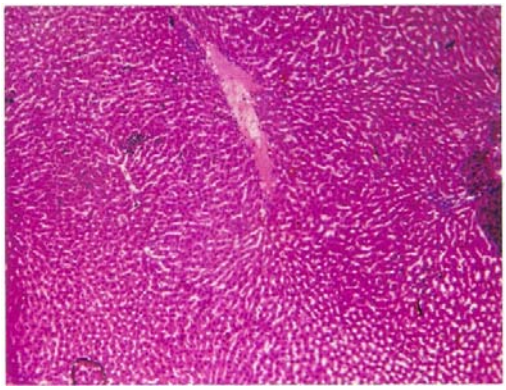
•P<0.01 compared with HFD

ΔP<0.05 compared with HFD

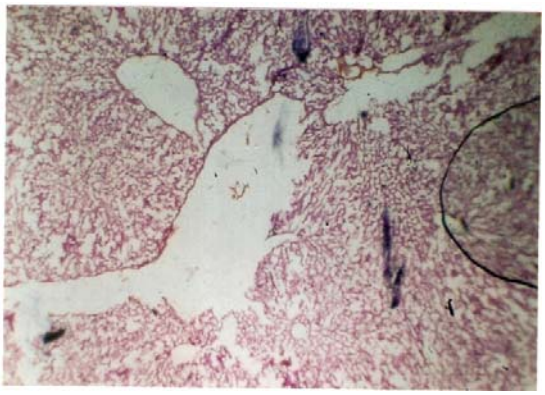
Results were expressed as mean ± SEM by using two- way ANOVA followed by Dunnett□s multiple comparisons test *versus* HFD, P <0.001 implies more significant.

Histological evaluation of the hepatic tissue (fig.1) revealed marked degenerative and fatty changes in the high fat diet fed animals. The animals treated with the aqueous extract of *M. maderaspatana* and Navaka guggulu showed micro vesicular fatty changes in the rat livers.

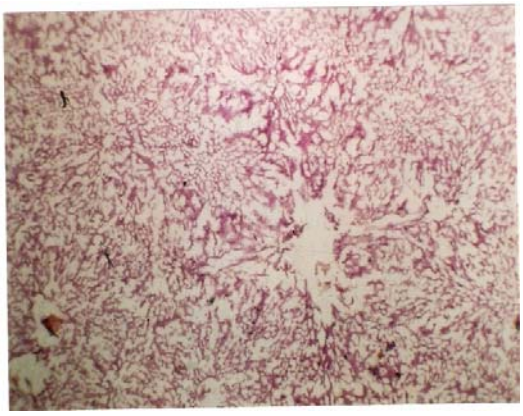
Figure 1: Histopathological studies in rats before and after treatment with Aqueous extract of *M. maderaspatana*



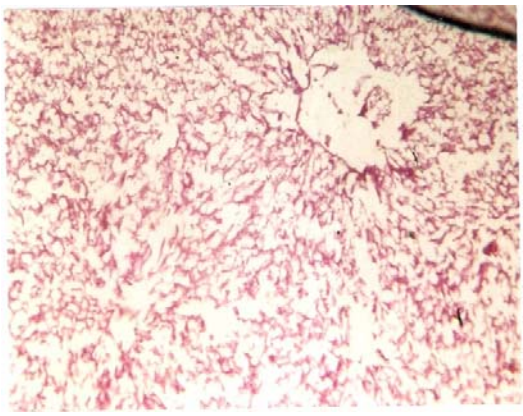
Normal rat liver



HFD treated rat liver



Navaka Guggulu (400mg kg^{-1},p.o.) treated rat liver



Aq.extract of *M.maderaspatana* (2gm kg^{-1}, p.o.) treated rat liver

Conclusions

Thus, considerable decline in the lipid level (hypolipidemic effect) has been investigated with aqueous extract of whole plant of *M. maderaspatana*, in high fat diet fed rats. The results were found more comparable with the Navaka guggulu in high fat diet induced rats. However, further studies have been required to evaluate long term uses and adverse and beneficial effects of this plant extracts. The present study would provide strong pharmacologic basis for the traditional use of this plant.

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References

1. Stoney CM. Gender and cardiovascular disease a psychobiological and integrative approach article in press doi: 10.1111/1467-8721.01247.
2. Singh BB, Vinjamury SP, Der-Martirosian C, Kubik E, Mishra LC, Shepard NP, Singh VJ, Meier M, Madhu SG. Ayurvedic and collateral herbal Treatments for hyperlipidemia: A systematic review of randomized controlled trials and Quasi-experimental designs. Altern Ther Health Med 2007; 13(4):22-28.
3. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlipidemic effect of *Lagenaria siceraria* (Mol.) fruit extracts. Indian journal of experimental biology 2006; 44:905-909.
4. Wu J, Xia C, Meier J, Li S, Hu X Lala DS. The hypolipidimic natural products guggulosterone acts as an antagonist of the bile acid receptor. Mol Endocrinol 2002; 19:1990-1997.
5. Shaila HP, Udupa SL, Udupa AL. Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis. Int J Cardiol 1998; 67:119-24.
6. Anila L, Vijayalakshmi NR. Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats. Food Chem 2003; 83:569-574.
7. Adeyemi DO, Komolafe OA, Adewole SO, Obuotor EM. Anti Hyperlipidemic Activities of Annona Muricata (Linn) The Internet Journal of Alternative Medicine 2009; 7(1):30-41
8. Kumar V, Singh S, Khanna AK, Khan MM, Mahdi RCF, Saxena JK, Singh R, Singh RK. Hypolipidemic activity of *Anthocephalus indicus (kadam)* in hyperlipidemic rats. Med Chem Res 2008; 17:152-158.
9. Pande VV, Dubey S. Antihyperlipidemic activity of *Sphaeranthus indicus* on atherogenic diet induced hyperlipidemia in rats. International journal of green pharmacy 2009; 3(2):159-161.
10. Adeneye AA, Olagunju JA. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. In Wistar rats. Biology and Medicine 2009; 1 (1):1-10.
11. Chattopadhyaya R, Pathak D, Jindal DP. Antihyperlipidemic agents. A review. Ind Drugs 1996; 33:85-98.
12. Kiritikar KR, Basu BD. Indian medicinal plants. 2nd ed. Deharadun: International book distributers.1987; 2:910.

13. Publication and Information Directorate. The Wealth of India. New Delhi, C.S.I.R., 1962:336.
14. Kirtikar KR, Basu BD. Indian medicinal plants 2nd ed. Vol. III. International book distribution, New Delhi India, 1975:1161-1162.
15. Nadkarani KN. Indian Meteria Medica, prakashan Pvt. Ltd., Bombay, 820. The wealth of India, Publication and information Directorate, New Delhi, CSIR 1971:336.
16. Sinha BN, Sasmal D, Basu SP. Pharmacological studies on Melothria maderaspatana. Fitoter 1997; 68:75-78.
17. Sinha BN, Thanigavelan J, Basu SP, et al. Studies on Melothria maderaspatana (Linn). Cogn Anci Sci Life 1996;15: 238-240.
18. Raja B, Kaviarasan K, Arjunan MM, Pugalendi KV. *Melothria maderaspatana* leaf extract for treating hypertension. Alternative and Complementary Therapies 2005; 11(5):264-268.
19. Ramakrishanamacharya CH, Krishnaswamy MR, Rao RB. Anti-inflammatory efficacy of Melothria maderaspatana in active rheumatoid arthritis. Clin Rheumatol 1996; 15:214-215.
20. Yugarani T, Tab B, Teh M, Das NP. Effects of polyphenolic natural products on lipid profile of rats fed high fat diet. Lipids 1992; 27:181-186.
21. Friedwald WT, Levy RI, Fredricson DS. Estimation of concentration of low-density lipoprotein in plasma, without use of preparative ultracentrifuge. Clin Chem 1972; 18:499-502.
22. Mathew BC, Daniel RS, Augusti KT. Hypolipidimic effect of garlic protein substituted for casein in diet of rats compared to those of garlic oil. Indian J Exp Biol 1996; 34:337-340.
23. Okhawa H, Ohishi N, Yogi K. Assay of lipid peroxide in animal tissue by thiobarbutaric acid reactions. Anal Biochem 1979; 95:351-358.
24. Lees Am, Mok HYI, Lees RS, Mc Cluskey MA, Grundy SM. Plant sterol as cholesterol loaring agents: Clinical trials in patients hypercholesterlemia and studies of sterol balance. Atherosclerosis 1977; 28: 325-338.
25. Mattson FH, Grundy SM, Crouse JR. Optimizing the effect of plant sterols on cholesterol absorption in man. American Journal of Clinical Nutrition 1982; 35:697-700.
26. Balaraman AK, Singh J, Dash S, Maity TK. Antihyperglycemic and hypolipidemic effects of *Melothria maderaspatana* and *Coccinia indica* in streptozotocin induced diabetes in rats. Saudi Pharmaceutical Journal 2010; 18:173-178.