

**ANTIHYPERLIPIDEMIC ACTIVITY OF CAMELLIA SINENSIS AND MACROTYLOMA UNIFLORUM ON HIGH FAT DIET INDUCED WISTAR ALBINO RATS**

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Corresponding author e-mail: dhanadlx@gmail.com*ABSTRACT**

Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of the various lipid and lipoprotein fractions, which is the key risk factor for cardiovascular disorders (CVD). *Camellia sinensis* and *Macrotyloma uniflorum* used as antihyperlipidemic drugs as per literature review. The aim of the present study was to evaluate the antihyperlipidaemic activity of polyherbal formulation of methanolic extract (*Camellia sinensis* and *Macrotyloma uniflorum*) in High fat diet (HFD) fed rats. Male Wistar albino rats were randomly assigned to five groups: Groups I normal control; Group II HFD control; Group III HFD+ standard drug (Atorvastatin 10 mg/kg), Group IV HFD + Polyherbal methanolic extract (200 mg/kg) and Group V HFD + Polyherbal methanolic extract (400 mg/kg). The whole study lasted for 28 days. Administration of HFD caused a significant ($p < 0.05$) rise in the serum total cholesterol (T.C), LDL-cholesterol, VLDL-cholesterol, triglycerides (T.G). Simultaneous administration of polyherbal methanolic extract of *Camellia sinensis* and *Macrotyloma uniflorum* significantly ($p < 0.05$) prevented the rise in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides. There was a significant decrease in body weight and increase in HDL-cholesterol was observed in polyherbal extract treated rats. Thus, the results indicate antihyperlipidaemic effect of Polyherbal formulation.

Key words: hyperlipidemia, High fat diet, Total cholesterol, LDL-cholesterol, Atorvastatin.**INTRODUCTION**

Dyslipidemia usually involve elevated plasma levels of triglycerides, total cholesterol, LDL- cholesterol and VLDL cholesterol and a low level of HDL-cholesterol^[1]. These lipoprotein abnormalities are held to be responsible for considerable cardiovascular disease-related morbidity and mortality. Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs^[2]. In spite of the availability of a number of drugs for the treatment of hyperlipidemia, antihyperlipidemic therapy

is still deprived of the efficiency, safety and finally “cost”. For example, there is risk of severe muscle damage with statins, which are particularly well suited for lowering LDL^[3]. Niacin a good drug for lowering triglycerides, may cause hyperglycemia and may also cause liver damage^[4]. Adverse reaction of Achilles tendon xanthomas have been reported after the addition of niacin and bile acid sequestrants to ongoing statin therapy in patients of hypercholesterolemia^[5]. Adverse effects due to the use of fibrates often relate to the skeletal muscles, kidneys or liver. Fenofibrate induced rhabdomyolysis which was complicated with acute renal

failure [6]. Thus, there is still need for development of better antihyperlipidemic agents. Herbs constitute a major part in all traditional systems of medicines. Because they have fitted the immediate personal need, are easily accessible and inexpensive. Plant products are frequently considered to be less toxic and free from side effects than synthetic ones. 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. There are number of plants reported to possess antihyperlipidemic activity in clinical studies such as *Allium sativum* [7], *Commiphora mukul* [8], soy or Glycine max [9], *Nigella sativa* [10], and *Plantago ovate* [11].

In the literature survey, it was found that flavonoids, phytosterols, tannins, and saponins shown promising effects to tackle dyslipidemia by various mechanisms [12]. It was reported that leaves of *Camellia sinensis* possess antioxidant, antidiabetic [13] activities and legume of *Macrotyloma uniflorum* possess antihyperlipidemic activities [14], But effect of combined extract on hyperlipidemic has not been explored. So the aim of my study was to explore *Camellia sinensis* and *Macrotyloma uniflorum* – combined extract on high fat diet induced hyperlipidemia in rats.

MATERIALS AND METHODS

Plant material: Dried leave of *Camellia sinensis* and legumes of *Macrotyloma uniflorum* was procured from Madhavachetti botanical garden, Thirupathi and was authenticated by Dr. K. Madhavachetti, Assistant Professor in Department of Botany at Sri Venkateswara University, Tirupathi.

Plant extracts preparation: The dried leaves are made into powder and then gone

for the Maceration with sufficient quantity of methanol for 7 days. During maceration, it was shaken twice daily. On 7th day it was filtered and the filtrate was concentrated. The remaining solvent was evaporated by heating on a water bath (50°C) to get methanolic extract and the extract was stored in desiccator.

Experimental Animals: Albino mice of either sex weighing between 16 - 25 g were procured from **albino research & training institute** for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry conditions.

Room temperature	-	26 ± 2 ⁰ C
Relative humidity	-	45 - 55%
Light/ dark cycle	-	12 : 12 h

The animals were fed with a standard diet purchased from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli, Maharashtra, India. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed as per the guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Approval Number: 1722/RO/ERe/S/13/CPCSEA.

Acute Toxicity Studies: Acute toxicity studies for the methanolic extract of polyherbal formulation were conducted as per OECD guidelines 423^[15] using Sprague–Dawley rats. Each animal was administered the methanolic extract of polyherbal formulation by oral route. The animals were observed for mortality up to 72 hours. The methanolic extract of polyherbal formulation was found to be safe up to 2000mg/kg body weight.

Preparation of High Fat Diet: Lard (275 g kg-1), casein (200 g kg-1), cholesterol (10 g kg-1), vitamin and mineral mix (60 g kg-1), dl-methionine (3g kg-1), sodium chloride (2

g kg⁻¹) and sucrose (150 g kg⁻¹) were added to normal diet as described by Srinivasan et al., 2005^[16].

Experimental Protocol: Thirty animals were divided into 5 groups with 6 animals per group. Group A receives standard pellet diet while Group B, C, D and E receive high fat diet followed by respective treatment with plant extract and standard drug as given below:

Group A: (Normal diet): Normal diet (15 g/day/rat) + Drug vehicle (1 ml/kg).

Group B: (HFD control): HF diet (15 g/day/rat) + Drug vehicle (1 ml/kg).

Group C: HF diet (15g/day/rat) + Methanolic Polyherbal Extract 200mg/kg.

Group D: HF diet (15g/day/rat) + Methanolic Polyherbal Extract 400mg/kg.

Group E: HF diet (15g/day/rat) + Atorvastatin (10 mg/kg).

The herbal extracts and standard drug atorvastatin were administered once daily as a single oral suspension for 28 days. The oral suspension was prepared with 0.5 % w/v carboxymethyl cellulose (CMC). All the experimental procedure was carried out between 7:30 am to 10:00 am. The standard and test substance were administered between 8:00 am to 9:00 am. At the end of 28 days, rats were fasted for 16 hrs, anaesthetized with ether by inhalation, blood was collected via retro orbital puncture and serum analyzed for lipid profile.

Biochemical Analysis of Serum: Serum samples were analysed for total cholesterol, high-density lipoproteins and triglyceride levels using standard enzymatic assay kits: Total cholesterol Kit, HDL-Precipitating RGT Kit, Triglyceride Kit. Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were calculated according to Friedwald formula^[17].

$LDL = TC - HDL - VLDL$

$VLDL \text{ cholesterol} = \text{Triglycerides} / 5.$

Statistical Analysis: Data were analyzed using one way ANOVA method using trail version of Graph Pad Prism 5 by Dunnet t test and $p < 0.05$ was considered significant. Values are expressed as Mean \pm SEM for six rats per group.

RESULTS AND DISCUSSION

Body weight: High fat diet group showed significant ($p < 0.05$) increase in body weight as compared to control group. The administration of methanolic Polyherbal extract prevented the gain in average body weight as compared to the HFD group ($p < 0.05$).

Effect of Polyherbal Formulation on Lipid Profile in Serum and Liver of High Fat Diet Induced Hyperlipidemic Rats:

Oral administration of methanolic extract of polyherbal formulation (200 mg/kg and 400mg/kg, p.o.) significantly reduced the serum cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), VLDL-cholesterol levels, but significantly increased serum HDL-cholesterol level as compared with high fat diet (HFD) treated rats.

DISCUSSION

Obesity is a major risk factor for augmented morbidity and mortality and is associated with various medical ailments^[18]. High fat diet-induced obesity has been considered as the most popular model among researchers due to its high similarity of mimicking the usual route of obesity episodes in human^[19] and so why it is considered as a reliable tool for studying obesity as they will readily gain weight when feed high-fat diets^[20]. Other studies have revealed that HFD promote hyperlipidemia^[21] and hyperglycemia^[22].

Thus, High-fat diets can be used to generate a valid rodent model for the analysis of the pathophysiology of dyslipidemia [23-25]. Therefore, in this study, high fat diet-induced dyslipidemia rat model was used to examine the combined effects of *Camellia sinensis* and *Macrotyloma uniflorum* dietary induced lipoprotein changes.

Increase in body weight and fat deposition is the chief indicators for the gradual progress of obesity. As the animals were fed with HFD, there was an increase in the adiposity, which in turn increased the fat cell mass. Thus, there was an overall increase in body weight. The increased body weight found in HFD rats might be due to the consumption of a diet rich in energy, in the form of saturated fats (lard) and its deposition in various body fat pads [26]. However, a significant decrease in body weight and was observed in polyherbal formulation treated rats.

It is well known that dyslipidemia is a highly predisposing condition for arteriosclerosis and other cardiovascular disease [27]. It is well documented that elevated total cholesterol and LDL-cholesterol levels promote atherosclerosis and cardiovascular complications [28].

The present study result supports the hypolipidemic property of polyherbal formulation. Findings of lipid profile of polyherbal formulation treatment in all the doses showed a significant decrease in triglyceride, total cholesterol, VLDL & LDL and significant increase in HDL values at 200 mg/kg and 400 mg/kg when compared with control group over a period of 28 days treatment. The methanoli extract of polyherbal formulation shows significant increase in HDL level and decrease in LDL, VLDL levels. Increase in LDL, VLDL levels are increase the risk of cardiovascular

diseases. Increases of HDL have cardioprotective effect and it was proved by various studies. [29-30].

CONCLUSION

Result of present study revealed that the leaves of methanolic polyherbal extract (*Camellia sinensis* and *Macrotyloma uniflorum*) improved the serum lipid profile in rats by decreasing serum TC, TG, LDL, VLDL and increasing serum HDL. This finding provides some biochemical basis for the use of leaves extract of *Camellia sinensis* and *Macrotyloma uniflorum* as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. Further, studies are required to gain more insight in to the possible mechanism of action.

Acknowledgments

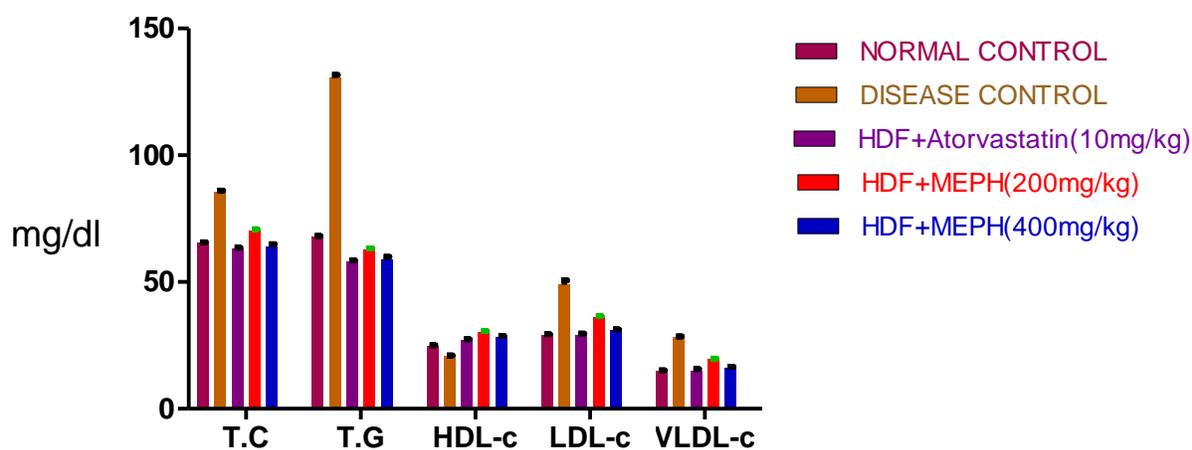
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Table 1 : Effect of methanolic polyherbal extract (MPHE) on animal Bodyweight

TREATMENT	T.C	T.G	HDL-c	LDL-c	VLDL-c
NORMAL CONTROL	65.32±0.3522	67.65±0.5527	24.87±0.2286	29.09±0.3532	14.87±0.3125
HDF CONTROL	85.32±0.7099	130.7±0.9670	20.65±0.3883	48.98±1.719	28.12±0.4885
ATORVASTATIN 10MG/KG	62.98±0.5383	57.89±0.7260	15.76±0.3973	28.98±0.6673	15.01±0.6643
MPHE (200Mg/Kg)	70.04±0.7742	62.76±0.5494	30.98±0.5996	35.98±0.5549	19.67±0.1734
MPHE (400Mg/Kg)	64.09±0.8967	58.76±1.199	52.98±0.7141	30.87±0.4753	16.16±0.3512

Table 2: Effect of methanolic polyherbal extract (MPHE) on lipid profile in HCD induced hyperlipidaemic rats.

TREATMENT	Initial Body Weight (gm)	Final Body Weight (gm)
NORMAL CONTROL	240.25 ± 2.63	245.23 ± 3.74
HDF CONTROL	230.92 ± 0.785	302.19 ± 1.38
ATORVASTATIN 10MG/KG	229.45 ± 0.68	234.76 ± 0.81
MPHE (200Mg/Kg)	228.68 ± 0.5498	232.08 ± 0.3764
MPHE (400Mg/Kg)	230.39 ± 1.79	235.68 ± 1.47

**Fig.No.1: Effect of methanolic polyherbal extract (MPHE) on lipid profile in HCD induced hyperlipidemic rats.**

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