ORIGINAL ARTICLE

Antihyperlipidemic activity of ethanolic extract of *Macrotyloma uniflorum* (Lam.) Verdc. leaves and stems on high-fat diet-induced hyperlipidemia

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Abstract

Background: Hypercholesterolemia is the state depicted by expanded greasy substances called lipids, rise in plasma total cholesterol (TC) and triglyceride (TG) levels, it is additionally called hyperlipoproteinemia. **Materials and Methods:** The present study was designed to investigate the hypolipidemic effect of ethanolic extract of *Macrotyloma uniflorum* leaves and stems (EMULS) in high-fat diet (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal laboratory feed) induced hyperlipidemia. EMULS was administered in three doses of 100 mg/kg/day *p.o*., 200 mg/kg/day *p.o*, and 400 mg/kg/day *p.o* each for 30 days. **Results:** Simultaneous administration of EMULS significantly ($P < 0.01$) prevents the rise in serum levels of Total Cholesterol (TCs), Triglycerides (TGs), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) whereas significant ($p<0.05$) increases in the level of High Density Lipoprotein Cholesterol (HDL-C). **Conclusion:** Based on the results we conclude the use of leaves and stems extract of *Macrotyloma uniflorum* has preventive and curative effect against hyperlipidemia.

Key words: Ethanolic extract of *Macrotyloma uniflorum* leaves and stems, high fat diet, hypercholesterolemia

INTRODUCTION

Hyperlipidemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart diseases (CHD).[1] Atherosclerosis is the most common cause of mortality and morbidity worldwide. Although several factors, such as diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle, play a significant role in causing heart failure, the high levels of cholesterol, particularly total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) cholesterol, are mainly responsible for the onset of CHDs. About 20% reduction of blood cholesterol level can decrease about 31% of CHD incidence and 33% of its mortality rate.[2-6]

Lipids play out some significant capacities in body. In addition, hyperlipidemia is induced by secondary effect of diabetes; therefore, the agent having some antioxidant and antidiabetic effect also showed favorable effect to hyperlipidemia. 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG Co–A) reductase inhibitor has been used in the treatment of hyperlipidemia, and atorvastatin is one of the most prevalently used HMG-CO A reductase inhibitors.[7-10]

Horse gram is an incredibly dry season safe yield. Warm and dry climatic conditions are appropriate for its ideal development. It can be developed up to a height of 1000 m over the sea level. The temperature range of 25–30°C and relative humidity in the range of 50–80% are the required conditions for its growth. The main constituents that were identified are reported as mome inositol, ethyl D-glucopyranoside,

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n-hexadecanoic acid, linoleic acid, its esters and ethyl derivatives, Vitamin E, stigmasterol, and 3stimast-5-en-3-ol. Hence, the plant can be medicinally useful as an antioxidant and also in diabetes and other disorders.[11,12]

Macrotyloma uniflorum (Fabaceae) are packed with powerful plant pigments called anthocyanins which are believed to be lowering the blood sugar levels. *M. uniflorum* are one of the richest sources of antioxidant substances that seek out and destroy free radicals, harmful molecules that circulate in the body. The chemicals are also found in red grapes, strawberries and sour cherry may acquire new interest, and mainly due to the fact that it can be considered as a "functional food" because of its high content of antioxidant compounds.[13-15]

However, the biggest insulin effects seem to come from the type of alkaloids, tannins, phenols, flavonoids, and glycosides in the plant. The consumption of these may play a significant role in preventing lifestyle-related diseases such as cancer, diabetes, and cardiovascular and neurological diseases. *M. uniflorum* contain various phytochemicals such as anthocyanins and phenols. Anthocyanins are flavonoid pigments in many plants as well as in *M. uniflorum*.[16,17] Cyanidin is the major aglycone in *M. uniflorum* and its glycosylated form provides the anthocyanins. All anthocyanins are derivatives of the basic flavylium cation structure. These anthocyanins have been found to be antioxidants of lipids, particularly in foods inhibit cyclooxygenase enzymes. Cyanidin was intermediate in the efficacy between aspirin and the nonsteroidal anti-inflammatory drug, Flurbiprofen. The anthocyanins on heating destroy their effectiveness.

Based on this information, the present study was designed to investigate the antihyperlipidemic effect of the extract of *M. uniflorum* leaves and stems (EMULS) on serum lipid and lipoprotein profile in High Fat Diet (HFD)-induced hyperlipidemia.

MATERIALS AND METHODS

Procurement and Authentication of Plant

The leaves and stems of *M. uniflorum* were collected from Guntur, Andhra Pradesh. The plant material was identified and authenticated by Dr. K. Madhava Chetty, Plant Taxologist, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Preparation of Plants Extract

The leaves and stems of *M. uniflorum* were collected and washed thoroughly with distilled water to make sure the leaves and stems are free of dust and are shade dried. The dried leaves and stems are then powdered finely using mechanical grinder. And then, required quantity of the powder is subjected to solvent extraction with ethanol. The obtained extract is dried completely using desiccators.[15]

Animals and Treatment

Animals

Healthy male Sprague Dawley rats weighing between 250 and 300 g were procured and maintained in polypropylene cages at ambient temperature of 22 ± 1°C and relative humidity of 50–60% with a 12 h light/dark cycle in registered animal house. The animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals approved under the number (1048/a/07/CPCSEA), India, and approved by the Institutional Animal Ethics Committee. Throughout the experimental period, the animals were fed with standard pellet diet and water *ad libitum*.

Experimental Design

Animals were randomly divided into six groups containing six ($n = 6$) animals each and treated as explained in Table 1.

Body weights were recorded once a week throughout the experimental study. At the end of each week, blood samples were collected from the retro-orbital plexus. Blood samples were kept aside for approximately 1 h at room temperature and centrifuged at 2500 rpm at 4°C for 15 min to separate the serum from blood. The serum samples were used for the estimation of biochemical parameters such as TC, high-density lipoprotein (HDL), LDL, very LDL (VLDL), and TG.

At the end of the study, animals were sacrificed by cervical dislocation and cut open to isolate liver and weighed immediately. Then, liver was fixed in 10% Formalin and was used for histopathological study.

Table 1: Treatment groups

Name of the group	Treatment
Normal	Received standard pellet diet for 30 days
HFD	Received HFD for 30 days
HFD+standard atorvastatin	Received HFD+standard atorvastatin (20 mg/kg b.wt, *p.o*) for 30 days
HFD+test dose 100 mg/kg	Received HFD+100 mg mg/kg b. wt, *p.o* of plant extract for 30 days
HFD+test dose 200 mg/kg	Received HFD+200 mg mg/kg b. wt, *p.o* of plant extract for 30 days
HFD+test dose 400 mg/kg	Received HFD+400 mg mg/kg b. wt, *p.o* of plant extract for 30 days

HFD: High-fat diet

Statistical Analysis

The results were expressed as Mean ± S.D (standard deviation). Statistical analysis was calculated using One-way Analysis of Variance (ANOVA) followed by *post hoc* Dunnett's test for multiple comparisons and statistical significance was set at $P < 0.05$. Values are represented as mean ± SEM; a (***) – $P < 0.0001$, b (**) – $P < 0.01$, and c (*) – $P < 0.05$.

RESULTS

Estimation of Serum Biochemical Parameters

The estimated values of biochemical parameters in all the experimental rats before and after the treatment period are summarized in Tables 2 and 3 and Figures 1 and 2.

Histopathological Findings

Histopathological changes in liver of HFD-induced hyperlipidemia after 30 days treatment are shown in Figure 3.

DISCUSSION

Hyperlipidemia, a well-known risk factor for cardiovascular disease, especially atherosclerotic coronary artery disease, is one of the major causes of premature death globally and it is expected to be the most important cause of mortality in India by the year 2020.[18]

It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Several animal and human studies have confirmed the hypercholesterolemia properties of saturated fatty acids and cholesterol which include increasing TC and altering lipoprotein pattern and whose mechanisms remain under study. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models.[1,2,19]

Rats fed with a diet supplemented with cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal laboratory feed for 30 days served as the experimental model. This is in accord with the previous finding reported by Yoon *et al*., 2008, who showed that feeding rats with high cholesterol diet (HCD) for 7 days induced hyperlipidemia. Similar results have been reported by Arafa, 2005, feeding rats with an HCD for 7 consecutive

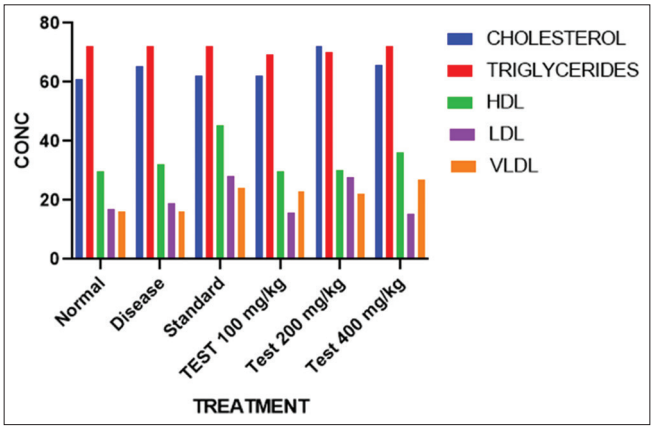

Figure 1: Blood parameters (total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein), body weight, before disease-induced hyperlipidemia

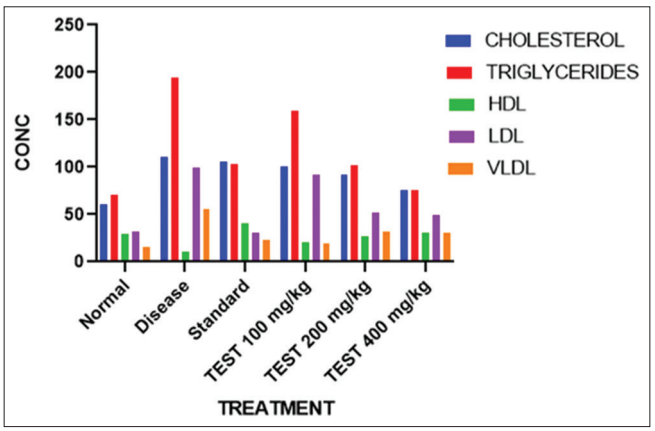

Figure 2: Effect of the extract of *Macrotyloma uniflorum* leaves and stems on various lipid parameters after the 30th day of treatment

Table 2: Blood parameters (TC, TG, HDL, LDL, and VLDL) and body weight, before disease induced hyperlipidemia in experimental rats

Group	Body weight	TC	TG	HDL	LDL	VLDL
Normal	152±0.12	61.12±2.8	72.25±1.9	29.65±1.6	17.02±2.4	16.08±3.7
HFD	150+1.12**	65.59+1.5**	72.12±1.0**	32.30±1.3**	18.88±3.2**	16.12±1.2**
HFD+atorvastatin 20 mg/kg	156±0.36*	62.32±3.8*	72.12±1.9*	45.25±2.3*	30.36±1.5*	24.23±1.5*
HFD+test dose 100 mg/kg	160±2.35*	62.32±1.6*	84.32±3.5*	29.68±1.9*	15.77±2.3	23.03±2.6**
HFD+test dose 200 mg/kg	158±1.22*	72.23±1.5*	70.1±2.3*	30.25±1.5*	27.96±2.9	22.6±2.06*
HFD+test dose 400 mg/kg	162±0.66*	65.75±1.2*	72.8±2.6*	36.2±2.1*	15.31±1.8*	27.1±2.3*

TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein

Table 3: Effect of EMULS on various blood parameters (TC, TG, HDL, LDL, and VLDL), after the 30th day of treatment

Group	TC	TG	HDL	LDL	VLDL
Normal	61.05 ± 0.5	71.06 ± 3.4	29.06 ± 2.6	32.30 ± 0.9	16.07 ± 1.3
HFD	110.02 ± 2.3**	194.04 ± 0.2**	11.08 ± 0.2**	99.14 ± 2.6**	56.4 ± 0.2**
HFD + atorvastatin (20 mg/kg)	105.03 ± 1.4*	103.0 ± 1.0*	40.46 ± 2.6**	30.32 ± 3.4*	23.08 ± 2.0*
HFD + test dose 100 mg/kg	101.06 ± 2.3*	159.4 ± 0.3**	20.14 ± 2.3	92.26 ± 0.1*	19.03 ± 1.2*
HFD + test dose 200 mg/kg	92.0 ± 0.6*	102.7 ± 0.9*	27.03 ± 2.6	52.14 ± 0.9*	32.06 ± 2.4*
HFD + test dose 400 mg/kg	76.04 ± 2.0*	76.03 ± 0.9*	30.06 ± 1.4	49.14 ± 0.9*	30.01 ± 2.6*

TC: Total cholesterol, TG: Triglyceride, EMULS: Extract of *Macrotyloma uniflorum* leaves and stems, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein

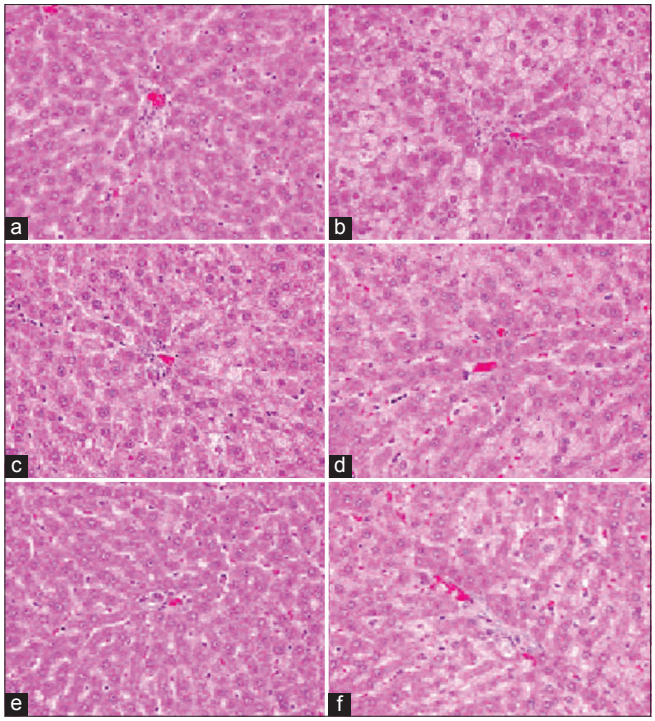

Figure 3: Effect of extract of *Macrotyloma uniflorum* leaves and stems and other treatment groups on histopathology of liver after the 30th day of treatment. (a) Liver of rat from Normal control group showing the normal histopathological structure of hepatocytes (H and E ×200). (b) Liver of rat from disease control group showing the fat deposits in histopathological structure hepatocytes (H and E ×200). (c) Liver of rat from standard control group showing almost normal histopathological structure of hepatocytes treated with standard drug atorvastatin (20 mg/kg) (H and E ×200). (d) Photomicrograph of liver section of rat subjected to hyperlipidemia and treated with low dose (100 mg/kg) of the extract of *Macrotyloma uniflorum* leaves and stems (EMULS) for 30 days showing necrosis and fat deposits (100 mg/kg) (H and E ×200). (e) Photomicrograph of liver section of rat subjected to hyperlipidemia and treated with medium dose (200 mg/kg) of EMULS for 30 days showing very few effects of necrosis and fat deposits. (f) Photomicrograph of liver section of rat subjected to hyperlipidemia and treated with high dose (400 mg/kg) of EMULS for 30 days showing significant effect with almost normal hepatocytes (h) but with necrosis (n)

days resulted in marked hypercholesterolemia. Furthermore, Varalakshmi *et al*., 2006, have demonstrated that feeding rats for 30 days an HCD increased the serum lipids. The mechanism of the action of HCD is twofold an increase in cholesterol absorption and a concomitant suppression of cholesterol 7α-hydroxlyase activity that results in decreased cholesterol excretion.[20] Cholic acid improves cholesterol absorption by its emulsifying property.

From obtained result, it was observed that keeping the animal on HFD significantly increased the TC, TG, and LDL-C level in serum ($P < 0.05$) as compared to rats on normal diet. When HFD was coadministered with EMULS, the elevated levels of TC, TG, and LDL-C condition have shown considerable decline. It was noted that TC, TG, and LDL-C lowering activity of EMULS (400 mg/kg) were more significant as compared to the other two lower doses. There was a significant elevation in plasma HDL- cholesterol (C) in EMULS-treated rats as compared to HFD rats, thus indicating the efficacy of EMULS in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Ample of evidence exists with respect to the fact that HDL-C is inversely related to total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis, leading to ischemic heart diseases, by impairing the clearing of cholesterol from the arterial wall.[5,18,21-23] Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats.[24,25] Flavonoids and polyphenols found in our EMULS could, therefore, be considered favorable in increasing HDL and decreasing LDL and VLDL in EMULS-treated rats.

Atorvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor. HMG-CoA reduces serum TG levels through the modulation of apolipoprotein C-III and lipoprotein lipase. Rats treated with atorvastatin showed marked reduction in all serum lipoproteins and increase in HDL level as compared with HCD group.[26,27]

CONCLUSION

The result of the present study revealed that the ethanolic extract of *M. uniflorum* leaves and stems improved the serum

lipid profile in rats by decreasing serum TC, TG, LDL-C, and increasing serum HDL-C. This finding provides some biochemical basis for the use of leaves and stems extract of *M. uniflorum* as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. Further studies are required to again more insight into the possible mechanism of action.

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