

Research Article

Anticholesteremic and Antilipidemic activity of Stem bark extracts of *Moringa oleifera* in Diet induced hyperlipidemia model in rats

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ABSTRACT

The present study was carried out to evaluate the anticholesteremic and Antilipidemic activity of ethanolic stem bark extracts of *Moringa oleifera* using Diet-induced hyperlipidemia model in Wistar albino rats. Albino Wistar rats of either sex weighing in between 200-350 gms were divided into 6 groups containing 6 animals in each group. All the groups received cholesterol (400mg/kg b.w. in 5ml coconut oil p.o) except saline control for 2 weeks and were tested orally at the dose of 150, 300 and 600 mg/kg body weight. The extract (300 mg/kg & 600 mg/kg) showed significant ($P < 0.05$) reduction in total serum cholesterol and lipid levels compared to vehicle control group. The serum Total cholesterol, Triglyceride, HDL, VLDL and LDL levels were analyzed. This present study proved that ethanolic stem bark extracts of *Moringa oleifera* shows significant Anticholesteremic and Antilipidemic activity against the Diet induced hyperlipidemia model by reducing the serum TC, TG, VLDL, LDL levels and increasing HDL level and insisting the use of *Moringa oleifera* stem bark extracts in traditional system of medicine.

Keywords: *Moringa oleifera*, Antilipidemic, Anticholesteremic TC, TG, HDL, VLDL, LDL.

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases¹. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death².

Hyperlipidemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease. The treatment of hyperlipidemia depends on the patient's cholesterol profile. Many anihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezetimibe etc reduce cholesterol level with different condition³. Hyperlipidemia characterized by elevated serum total cholesterol, low density, very low density lipoprotein and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease⁴. Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated using anti-lipidemic drugs but the secondary type

originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia⁵. The Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (i.e., statins) profoundly lower LDL cholesterol⁶. It was then decided to resolve this claim by investigating the effects of the crude extract of stem barks of *Moringa oleifera*.

Moringa oleifera (Family: Moringaceae) is commonly known as drumstick. *Moringa*, Horseradish tree, Drumstick tree, Saijan etc. It is a slender, semi-deciduous, perennial tree that grows to about 10 meters tall, with drooping branches. Internally stimulant and antilithic agent. Bark is emmenagogue and abortifacient. Flowers are stimulant, tonic and diuretic and increase the flow of bile. The leaves are tasty, cooling; removes all kinds of excessive pain & inflammation. Used in Fattening, anthelmintic, cures hallucinations, dry tumors, hicough, asthma⁷. The root is bitter; tonic to the body and the lungs; emmenagogue, laxative, expectorant, diuretic; enriches the blood, cures stomatitis, gleet, urinary discharges, obstinate asthma, the roots and seeds are prescribed for treatment of snake bite (Charaka, Sushruta) and scorpion-sting. (Sushruta)⁷. The root is used

for soreness of mouth and throat; and gum for dental carries. Seeds are used in venereal infections in Sind⁷. There are some reports on Anticholesteremic and Antilipidemic activity of this plant. *Moringa oleifera* Lam. plant is claimed to possess cholesterol-reducing effect and is used to treat patients with heart disease and obesity.

MATERIALS AND METHODS

Collection of plant materials

The stem barks of *Moringa oleifera* were collected in June 2011 from different localities of Mangalore district, Karnataka state. The plant materials authenticated by botanist Dr. Neoline J.Pinto, Professor & head of Department of botany, St. Agnes College, Mangalore. The stem barks were cut, washed with distilled water and dried in shade, pulverized by mechanical grinder to get course powder and stored in an airtight container.

Preparation of Plants Extracts

The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using ethanol solvent in a soxhlet extractor. The different extracts obtained were evaporated at 45°C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of alcoholic extract was found to be 45.50 %w/w and the ethanolic extract was used for further studies⁸.

Preliminary phytochemical screening

The phytochemical examination of the *Moringa oleifera* extract was performed by the standard methods.^{9, 10}

Acute toxicity test LD₅₀^{11, 12}

Adult male and female wistar albino rats (6 - 8 weeks old) were weighed between 150 - 180 gm. The animals were given standard rat pellets and tap water and *libitum*. The acute toxic study was used to determine a safe dose for the stem bark extract. Eighteen rats (9 males and 9 females) were assigned equally each into 3 groups labelled as vehicle (CMC, 0.25% w/v, 5 ml/kg); 2000 and 5000 mg/kg of *Moringa oleifera* stem bark extract preparation, respectively. The animals were fasted overnight (water but not food) prior to dosing. Food was withheld for a further 3 to 4 h after dosing. The animals were observed for 30 min and 2, 4, 8, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The acute toxicity LD₅₀ was calculated as the geometric mean of the dose that resulted in 100%

lethality and that which caused no lethality at all.

Experimental Animals

Wistar albino rats of either sex weighing around 200-350gm were taken from inbreeds colony animals, which were housed in polypropylene cages under standard laboratory conditions (light period 7.00 A.M. to 7.00 P.M., 21±2 °C, and relative humidity 55%). The animals were given standard rat pellets and tap water *ad libitum*, but they were deprived of food 36 h before the experiments. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animal treatment^{13, 14, 15]}

A high fat / cholesterol supplement to normal diet was administered separately incorporated into diet pellets for 1-2 weeks (like 400 mg/kg cholesterol in 5ml coconut oil). The high fat diet was prepared by mixing calculated amounts of 2% cholesterol, 1% cholic acid and 1 mL coconut oil. The Parachute coconut oil was chosen because of its high saturated fat content which aggravates the atherogenic profile in the rats (Hassarajani et al., 2007) with slight modification. Hyperlipidemic control group was orally administered with vehicle (carboxymethyl cellulose, CMC, 0.25% w/v, 5ml/kg). The reference group received oral doses of 4 mg/kg simvastatin in CMC (5 ml/kg) as positive controls. Experimental groups were orally administered with 150, 300 and 600 mg/kg of ethanol extract of stem bark of *Moringa oleifera* in distilled water (5 ml/kg), respectively. The biochemical parameters (HDL, LDL, VLDL, TC and TG) have been investigated in serum after 2 weeks during the treatment with Simvastatin and alcoholic extract of the plant. Blood was withdrawn using the heparanised capillaries from the retro-orbital sinus in the overnight fasted animals. The serum was obtained after centrifuging the blood, which was used to estimate the concentration of biochemical parameters using the semi auto analyser and relevant lipid profile kits. Total cholesterol, triglyceride level, and were estimated from serum by CHOD-PAP method and GPO-PAP method and HDL by the precipitation method using phosphotungstate magnesium acetate reagent. LDL and VLDL-cholesterol were

calculated following the method by Johnson et al. (1997).

Thus obtained data's were tabulated and checked for the statistical tools like one way analysis of variance (ANOVA). Finally Dunnett's test was applied to find out difference between groups.

Statistical analysis

The values are represented as mean \pm S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the ethanolic extract of *M.oleifera* revealed that presence of alkaloids, flavonoids, terpenoids. (Table-1)

Acute toxicity study

An Acute toxicity study was carried out in which the animals were treated with the stem bark extracts at a dose of 2000 and 5000 mg/kg of *Moringa oleifera* and were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. There were no abnormal signs, behavioural changes, body weight changes, or macroscopic finding at any time during the observation period.

Diet induced hyperlipidemia

There was a significant increase in the serum cholesterol, triglyceride, LDL, VLDL and decrease in the levels of HDL in the high fat diet fed animals when compared to normal fed rats. Treatment with ethanolic extract, at three different doses, decreased the serum level of cholesterol, triglyceride, LDL and VLDL and increased the serum HDL levels as compared to hyperlipidemic control group. (Table-2), Figure: (1-5)

DISCUSSION

The present study was under taken to evaluate the Anticholesteremic and Antilipidemic activity of ethanolic extract of stem bark of *Moringa oleifera*. The study was conducted by using Diet induced hyperlipidemia model. The parameters used for the assessment of Anticholesteremic and Antilipidemic activity are Total Cholesterol, Triglycerides, High density Lipoproteins, Very Low Density Lipoproteins, and Low Density Lipoproteins.

Flavonoids may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the inco-

operation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells.

Saluja et al. (1978) reported isolating β -sitosterol from the stem of a hybrid variety of *Moringa oleifera* Lam. β -sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain^[16]. It is believed to lower cholesterol by lowering plasma concentrations of LDL (Kane and Malloy, 1982)¹⁷. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of sterols that results in decrease of body lipids.

We can suggest that it may be possible to use plant stem bark extracts as remedy to prevent hyperlipidemia. However, further investigations are required to elucidate their exact mechanism of action of Antihyperlipidemic activity.

CONCLUSION

The present study was under taken to assess the Anticholesteremic and Antilipidemic activity of ethanolic extracts of stem bark of *Moringa oleifera*. The ethanolic extracts of stem bark of *Moringa oleifera* showed marked decrease in the serum Total Cholesterol, Triglyceride, VLDL, LDL levels and increase in HDL level in diet induced hyperlipidemia model in a dose dependent manner. Ethanolic extracts of stem bark of *Moringa oleifera* at 600 mg/kg reduced the lipids and cholesterol significantly, thus showing the Anticholesteremic and Antilipidemic mechanism involved. So, ethonolic extract at 600 mg/kg exhibited almost equipotent effect as that of simvastatin and these results offer pharmacological evidence and support on the folkloric use of *Moringa oleifera* stem bark as an Anticholesteremic and Antilipidemic agent. (Table-2), Figure: (1-5)

It leads to the conclusion that the stem bark of *Moringa oleifera* can be utilized for its Anticholesteremic and Antilipidemic activity and in future, this work has been extended by including more hyperlipidemic models for meaningful and tangible conclusion. Toxicological studies can also be carried out to know about the toxic and non-toxic nature of the drug. Isolation and characterization of the phyto-constituents responsible for pharmacological activity can also be attempted in future.

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Table 1: Qualitative analysis of ethanolic extract of *Moringa oleifera*

S. No.	Test	Ethanolic Extract
1.	Alkaloids	
	a) Dragendroff's test	+ve
	b) Hager's test	+ve
	c) Wagner's test	+ve
2.	Carbohydrates	
	a) Molisch's test	-ve
	b) Benedict's test	-ve
	c) Fehling's test	-ve
3.	Flavonoids	
	a) Shinoda test	+ve
4.	Saponins	
5.	Tannins	
6.	Steroids	
	a) Libermann Burchard's test	-ve
7.	Proteins	
	a) Biuret test	+ve
8.	Triterpenoids	
	a) Libermann Burchard's test	+ve

Table 2: Effect of *Moringa oleifera* stem bark extract on various parameters in Diet induced Hyperlipidemia model

Treatment Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal	82.17±3.78	125.5±4.11	44.50±2.44	25.10±0.82	12.50±2.12
Control(HFD)	190.3±4.24	197.8±6.22	39.17±3.0	39.57±1.24	111.6±5.08
Ethanolic Extract 150mg/kg	183.8±3.87	188.8±3.27	41.67±1.33	37.77±0.65	104.5±4.44
Ethanolic Extract 300mg/kg	143.2±2.66**	165.8±4.16 *	47.83±2.12**	33.17±0.83 *	62.17±2.82 *
Ethanolic Extract 600mg/kg	113.2±4.51***	140.8±5.83 **	51.67±2.55***	28.17±1.16***	33.33±6.58**
Simvastatin (standard)	96.50±3.24***	128.0±3.14***	57.67±2.31***	25.60±0.62***	13.23±4.23***

Values are expressed as (mean±SEM) P***<0.001, P**<0.01, P*0.05 (When compared with hyperlipidemic control) P<0.001 (When compared with control). Values are expressed in mean±SEM

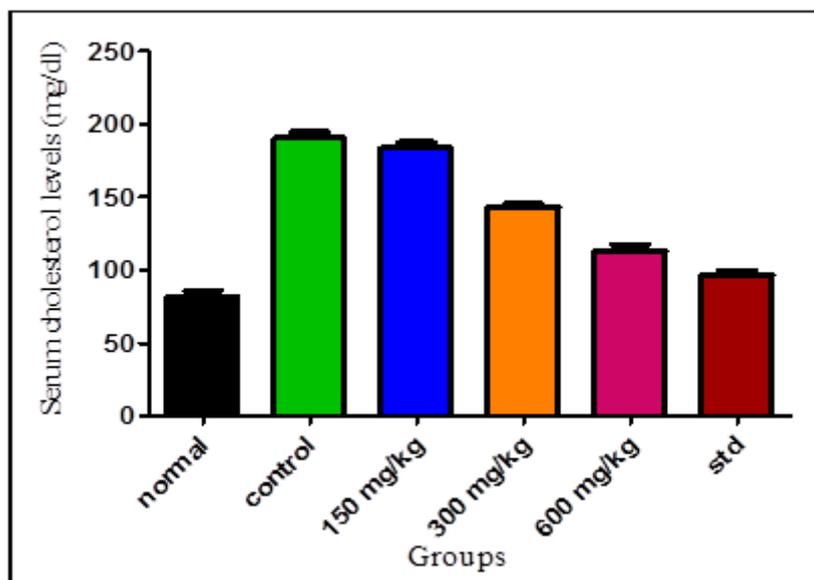


Fig. 1: The serum Cholesterol (mg/dL) levels in Diet induced hyperlipidemia

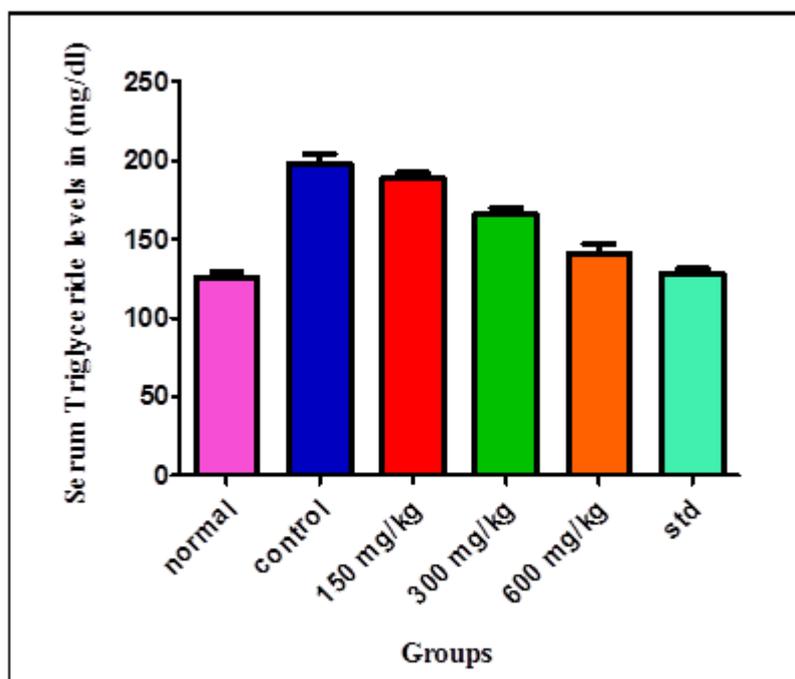


Fig. 2: The serum Triglyceride (mg/dL) levels in Diet induced hyperlipidemia

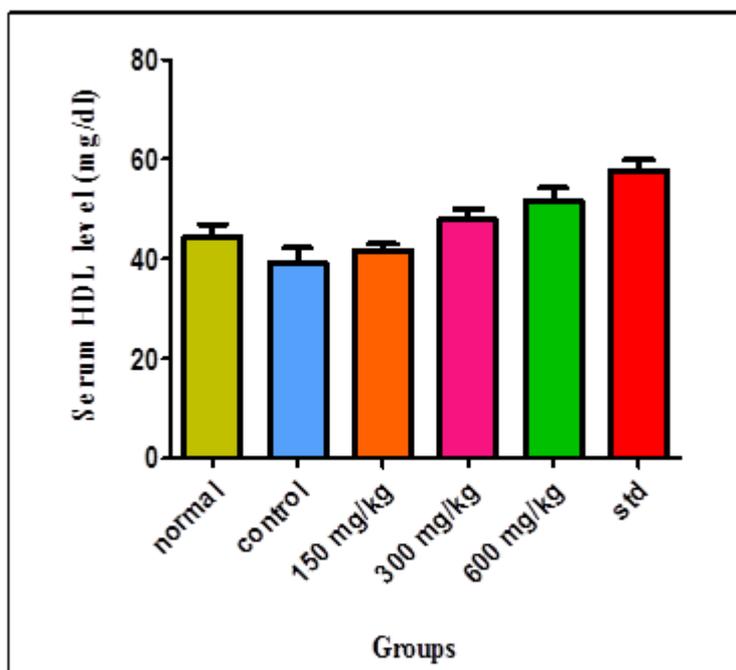


Fig. 3: The serum HDL (mg/dL) levels in Diet induced hyperlipidemia

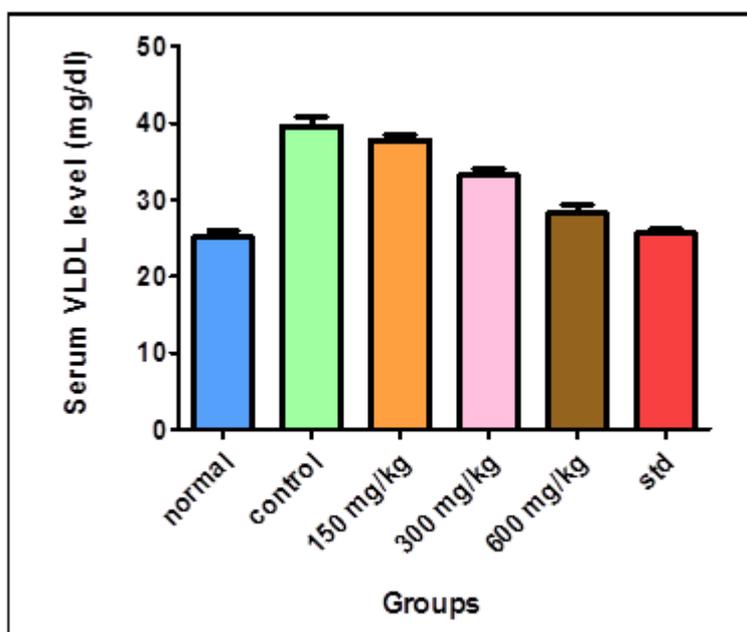


Fig. 4: The serum VLDL (mg/dL) levels in Diet induced hyperlipidemia

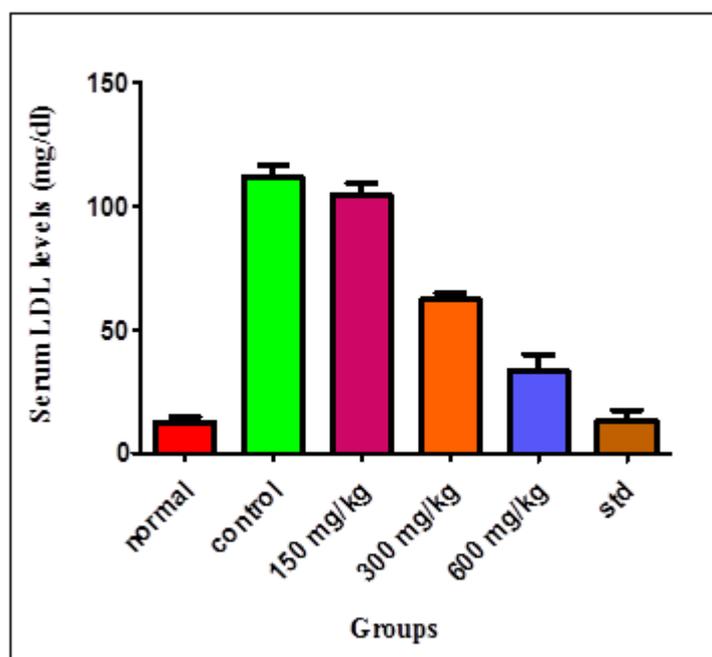


Fig. 5: The serum LDL (mg/dL) levels in Diet induced hyperlipidemia

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