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ANITHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF LIMONIA ACIDISSIMA ROOTS IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

The present study was conducted to evaluate the antihyperlipidemic activity of ethanolic root extract of *Limonia acidissima* in high fat diet induced hyperlipidemia rats. The rats were divided into 5 groups of 6 animals each. The animals of all the groups except normal group were given a high cholesterol diet consisting of 2% cholesterol, 1% cholic acid and 2 ml coconut with standard pellet diet for 30 days. The first group served as normal control received 0.5% Carboxy Methyl Cellulose orally for 30 days. The second group served as hyperlipidemic control, was given High cholesterol diet while the third group was treated with ethanolic extract of *Limonia acidissima* root (200 mg/kg, p.o.), once a day for 30 days. The fourth group was treated with Atorvastatin suspension prepared with 0.5% CMC (10mg/kg; p.o.), once a day for 30 days. Hyperlipidemia was induced by high cholesterol diet consisting of 2% cholesterol, 1% cholic acid and 2 ml coconut oil with standard pellet diet for 30 days. On 31st day the serum was subjected to various biochemical tests like Total Choleserol, Triglyceride, HDL-C, and LDL-C, VLDL-C. The results showed that there was significant decrease in Total Choleserol, Triglyceride, LDL and VLDL and increase in HDL. From the result it was concluded that the ethanolic root extract of *Limonia acidissima* exhibit antihyperlipidemic activity.

Key words: *Limonia acidissima*, Hyperlipidemia, Atorvastatin.

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases and believed to be the primary cause of death. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease [1].

In India, approximately 53% of CVD deaths are in people younger than 70 years of age; in China, the corresponding figure is 35%. About 29.8 million people were estimated to have CHD in India in 2003; 14.1 million in urban areas and 15.7 million in rural areas. According to National Commission on Macroeconomics and Health, a Government of India undertaking, there would be around 62 million patients with CAD by 2015 in India and of these, 23 million would be patients younger than 40 years of age [2].

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Currently used modern hypolipidemic drugs are associated with so many adverse effects and withdrawal is associated with rebound phenomenon which is not seen with herbal preparations. Plant parts or plant extract are sometimes even more potent than known hypolipidemic drugs. Taking these finding forward is mandatory to develop new drugs in this area.

Limonia acidissima L. Swingle Syn. *Feronia elephantum* Correa, *Schinus Limonia* L. (Rutaceae), is a tropical plant species, indigenous to India and locally known as elephant apple. *Limonia acidissima* is native to India and also cultivated in Bangladesh, Pakistan and Srilanka. The wood-apple is native and common in dry plains. It prefers a monsoon climate with a distinct dry season. The tree grows up to an elevation of 450 m in the western Himalayas. It is apparently drought tolerant and best adapted to light soils [3]. All the parts of *Limonia* are prescribed in indigenous system of medicine for the treatment of various ailments. Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs, cures cough, hiccup and good for asthma, consumption, tumours, opthalmia and leucorrhoea [4]. Unripe fruit is astringent while seeds are used in heart diseases. The fruits are used as a substitute for bael (*Eagle marmelos*) in diarrhea and dysentery [5]. The bark and leaves are used for vitiated conditions of vata and pitta [6]. Leaves are astringent and carminative, good for vomiting, indigestions, hiccup and dysentery. The leaves have hepatoprotective activity [8].

The present study is aimed to evaluate the anithyperlipidemic activity of ethanolic root extract of *Limonia acidissima* in high fat diet induced hyperlipidemic rats.

MATERIALS & METHODS

Plant Material

The roots of *Limonia acidissima*, Linn. were collected from the outskirts of Salem, in the month of February. The plant samples were identified and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore, India. The voucher specimen (A/12786) has been deposited in Herbarium for further reference.

Preparation of Extract

The roots were washed with water and dried in sunlight for one hour and then it was dried under shade. By the help of grinder the dried roots were powered to get coarse. Dried course powders of the roots were extracted with ethanol (90%) by using soxhlet apparatus. The extracts were then concentrated, dried and stored in desiccators. Obtained dark green alcoholic extract were used for the pharmacological study.

Animals: Healthy male Sprague – Dawley rats weighing between 200 – 250 gm were used for this study. The animals were obtained from animal house, Sri Lakshminarayana Institute of Medical Sciences, Pondicherry, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (932/a/06/CPCSEA) and were in accordance with the Institutional ethical guidelines.

High Fat Diet induced Hyperlipidemic Model

The rats were divided into 4 groups of 6 animals each. The animals of all the groups except normal group were given a high cholesterol diet consisting of 2% cholesterol, 1% cholic acid and 2 ml coconut oil [9] with standard pellet diet for 30 days. The first group served as normal control received 0.5% Carboxy Methyl Cellulose (CMC) orally for 30 days. The second group served as hyperlipidemic control, was given High cholesterol diet while the third group was treated with ethanolic extract of *Limonia acidissima* roots (200 mg/kg, p.o.), once a day for 30 days. The fourth group was treated with Atorvastatin suspension prepared with 0.5% CMC (10mg/kg; p.o.), once a day for 30 days. On 31st day blood was collected in a non-heparinized tube by retro orbital sinus puncture, under mild ether anaesthesia. The collected blood samples were centrifuged for 10 minutes at 2000 r.p.m. and serum was separated. The separated serum was subjected to various biochemical tests like Total Choleserol [10], Triglyceride [11], HDL-C [12] and LDL-C, VLDL-C [13].

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's t test. P values < 0.05 were considered as significant.

RESULTS

The effect of ethanolic root extract of *Limonia acidissima* on lipid parameters in high fat diet induced hyperlipidemic rats were shown in the Table. No. 1.

In the animals of normal control the total cholesterol was 67.98±5.97 mg/dl, where as the total cholesterol was enhanced by high fat diet up to 109.87±8.98mg/dl, in hyperlipidemic control animals. The 200mg/kg of ethanolic root extract of *Limonia acidissima* significantly (P<0.001) decreased the total cholesterol to 79.03±4.77 mg/dl. The reference control Atorvastatin also significantly (P<0.001) reduced the total cholesterol to 83.99±4.06 mg/dl. The effect produced by

the ethanolic root extract of *Limonia acidissima* is equipotent as that of the reference control Atorvastatin.

In the animals of normal control the triglyceride was 53.90±3.22mg/dl, whereas the triglyceride was increased in hyperlipidemic control due to high fat diet up to 104.67±7.98mg/dl, The 200mg/kg of ethanolic root extract of *Limonia acidissima* significantly (P<0.01) decreased by reversed the elevated triglyceride to 66.99±6.90mg/dl. The reference control Atorvastatin also significantly (P<0.01) reduced the triglyceride to 75.64±4.78mg/dl. The effect produced by the ethanolic root extract of *Limonia acidissima* was comparable with the reference control Atorvastatin.

In the animals of normal control the HDL-Cholesterol was 31.22±2.90mg/dl, whereas the it was decreased in hyperlipidemic control up to 22.95±1.88mg/dl, The 200mg/kg of ethanolic root extract of *Limonia acidissima* and the reference control Atorvastatin significantly (P<0.01) enhanced the HDL – Cholesterol to the level of 24.74±1.98and 23.61±1.66 respectively.

In the animals of normal control the LDL-Cholesterol was 21.05±1.94mg/dl, whereas the it was increased in hyperlipidemic control up to 78.02±2.98 mg/dl, The 200mg/kg of ethanolic root extract of *Limonia acidissima* and the reference control Atorvastatin significantly (P<0.001) reduced the LDL – Cholesterol to the level of 42.87±2.70 and 42.42±3.74respectively as compared to hyperlipidemic control.

In the animals of normal control the VLDL - Cholesterol was 9.95±0.86mg/dl, whereas the VLDL - Cholesterol was elevated in hyperlipidemic control due to high fat diet up to 21.72±2.07 mg/dl, The 200mg/kg of ethanolic root extract of *Limonia acidissima* significantly (P<0.0001)decreased the elevated VLDL - Cholesterol to 12.97±1.05mg/dl. The reference control Atorvastatin also significantly (P<0.001) reduced the VLDL - Cholesterol to 13.98±0.09 mg/dl. The effect produced by the ethanolic root extract of *Limonia acidissima* was comparable with the reference control Atorvastatin.

Table 1. The effect of ethanolic root extract of *Limonia acidissima* on lipid parameters in high fat diet induced hyperlipidemic rats

*P<0.05, ** P<0.01 and** *P<0.001 Vs Control

S.No	Drug Treatment	Lipid Profiles (mg/dl)				
		Total Cholesterol	Triglycerols	HDL - Cholesterol	LDL - Cholesterol	VLDL Cholesterol
1	Group I Normal Control 0.5% CMC	67.98± 5.97***	53.90± 3.22***	31.22± 2.90***	21.05± 1.94***	9.95± 0.86***
2	Group II Hyperlipidemic Control (HCD)	109.87± 8.98	104.67± 7.98	22.95± 1.88	78.02± 2.98	21.72± 2.07
3	Group III Reference Control Atorvastatin (10mg/Kg) + HCD	79.03± 4.77***	66.99± 6.90**	24.74± 1.98**	42.87± 2.70***	12.97± 1.05***
4	Group IV *Limonia acidissima* (200mg/Kg) + HCD	83.99± 4.06***	75.64± 4.78**	23.61± 1.66**	42.42± 3.74***	13.98± 0.09**

Values are in Mean ±SEM.

CONCLUSION

From the above it was concluded that, the ethanolic root extract of aqueous leaf extract of *Limonia acidissima* exhibited antihyperlipidemic activity in high fat diet induced hyperlipidemic rats.

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