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LIMONIA ACIDISSIMA: A POTENTIAL ANTIHYPERLIPIDEMIC HERB AGAINST HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Currently used modern medicines for various ailments were associated with adverse effects and withdrawal leads to rebound phenomenon which is not seen with herbal preparations. Plant parts or plant extract are sometimes even more potent than known modern drugs. Taking these finding in considerations its mandatory to develop an alternate to modern drugs in plant origin. *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. All the parts of *Limonia* are prescribed in indigenous system of medicine for the treatment of various ailments. The present study was conducted to evaluate the antihyperlipidemic activity of ethanolic leaf 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 leaf extract of *Limonia acidissima* (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 leaf extract of *Limonia acidissima* exhibit antihyperlipidemic activity.

KEY WORDS: *Limonia acidissima*, Hyperlipidemia and 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]. Hyperlipidemia is a condition when abnormally high levels of lipids i.e. the fatty substance is found in the blood. This condition is also called hypercholesterolemia /hyperlipoproteinemia [2].

It has long been known that lipid abnormalities are major risk factors for premature coronary artery disease (CAD) [3]. Nearly 85% of the global mortality and disease burden from cardiovascular disease (CVD) is borne by low and middle-income countries. South Asians around the globe have the highest rates of CAD. 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.

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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 [4].

Currently used 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. 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 [5]. 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 [6]. The bark and leaves are used for vitiated conditions of vata and pitta [7]. Leaves are astringent and carminative, good for vomiting, indigestions, hiccup and dysentery. The leaves have hepatoprotective activity [8]. The gum is demulcent and constipating, and is useful in diarrhoea, dysentery, gastropathy, haemorrhoids and diabetes [9].

MATERIALS AND METHODS

Plant Material

The leaves of *Limonia acidissima*, Linn. were collected from Ammapattai, Bhavani Taluk, Tamilnadu the outskirts of Bhavani, Erode District, Tamilnadu in the month of July 2014. The Plant was identified and authentified as *Limonia acidissima*, Linn. by Botanist Dr. Saravana Babu, Department of Botany, Chikkaiah Naicker College, Erode. The voucher specimen was kept in the laboratory (Specimen No: CNC/ERD/01/30/15 for future reference.

Preparation of Extract

The leaves 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 leaves were powered to get coarse. Dried course powders of the leaves were extracted with alcohol (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.

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, IRT Perundurai Medical College, Erode, Tamilnadu, 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 (688/2/C-CPCSEA) and were in accordance with the Institutional ethical guidelines.

High Fat Diet induced Hyperlipidemic Model

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 oil [10] 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* leaves (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 [11], Triglyceride [12], HDL-C [13] and LDL-C, VLDL-C [14]. Atherogenic index (A.I) was also calculated as per the method of Mangathayaru *et al*., 2009 [15].

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 using GraphPad version 3. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Pharmacological Activity

High Fat Diet induced Hyperlipidemic Model

The effect of ethanolic leaf 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 65.45±2.67 mg/dl, whereas the total cholesterol was enhanced by high fat diet up to 112.45±5.52 mg/dl, in hyperlipidemic control animals. The 200mg/kg of ethanolic leaf extract of *Limonia acidissima* significantly (P<0.001) decreased the total cholesterol to 85.62±3.97 mg/dl. The reference control Atorvastatin also significantly

(P<0.001) reduced the total cholesterol to 82.86±3.66 mg/dl. The effect produced by the ethanolic leaf extract of *Limonia acidissima* is equipotent as that of the reference control Atorvastatin.

In the animals of normal control the triglyceride was 52.14±2.56 mg/dl, where as the triglyceride was increased in hyperlipidemic control due to high fat diet up to 94.78±4.83 mg/dl, The 200mg/kg of ethanolic leaf extract of *Limonia acidissima* significantly (P<0.01) decreased by reversed the elevated triglyceride to 72.32±2.16 mg/dl. The reference control Atorvastatin also significantly (P<0.01) reduced the triglyceride to 69.91±4.99 mg/dl. The effect produced by the ethanolic leaf extract of *Limonia acidissima* was comparable with the reference control Atorvastatin.

In the animals of normal control the HDL-Cholesterol was 32.17±1.98 mg/dl, where as the it was decreased in hyperlipidemic control up to 19.64±0.97 mg/dl, The 200mg/kg of ethanolic leaf extract of *Limonia acidissima* and the reference control Atorvastatin significantly (P<0.01) enhanced the HDL – Cholesterol to the level of 26.97±0.17 and 24.54±0.96 respectively.

In the animals of normal control the LDL-Cholesterol was 22.85±0.02 mg/dl, where as the it was increased in hyperlipidemic control up to 73.45±1.66 mg/dl, The 200mg/kg of ethanolic leaf extract of *Limonia acidissima* and the reference control Atorvastatin significantly (P<0.001) reduced the LDL – Cholesterol to the level of 41.47±0.87 and 46.62±0.34 respectively as compared to hyperlipidemic control.

In the animals of normal control the VLDL - Cholesterol was 10.43±0.32 mg/dl, where as the VLDL - Cholesterol was elevated in hyperlipidemic control due to high fat diet up to 18.96±0.25 mg/dl, The 200mg/kg of ethanolic leaf extract of *Limonia acidissima* significantly (P<0.0001)decreased the elevated VLDL - Cholesterol to 14.46±0.16 mg/dl. The reference control Atorvastatin also significantly (P<0.001) reduced the VLDL - Cholesterol to 13.98±0.13 mg/dl. The effect produced by the ethanolic leaf extract of *Limonia acidissima* was comparable with the reference control Atorvastatin.

The athrogenic index was calculated from Total Cholesterol and HDL – Cholesterol. The atherogeinc index was more in the hyperlipidemic control as compared to normal control. The same was decreased in reference control and ethanolic leaf extract of *Limonia acidissima* control as compared to hyperlipidemic control.

Table.No: 1. The effect of ethanolic leaf extract of *Limonia acidissima* on lipid parameters in high fat diet induced hyperlipidemic rats

S.No	Drug Treatment	Lipid Profiles (mg/dl)					Atherogenic index
		Total Cholesterol	Triglycerols	HDL - Cholesterol	LDL - Cholesterol	VLDL Cholesterol	
1	Group I Normal Control 0.5% CMC	65.45± 2.67***	52.14± 2.56***	32.17± 1.98***	22.85± 0.02***	10.43± 0.62***	1.03
2	Group II Hyperlipidemic Control (HCD)	112.05± 5.52	94.78± 4.83	19.64± 0.97	73.45± 1.66	18.96± 0.25	4.71
3	Group III Reference Control Atorvastatin (10mg/Kg) + HCD	82.86± 3.66***	69.91± 4.99**	26.97± 0.17**	41.47± 0.87***	13.98± 0.13***	2.07
4	Group IV *Limonia acidissima* (200mg/Kg) + HCD	85.62± 3.97***	72.32± 2.16**	24.54± 0.96**	46.62± 0.34***	14.46± 0.16**	1.94

The values were expressed as Mean ± SEM (n=6)
*P<0.05,**P<0.001 & ***P<0.001 Vs Hyperlipidemic Control

CONCLUSION

The antihyperlipidemic activity of ethanolic leaf extract of *Limonia acidissima* (200mg/kg) was evaluated to validate the scientific proof on the basis of ethnobotanical information. The Result of present study revealed that the oral administration of ethanolic leaf extract of *Limonia acidissima* (200mg/kg) improved the serum lipid profile in rats by decreasing serum TC, TG, LDL-C, VLDL - C and increasing serum HDL-C, thus improving the atherogenic index. This finding provides some biochemical basis for the use of *Limonia acidissima* leaves as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. Further, studies are required to again more insight in to the possible mechanism of action of *Limonia acidissima* leaves. Hence further research is needed in identifying the active principle and to conduct preclinical studies on this plant.

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