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ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC LEAF EXTRACTS OF THREE DIFFERENT PLANTS ON HIGH CHOLESTEROL DIET INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Coronary artery disease (CAD) is one of the most important causes of death all over the world. Hyperlipidaemia is one of the major risk factor for CAD. In most of the developed countries hyperlipidaemia and atherosclerosis is one of the leading cause of cardiac illness and death. In this study, methanolic leaf extracts of *Lactuca serriola*, *Basella alba* and *Celosia argentea* were screened for Anti hyperlipidemic activity. Atorvastatin was used as standard drug for comparing test extracts towards hyperlipidemic activity after induction of dyslipidaemia in the rats using high cholesterol diet for seven days. Treatment with methanolic plant extracts showed significant decrease in elevated Serum Triglyceride, TC, LDL-C, VLDL-C with significant increase in HDL-C when compared to Dyslipidaemic control. Low level of HDL is associated with high risk of coronary artery disease.

Key Words: Anti hyperlipidemic, *Lactuca serriola*, *Basella alba* and *Celosia argentea*.

INTRODUCTION

Lactuca serriola Linn, *Basella alba* Linn and *Celosia argentea* Linn are traditionally used for various ailments like sedative, hypnotic, expectorant, cough suppressant, purgative, demulcent, diuretic, antiseptic, vasorelaxant, antispasmodic, bronchitis, asthma, pertussis, gastrointestinal infection[1], constipation, sores, urticaria, gonorrhea, intestinal complaints inflammations, fever, blood diseases[2], mouth sores and in diarrhea. According to folklore usages *Lactuca serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn were selected for the study.

Hyperlipidaemia is defined as an elevation of one or more cholesterol, cholesterol esters, phospholipids or triglycerides. Cholesterol and triglycerides, as the major plasma lipids, are essential substrates for cell membrane formation and hormone synthesis and provide a source of free fatty acids [3]. Today in most of the developed and developing countries, hyperlipidemia and thereby

atherosclerosis is the leading cause of cardiac illness and deaths [4]. Epidemiologic data is reported that almost 12 million people die of cardiovascular diseases and cerebral apoplexy each year all over world [5]. Cholesterol, triglycerides and phospholipids are the major lipids in the body, and they are transported as complexes of lipid and proteins known lipoproteins. Plasma lipoproteins are spherical particles with surfaces that consist largely of phospholipid, free cholesterol, and protein and cores that consist mostly of triglyceride and cholesterol ester. The three major classes of lipoproteins found in serum are low-density lipoproteins (LDLs), high density lipoproteins (HDLs), and very low density lipoproteins (VLDLs). Abnormalities of plasma lipoproteins can result in a predisposition to coronary, cerebrovascular, and peripheral vascular arterial disease [6]. Accumulating evidence over the last decades had linked elevated total and LDL and reduced HDL to the development of coronary heart disease. Premature coronary atherosclerosis, leading to the manifestations of ischemic heart disease, is the most common and significant consequence of hyperlipidemia [7]. Currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to

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hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. Any herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system. Medicinal plants play a major role in antihyperlipidemic activity [8].

In the present work anti hyperlipidemic activity of *Lactuca Serriola* Linn (LSME), *Basella alba* Linn (BAME) and *Celosia argentea* Linn (CAME) methanolic leaf extracts were tested against High Cholesterol Diet Induced Hyperlipidemic rats. Atorvastatin used as standard is a well-known drug used in the treatment of Hyperlipidaemia, it is known to raise the level of HDL and reduce the levels of total cholesterol and LDL.

MATERIALS AND METHODS

Collection and Authentication of Plant materials

Leaves of *Lactuca Serriola* were collected from Ravor village, Gulbarga district, Karnataka. Leaves of *Basella alba* and *Celosia Argentea* was collected from KR vegetable market, Bangalore and were authenticated by Dr P Santhan, botanist, Natural Remedies private limited, Bangalore.

Preparation of extract

Plant materials obtained were shade dried, made into coarse powder and passed through #40 and were successively extracted with methanol by Soxhlet extraction method.

Preliminary phytochemical screening

Preliminary phytochemical screening of methanolic extract of all three plants was carried out for detection of various plant constituents [9-12].

Experimental animals

Wistar albino rats weighing 150-200g of either sex were procured from Venkateshwara Enterprises, Bangalore. Housing was provided in the form of polypropylene cages with paddy husk as bedding material. The animals were fed with balanced diet in the form of pellets (Amrut laboratory animal feed, sangli) and free access to water was provided throughout the experimental period. The animals were maintained at $24 \pm 2^\circ\text{C}$ under 12 h light dark cycles. An acclimatization period of 15 days was allowed before the start of the experiment. Approval to carry out the experiment was obtained from institution Animals Ethics Committee vide their certificate no (IAEC/NCP/78/13) and all the procedures were performed in accordance with the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Induction of Dyslipidaemia

High cholesterol diet was prepared by mixing 100g cholesterol, 30g propylthio-uracil, and 100g cholic acid in one liter of peanut oil was administered orally as a daily single dose of 1ml/100gm body weight to rats in addition to a normal diet for seven days [13]. Dyslipidaemic condition developed within seven days. The increase in dietary cholesterol, inhibition of thyroxine production due to the propylthiouracil and the presence of cholic acid in the diet contribute to the development of the dyslipidemic condition [14, 15].

Dose preparation and administration of standard atorvastatin and extracts

Standard Atorvastatin at a dose of 10 mg kg⁻¹ was prepared by suspending Atorvastatin in 0.5% CMC. The methanolic extracts of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn 200 mg and 400mg/kg, b.w respectively was dissolved in 0.5% CMC and was given to the rats once in a day along with high cholesterol diet orally. Treatment was given daily for seven days.

Experimental protocol for antihyperlipidemic activity

The experimental animals were divided into nine groups with six animals in each group:

Group I: Non-dyslipidaemic control (0.5% CMC)

Group II: Dyslipidaemic control (0.5% CMC)

Group III: Dyslipidaemic- Standard Atorvastatin (10mg/kg b.w)

Group IV-V: Dyslipidaemic treated with LSME (200mg/kg and 400mg/kg respectively)

Group VI-VII: Dyslipidaemic treated with BAME (200mg/kg and 400mg/kg respectively)

Group VIII-IX: Dyslipidaemic treated with CAME (200mg/kg and 400mg/kg respectively)

Treatment was given daily for seven days orally.

Collection of blood

On the 8th day, under light ether anaesthesia, blood sample was collected from retro orbital puncture after 8 hr fasting. The blood was allowed to clot, serum was separated by centrifuging at 2500 rpm for 15 minutes and analysed for various biochemical parameters.

Biochemical analysis

The Serum samples were analyzed using auto biochemistry analyser for Serum Triglyceride, Total Cholesterol, HDL- Cholesterol, LDL-Cholesterol and VLDL- Cholesterol using diagnostic kits which were procured from Pericugent, Pinnacle biotechnologies Ltd. Mumbai. Very low density lipoprotein (VLDL-C), High density lipoprotein ratio (HDL-Cratio), and low density lipoprotein cholesterol (LDL-C) were calculated by using formula.

Statistical analysis of data

Results were expressed as Mean \pm (n=6). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test p values less than <0.05 was considered to be statistically significant, *p<0.05, ** <0.01 and *** <0.001, when compared with Dyslipidaemic control as applicable.

RESULTS AND DISCUSSION

The Phytochemical tests with methanolic leaf extracts of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn indicated the presence of Glycosides, saponins, phenolic compounds, flavanoids.

The rats fed with high cholesterol diet for seven days exhibited significant increase in Serum Triglyceride, TC, LDL-C, VLDL-C and significant decrease in HDL-C as compared to Non-dyslipidaemic control. Treatment with atorvastatin showed significant decrease in elevated Serum

Triglyceride, TC, LDL-C, VLDL-C with significant increase in HDL-C as compared to Dyslipidaemic control. Whereas treatment with methanolic leaf extracts of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn at a dose of 200,400mg/kg b.w showed significant decrease in elevated Serum Triglyceride, TC, LDL-C, VLDL-C with significant increase in HDL-C as compared to Dyslipidaemic control. (Table & Figure 1).

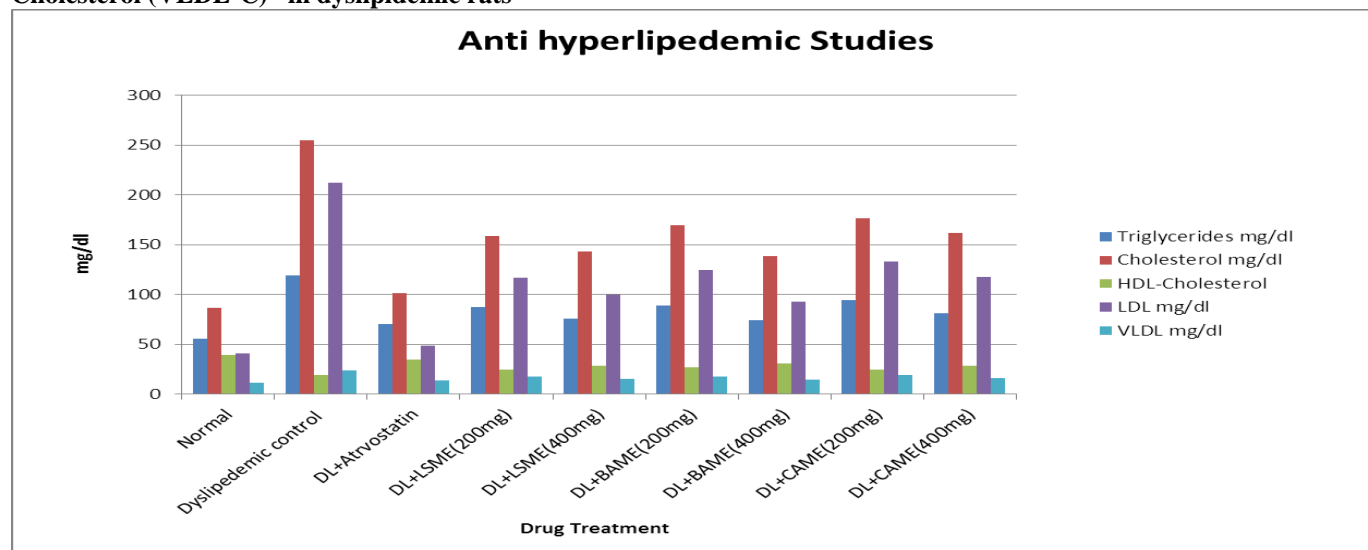
Low level of HDL is associated with high risk of coronary artery disease. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Antihyperlipidemic activity of certain phytoconstituents like flavanoids, phenolic compounds Tannins, carbohydrates, saponins have well been documented in the literature. The phytoconstituents alone or in combination may be responsible for the anti hyperlipidemic activity of the selected plants.

Table 1. Antihyperlipidemic activity results of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn leaf extracts on serum triglyceride, Total Cholesterol(TC), HDL- Cholesterol(HDL-C), LDL-Cholesterol (LDL-C) and VLDL- Cholesterol (VLDL-C) in dyslipidemic rats

Group	Triglycerides	TC	HDL-C	LDL-C	VLDL-C
I.	55.23 \pm 1.538	86.69 \pm 1.654	34.56 \pm 1.366	41.09 \pm 1.432	14.016 \pm 1.432
II.	119.1 \pm 1.419	255.2 \pm 1.631	19.21 \pm 0.2778	212.17 \pm 0.2902	23.82 \pm 0.2902
III.	70.08 \pm 1.143	101.6 \pm 1.932	39.2 \pm 0.4675	48.38 \pm 0.6723	11.04 \pm 0.6723
IV.	86.95 \pm 2.643***	158.7 \pm 2.667*	24.14 \pm 0.7233***	117.17 \pm 0.832***	17.39 \pm 0.789***
V.	76.06 \pm 1.712**	142.9 \pm 1.686***	28.09 \pm 0.4841***	99.598 \pm 0.7092**	15.212 \pm 0.9923**
VI.	89.21 \pm 1.718**	169.8 \pm 2.066**	27.21 \pm 0.738***	124.74 \pm 0.5643**	17.842 \pm 0.7022**
VII.	73.87 \pm 1.051***	138.3 \pm 1.895**	31.06 \pm 0.341**	92.466 \pm 0.553**	14.774 \pm 0.8662**
VIII.	94.41 \pm 1.754***	176.2 \pm 1.751***	24.47 \pm 0.6154**	132.848 \pm 0.8387**	18.882 \pm 0.9024**
IX.	81.36 \pm 0.6174***	162.1 \pm 1.965**	28.37 \pm 0.3705**	117.458 \pm 0.4208**	16.272 \pm 0.7022**

Values are mean of \pm SEM of 6 rats Significant *p<0.05, **p<0.01, ***p<0.0001 compared with standard control

Figure 1. Graphical representation of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn leaf extracts on serum triglyceride, Total Cholesterol (TC), HDL- Cholesterol (HDL-C), LDL-Cholesterol (LDL-C) and VLDL-Cholesterol (VLDL-C) in dyslipidemic rats



CONCLUSION

In the present study, methanolic leaf extracts of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn showed significant antihyperlipidemic activity. Anti-hyperlipidemic activity was evaluated by using High Cholesterol-induced diet model. It was found that methanolic leaf extracts significantly decreased the elevated Serum Triglyceride, TC, LDL-C VLDL-C with significant increase in HDL-C as compared to

Dyslipidaemic control. In comparison to standard drug atorvastatin effect of methanolic extract of *all three plants* was less.

But comparable notably, present studies reveal that methanolic extracts of *Lactuca Serriola* Linn, *Basella alba* Linn and *Celosia argentea* Linn can be used as effective antihyperlipidemic agent and can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

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