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# EVALUATION OFANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *SAUSSURAE LAPPA* IN RATS

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## ABSTRACT

Hyperlipidemia is an excess of fatty material called lipids, largely cholesterol and triglycerides, in the blood. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease. The aim of the study is to reduce the levels of triglycerides by using herbal plant ethanolic extract of *Saussurae Lappa* (EESL). The food intake and body weight was increased in high cholesterol fed diet rats as compared to normal control. Treatment with ethanolic extract of *Saussurae Lappa* showed no change in food intake but there was significant decrease in weight gain as compared to high cholesterol fed diet rats. The antihyperlipidemic activity may be due to presence of tannins, triterpenes, alkaloids, inulin, essential oil present in the roots of *Saussurae Lappa*.

## KEYWORD

Hyperlipidemia, Atherosclerosis, *Saussurae Lappa*, Triglycerides, Flavonoids.

## INTRODUCTION

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as Coronary Heart Disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease. Hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease. Reduction in serum cholesterol levels reduces the risk for CHD. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease. Hyperlipidemia includes several conditions, but it usually means that the high cholesterol and high triglyceride levels. High lipid levels can speed up a process called atherosclerosis, or hardening of the arteries. Our arteries are normally smooth and unobstructed on the inside, but as our age, a sticky substance called plaque forms in the walls of our arteries. Hyperlipidemia is an abnormally high level of fatty substance called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipiproteinemia, because these fatty substances travel in the blood by attaching to proteins forming large molecules. Triglycerides also known as triacylglycerol or triacylglycerides are glycerides in which the glycerol is esterified with three fatty acids. They are the main constituents of vegetable oil and animal fats. They play an important role in the metabolism as energy source. They contain a bit more than twice as much as energy 9k.cal/gm as carbohydrates and proteins[1]. Cholesterol is essential for development and maintenance of cell membranes helps the cells to resist changes in temperature and protects and insulates nerve fibers. Formation of sex hormones progesterone, testosterone, estradiol, cortisol, Production of bile salts, helps to digest food, Conversion into vitamin D in the skin when exposed to sun light. The enzymes required for fatty acid synthesis are present in the cytosomal fraction of the cell. Acetyl CoA is the source of carbon atoms, NADPH provides the reducing equivalent and ATP supplies energy for fatty acid formation[2]. Phospholipids are triglycerides that are covalently bonded to a phosphate group by an ester linkage. The diglyceride is composed of a glycerol backbone that has esterified to two fatty acids. The result is an amphophilic molecule[3]. Lipoproteins are the “packages” in which cholesterol and triglycerides (lipids) are transport throughout the body by combining with proteins. Lipoproteins contain cholesterol, phospholipids and triglycerides at the core and an outer layer of protein called “apolipoproteins/apoproteins”. The lipid constituents are water insoluble and apoproteins are water soluble[4].Depending on upon their lipid carrying capacity Lipoproteins are classified into[5].Depending on the complexity of the disease, hyperlipidemia is classified into two types. Primary Hyperlipidemia and Secondary / Acquired Hyperlipidemia[6].

## MATERIALS AND METHODS

***(i) Selection and acclimatization of animals***

Male Wister rats (150-180gm) were housed in polypropylene cages and fed with standard rodent pallet diet and water *ad libitum*. The animals were exposed to alternate cycle of l2hrs of darkness and l2hrs light. Before each test, the animals were fasted for at least 12hrs and the experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee and were cleared by the same. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental

pain in conscious animals. The standard syringe was used for drug administration in experimental animals. Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. All the animals have been divided into five groups and placed in separate cages, each consisting of six animals. The experimental protocol was approved by CPCSEA. (Ref: XII / VELS / PCL / 26 / 2000 / CPCSEA/11.03.11).

***(ii) Collection of plants***

Plants were collected from kandaswamy herbal store at Broadway in Chennai. Authentication is done by Prof. P. Jayaraman, Ph.D, Botonist, PARC, Chennai.

***(iii) Extraction by simple maceration***

The dried powdered roots of *Saussurea Lappa* were mixed with twice the amount of ethanol (70%) and macerated for 72 hours, and then it was filtered three times and evaporated to get a crude extract. Then the crude extract was subjected to phytochemical screening. The percentage yield of the extract was found to be 12.8%.

***(iv) Preliminary phytochemical screening***

The crude ethanolic extract of *Saussurea Lappa* were tested for its different chemical groups such as alkaloids, flavonoids, tannins, steroids, saponins, iridoid glucosides, fixed oils, gums and mucilages, tri-Terpenoids, carbohydrates and glycosides, phytosterols.

***(v) Acute toxicity study***

The acute oral toxicity study was carried out as per the OECD guidelines-423. Upto 2000mg/kg the extract did not produce any toxic symptoms on oral administration. Hence, One-tenth and one-fifth of the maximum dose used in the toxicity study was considered as therapeutic lower and higher dose for further pharmacological study.

***(vi) Treatment protocol***[7]

Male wistar rats were induced for hyperlipidemic by the oral administration of cholesterol (400 mg/kg) along with cholic acid (50 mg/kg) in coconut oil for 20 days, once daily. The rats with elevated cholesterol level were divided into 5 groups of 6 animals each and given drug/ vehicle treatment for 7 days. During these 10 days all groups also received cholesterol in same dose as earlier. In each group six animals were taken. After the experimentation period, blood sample was collected by retro orbital sinus puncture under mild ether anaesthetic condition and allowed to clot for 30 minutes at room temperatur. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out. Group – I: Normal control **-** received vehicle (Acacia 4%) 5 ml/kg.p.o., Group – II: Hyperlipidemic Control **-** received vehicle (Acacia 4%) 5 ml/kg. p.o., Group – III: Standard **-** received atorvastatin10mg/kg p.o.; Group- IV: received EESL (200 mg/kg; p.o.)**,** Group- V: received EESL (400 mg/kg; p.o.) and the biochemical parameters estimated were total cholesterol, triglycerides[10], LDL, VLDL, HDL[8] using standard methods with the help of clinical chemistry analyzer (METRO LAB 1600-DR-K).

***(vii) Statistical analysis***

Data were expressed as mean ±SEM (n=6) for statistical evaluation of data. One-way ANOVA and student t-test were performed by using PRISM PAD statistical software program.

## RESULTS AND DISCUSSION

Lipid metabolism normally maintains elegant balance between synthesis and degradation of biological tissues. Coronary heart diseases (CHD) are the clinical manifestation of atherosclerosis. It is most common cause of morbidity and mortality in developed and developing counties.Development of hyperlipidemia disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries & other major arteries within the body. A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity. The weight gain in high cholesterol diet (HCD) group of rats was significantly

higher than control rats reflecting the influence of high cholesterol diet.[11] High fat diet fed rats showed significant increase ($p<0.001$) in Serum cholesterol, triglyceride, LDL-C, but significant decrease ($p<0.001$) in HDL-C level as compared to 0 day and normal control rats. Treatment with EESL (200mg/kg) exhibited the significant reduction ($p<0.05$) in triglyceride, LDL-C. Treatment with EESL (400mg/kg) showed significant reduction ($p<0.001$) TC, LDL, and Triglyceride ($p<0.001$), but significant increase ($p<0.01$) in HDL-C as compared to high cholesterol diet fed rats.

**Table 1**
***Influence of Saussurea Lappa on body weight gain of hyperlipidemic rats.***

| | | Effect on weight gain (grams) | | | | |
|---|---|---|---|---|---|---|
| **S.No** | **Groups** | **0th Day** | **8th Day** | **16th Day** | **24th Day** | **28th Day** |
| 1 | Normal | 180±13.40 | 180±13.40 | 180±13.40 | 180±13.40 | 180±13.40 |
| 2 | Control | 175±12.58# | 190±14.06# | 230 ± 15.58# | 260±15.79# | 290±15.58# |
| 3 | Test 1 | 185±14.02** | 175±13.02** | 175±11.02** | 165±10.06** | 165±10.06** |
| 4 | Test 2 | 190±15.03** | 185±12.04** | 170±10.05** | 170±10.05** | 167±10.07** |
| 5 | Atorvastatin | 185±14.20** | 170±12.03** | 145±12.36** | 125±11.02** | 125±11.02** |

***Values are as mean ± SEM, #P < 0.05, **P < 0.01, Vs Control. (n=6)***

**Table 2.**
***Effect of ethanolic extract of Saussurea Lappa on lipid profile in Hyperlipidemic rats.***

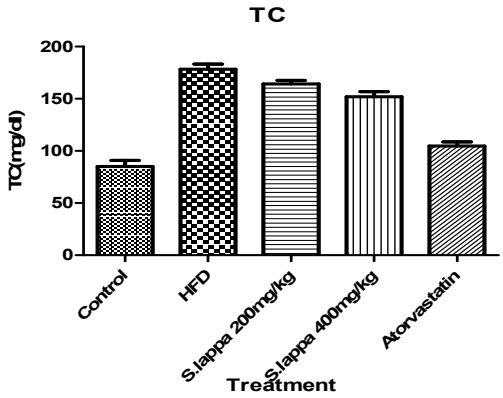
| S.No | Groups | TC (mg/dl) | HDL (mg/dl) | TGL (mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|---|
| 1. | Normal | 85±5.857 | 38±1.158 | 70.92±1.480 | 32±1.304 |
| 2. | Control | 178.4±4.642### | 15.6±1.288### | 148.8±2.104### | 127.8±4.532### |
| 3. | Test 1 | 164.2±3.399 | 20.6±2.135 | 132.4±3.4* | 120.2±5.953* |
| 4. | Test 2 | 152±4.743** | 23.6±2.135* | 108.4±2.78*** | 101.8±5.508** |
| 5. | Atorvastatin | 104.6±4.014** | 32.8±1.393*** | 92.8±5.643*** | 88.6±3.6*** |

***Values are as mean ± SEM, ###P < 0.05Vs Normal; *P<0.05,**P<0.01, ***P<0.001 Vs Control.***

Similarly, in this study there was also a significant weight gain in cholesterol control as compared to normal control group. Treatment with ethanolic extract of *Saussurea Lappa* significantly reduced the weight gain. Lowering high cholesterol levels significantly reduced the risk of heart attacks, strokes, and death. Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increases the catabolic conversion of cholesterol to bile acids in liver. High cholesterol diet increased serum cholesterol and LDL-C level significantly[12]

**.Graph 1**

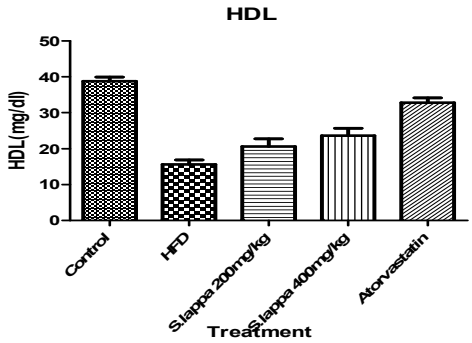
***Effect of ethanolic extract of Saussurea Lappa on Total Cholesterol profile in HFD induced Hyperlipidemic rats.***




A rise in LDL may cause deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease.[13]Studies show that both LDL and VLDL have a positive role in atherogenesis.[14] In the present study, there was an elevation in serum and tissue cholesterol, LDL-C, in response to high cholesterol diet as compare to normal control group. Treatment with ethanolic extract of *Saussurea Lappa* significantly reduced serum and tissue cholesterol, LDL-C. The decrease in serum triglyceride level is an important finding of this experiment.

**Graph 2**

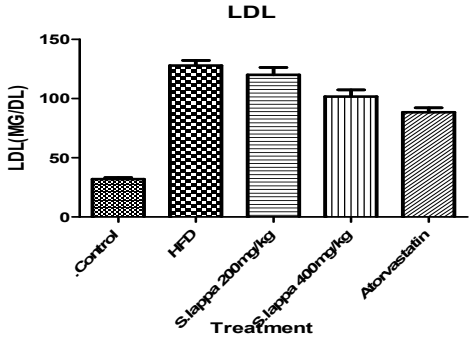
***Effect of ethanolic extract of Saussurea Lappa on HDL profile in HFD induced Hyperlipidemic rats.***




Recent studies show that triglycerides are independently related with coronary artery disease.[15] Treatment with ethanolic extract of *Saussurea Lappa* shows significant decreased in triglyceride. HDL is synthesized mainly in intestine and liver. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease. In the present investigation, HDL-C level in both serum and tissue were significantly increased by ethanolic extract of *Saussurea Lappa*.

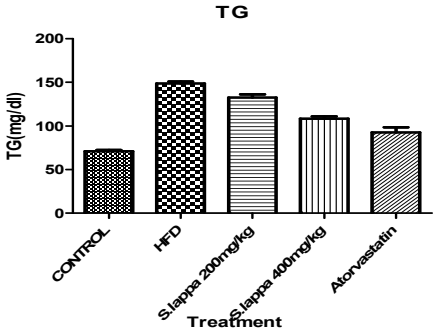
**Graph 3**

***Effect of ethanolic extract of Saussurea Lappa On LDL in HFD induced Hyperlipidemic rats***




**Graph 4**

***Effect of ethanolic extract of Saussurea Lappa on TG in HFD induced Hyperlipidemic rats.***

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