Research Article

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EFFICACY OF LACTUCA SATIVA IN HYPERLIPIDEMIA: A PILOT STUDY

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Abstract

Aim: Lactuca sativa has anti toxidant, anti inflammatory and anti diabetic activities. In the present pilot study is that efficacy of lactuca sativa in hyperlipidemia. Hyperlipidemia patients meeting the inclusion criteria were randomly assigned in to the treatment and placebo. **Methods**: 40 all randomized patients in treatment group took lettuce seed capsule (1000 mg, once per day); whereas the patients in placebo group took placebo capsule (once per day) for 12 weeks. The lipid profile, blood pressure, body mass index and liver enzymes of the patients were evaluated at baseline, and after 6 and 12 weeks of the clinical trial. **Results:** The final results showed that lactuca sativa capsules significantly p value 0.05 improved LDL, HDL, cholesterol, compared to the placebo group. It had no effect on systolic or diastolic blood pressure and the effect on BMI reduction was not significant (P>0.05) compared to placebo. The lettuce showed an inhibitory effect on atorvastatin-induced elevation of hepatic enzymes and possible liver toxicity. No serious side effect was reported for lettuce seed extract administration. Therefore, lettuce seed extract could be considered as a supplement for treatment of dyslipidemia.

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INTRODUCTION

For most essential consideration suppliers, hyperlipidemia is characterized as rises of fasting all out cholesterol focus which could conceivably be related with raised TG fixation [1,2]. Be that as it may, lipids are not solvent in plasma, however are rather shipped in particles known as lipoproteins [3].

CLASSIFICATION OF APOLIPOPROTEIN

- Chylomicrons – Triglyceride rich bearer of dietary fats.
- Very Low Density Lipoprotein (VLDL) – Triglyceride rich bearer of hepatic blended triglycerides (TG)
- Intermediate and Low Density Lipoprotein (IDL & LDL) – Cholesterol rich leftover particles got from lipolysis of triglycerides in VLDL
- High Density Lipoprotein (HDL) – Cholesterol rich molecule that transports cholesterol to liver for removal or reusing [4].

Human services suppliers are worried about hyperlipidemia in light of the settled relationship

between lipid focuses and the danger of CVD, the main source of death in the India, United State of America [5,6]. Hyperlipidemia is an expansion in one or a greater amount of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and additionally plasma lipoproteins including very low density lipoprotein and low-thickness lipoprotein, and decreased high-thickness lipoprotein levels [7,8]. Hypercholesterolemia and hyper triglyceridemia are the fundamental driver of atherosclerosis which is emphatically identified with ischemic coronary illness. Atherosclerosis is a procedure of supply routes solidifying due to deposition of cholesterol in the blood vessel divider which causes narrowing of the arteries. Atherosclerosis and atherosclerosis associated messes like coronary, cerebro vascular what's more; fringe vascular infections are quickened by the nearness of hyperlipidemia. Practically all the dietary fats are ingested from the intestinal lumen into the intestinal lymph what's more, stuffed into chylomicrons [9,10]. These lipoproteins move into the circulation system where they got hydrolyzed by endothelial lipoprotein lipase which hydrolyzes the triglyceride into glycerol and non esterifies unsaturated fats. After which the chylomicron remainders are invested in the liver and bundled with cholesterol, cholesterol esters and ApoB100 to shape VLDL. After the arrival of VLDL into the circulation system it will be changed over into IDL by the activity of lipoprotein lipase and hepatic lipase, where phospholipids and apolipoproteins moved back to HDL. Besides, after the hydrolysis by hepatic lipase, IDL will be changed over to LDL and misfortune more apolipoproteins [11,12]. Fringe cholesterol is come back to the liver by invert cholesterol transport pathway utilizing HDLs which are initially orchestrated by the liver what's more, discharged into the blood. In the blood, HDL cholesterol is esterifies by LCAT to cholesterol ester what's more, moved to VLDL and chylomicrons to return to the liver through LDL receptor [13,14]. Cholesterol ester are moved to LDL particles by CETP and afterward exposed to LDL-receptors interceded endocytosis. At long last, cholesterol esters are hydrolyzed to cholesterol and removed from the body as bile corrosive [15].

MATERIAL AND METHODS

PREPARATION OF LETTUCE EXTRACT

Lactuca sativa seeds were obtained from Belagavi Pharmacy, Belagavi, Karnataka, India. The Lactuca sativa seeds were rinsed with clean water and then dried in the shade at room temperature. The dried seeds were ground into a fine powder by a laboratory grinder. The dry powder (50g) was soaked in 250 ml solvent [aqueous-ethanolic (20/80, v/v)] and concentrated using a rotary evaporator at room temperature. The samples were centrifuged (4000 rpm for 10min) and the supernatant was collected. Then the hydroethanolic extract was filtered by Whatman filter paper No. 1 and the solution was evaporated in incubator at 40-45°C until dryness. The dried extract was kept at room temperature in darkness until use.

PREPARATION OF CAPSULES

Extraction of Lactuca sativa seed was prepared as mentioned above. Lactuca sativa seed extract and placebo were formulated into capsules in Department of pharmacy, Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi. The plant sample was identified at the department of Pharmacy, BHMCH&RC. The placebo capsule was filled with neutral and inert additive substance whereas; each lettuce capsule was filled with 1000 mg seed extract. There were no in clinical studies on the anti hyperlipidemic effect of Lactuca sativa seed extract. In the abovementioned works, administration of 1000mg/day Lactuca sativa seed extract was safe and effective.

STUDY DESIGN

The study was fully conducted in accordance with the Ethical Committee of the Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi and written informed consent was obtained from all patients before their inclusion in the pilot study. The study was a randomized, double-blind, placebo controlled, three months, clinical trial which was carried out on 110 hyperlipidemic outpatients of Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi. The authors applied inclusion and exclusion criteria for patients to improve the quality of the results in this study.

INCLUSION CRITERIA

Male and female outpatients aged 25 to 65 years; incidence of hyperlipidemia with at least one of the following factors: cholesterol level >200 mg/dl or TG level higher than 150 mg/dl or LDL-C level higher than 130mg/dL or HDL-C level <40 mg/dl.

EXCLUSION CRITERIA

The patients who had a history of chronic or metabolic diseases such as diabetes, Ischemic heart disease, hypertension, tachycardia, peripheral vascular disease, coronary artery disease, thyroid dysfunction, hospitalized, cannot follow therapeutic lifestyle modification and pregnancy. In addition, the exclusion criterion was a recent change in dosage of antilipemic agents such as hydroxymethylglutaryl coenzyme A (HMG-COA) reductase inhibitor, or adding hypoglycemic agents such as first and second-generation sulfonylureas or supplements or drugs known to affect the blood lipids, presence of side effects and unwillingness to participate in study. Other exclusion criteria were: LDL level more than 190 in patients who need medical treatment (for healthy people or with one risk factor); LDL ≥ 160 in patients who need drug treatment (for those with two or more risk factors of the following:

- Smoking.
- Hypertension.
- Low HDL level (less than 40).
- History of coronary artery disease at an early age in the household (less than 55 years in males and in females under age 65 years old), 5. Age above 65 years old).

SAMPLE SIZE

To have a power of 90%, a two-sided test was used, with a significance level of 0.05, and a 20% minimum detectable mean difference changes for LDL-C and SD 20.5% between treatment and placebo group. Finally, minimum sample size of 30 patients for each arm was calculated. Because of expected dropout, we considered 70 patients in each group.

40 were enrolled in this pilot study. The patients were randomly divided into the treatment (20 patients) treatment group and the placebo (20 patients) groups. Ten patients were excluded. Finally, 30 patients successfully completed the pilot study.

INTERVENTIONS

Participants were randomized to 2 intervention groups of 15 patients. The patients in the treatment group were taking Lactuca sativa extract (one capsule per day, 1000 mg/day), for 12 weeks; whereas the patients in placebo group were taking placebo (one placebo capsule per day) for 12 weeks. Participants did not receive any other hypocholesterolemic drugs during the pilot trial. The patient's compliance and medication adherence were confirmed through checking with the patient and his/her caregiver along with a capsule count at each visit.

OUTCOME MEASURES

Lipid profile (Cholesterol, TG, HDL and LDL), blood pressure (SBP and DBP), BMI index and liver enzymes (ALT, AST, ALP) were measured at baseline, 6 weeks and 3 months after intervention in treatment and placebo group.

MASKING

The enrolled participants were assigned using a stratified randomization and all of them received Lactuca sativa or placebo capsules, which were prepared in the same way. For randomization, a randomized code number was obtained from Microsoft Excel for each pillbox (treatment and control groups). All capsules had similar colour, shape, size, texture and odour. The capsules were stored in a dark container and coded by a pharmacist. The participants and those assessing outcomes were blinded until all participants finished the protocol.

SAFETY

The patients were requested to inform investigators about any adverse events or complaints for all illnesses, and hospitalizations that occurred during the trial. The symptoms were checked and recorded at the beginning and at each visit by general physician, cardiologist. Also, possible side effects were checked and recorded via telephone call every week and the general physician/homoeopathy physician was responsible for continuing or discontinuing the drugs.

STATISTICAL ANALYSIS

Baseline characteristics were analyzed using independent t-test or $\chi 2$ tests. The significant differences at various time points were assessed by

repeated measures of ANOVA, and followed by Tukey post hoc test before and after treatment. Demographic data were compared using Fisher's exact test. The variables were reported as mean and standard deviation (Mean ± SD). P value less than 0.05 was considered statistically significant.

Table 01: Demographic data of the patients in both study groups (M±SD)

VARIABLE	CONTROL GROUP	TEST GROUP	P_{value}
Age Years	0.2±0.56 0.8±0.77	0.33±0.61 0.61±0.81	0.290 0.315
Gender Female Male	 0.33±0.48 0.48±0.61	 0.53±0.51 0.6±0.73	 0.135 0.375
Marital status (n) Married Single	 0.53±0.63 0.46±0.63	 0.4±0.63 0.64±0.63	 0.32 0.272
Level of education (n) Diploma Under graduate Post graduate	 0.26±0.59 0.33±0.617 0.4±0.73	 0.26±0.593 0.26±0.59 0.46±0.83	 0.5 0.39 0.42

Table 02: The measurements of lipid profile between two groups (M±SD)

VARIABLE	CONTROL GROUP	TEST GROUP	P Value
Cholesterol			
At base line	209.9±0.2	209.8±0.6	0.33
After 6 weeks	209±0.3	179.9±0.2	0.001
After 12 weeks	208.0±0.2	139.9±0.2	0.0001
TG			
At base line	149.86±0.51	159.9±0.2	0.16
After 6 weeks	137.9±0.2	132.8±5.9	0.001
After 12 weeks	137.0±0.2	97.6±1.2	0.001
LDL			
At base line	32.6±1.7	149.93±0.2	0.33
After 6 weeks	31.9±0.2	130.6±1.6	0.001
After 12 weeks	30.9±0.2	100.1±0.5	0.0001
HDL			
At base line	32.6±1.7	32.06±2.2	0.17
After 6 weeks	31.96±0.2	44.8±0.7	0.001
After 12 weeks	30.93±0.2	50.4±1.8	0.0001

Table 03: The results of blood pressure in both groups (M±SD)

VARIABLE	CONTROL GROUP	TEST GROUP	P Value
SBP (mm of Hg)			
At base line	139.4±0.62	139.0±2.0	0.20
After 6 weeks	139±0.76	129.8±0.50	0.001
After 12 weeks	138.06±0.5	120.6±1.6	0.0001
DBP (mm of Hg)			
At base line	89.6±0.8	89.7±0.7	0.29
After 6 weeks	86.9±0.2	83.8±0.5	0.001
After 12 weeks	86.0±0.2	80.0±0.2	0.0001

Table 04: The anthropometric measurement of BMI between two groups (M±SD)

VARIABLE	CONTROL GROUP	TEST GROUP	P Value
BMI (kg/m^2)			
At base line	39.93±0.25	39.9±0.25	0.5
After 6 weeks	39.06±0.25	35.06±0.25	0.01
After 12 weeks	38.06±0.25	25.06±0.25	0.0001

Table 05: The amounts of liver enzymes between two group (M±SD)

VARIABLE	CONTROL GROUP	TEST GROUP	P Value
AST			
At base line	56.86±0.35	56.86±0.35	0.5
After 6 weeks	56.2±0.41	45.7±1.9	0.01
After 12 weeks	55.2±0.41	37.06±0.88	0.0001
ALT			
At base line	80.2±0.5	80.3±0.7	0.38
After 6 weeks	78.8±0.4	70.8±2.3	0.01
After 12 weeks	77.9±0.2	63.2±0.56	0.0001
ALKP			
At base line	145.86±0.3	145.93±0.2	0.16

	5	5	
After 6 weeks	144.93±0.25	136.2±0.56	0.001
After 12 weeks	143.93±0.25	126.06±0.25	0.0001

Among 30 type 2 hyperlipidemia patients with mean ± SD, age group cases were observed 0.2±0.56 in control group and 0.33±0.61 in a test group, P_{value} showed 0.290. Patient years mean ± SD were 0.8±0.77 in control group and test group was 0.61±0.81, P_{value} showed 0.315 (not significant). The Male 30 hyperlipidemia patients were 0.48±0.61 in control group and 0.6±0.73 in test group, female patients were 0.33±0.48 ain control group and 0.53±0.51 in test groups, P_{value} was 0.135. Marital status of married patients mean ± SD were 0.53±0.63 in control group and 0.4±0.63 in test group, P_{value} showed 0.32. In single patients values were 0.46±0.63 in control group and 0.64±0.63 in test group, P_{value} showed 0.272. Level of education in diploma mean and SD values were 0.26±0.59 under control group, 0.26±0.593 under test group, P_{value} showed 0.5 (Table 01).In 30 hyperlipidemia patient mean ±SD of cholesterol is 209.9±0.2 in control group, 209.8±0.6 in test group, P value is 0.33 at baseline. After 6 weeks of the cholesterol mean and standard deviation values are 209±0.3 in control group, 179. ±0.2 in test group, p_{value} is 0.001. After 12 weeks mean ±SD values 208.0±0.2 in control group, 139.9±0.2 in test group, P_{value} is 0.0001. P value is very signification in cholesterol variable. Triglycerides base line mean ±SD values are 149.86±0.51 in control group, 159.9±0.2 in test group, P_{value} is 0.16. After 6 weeks mean ± SD values were 137.9±0.2 in control group, 132.8 ±5.9 in test group, P_{value} is 0.001. After 12 weeks mean ± SD values were 137.0 ±0.2 in control group, 97.6 ±1.2 in test group, P_{value}e is 0.001. In low density lipoprotein base line values were 32.6±1.7 in control group, 149.93±0.2 in test group, P_{value} is 0.33. After 6 weeks mean ± SD values were 31.9 ±0.2 in control group, 130.6 ± 1.6 in test group, P_{values} 0.001. After 12 weeks mean ± SD values were 30.9 ± 0.2 in control group, 100.1 ± 0.5 in test group, P_{value} is 0.0001. HDL base line mean ± SD values were 32.6 ±1.7 in control group, 32.06 ± 2.2 in test group, P_{value} 0.17. After 6 weeks mean ± SD values were 31.96±0.2 in control group, 44.8±0.7 in test group, P_{value} is 0.001. After 12 weeks mean ± SD value is 30.93 ± 0.2 in control group, 50.4 ± 1.8 in test group, P_{value} is 0.0001 (Table 02).

Results of systolic blood pressure (mm of Hg) mean ± SD values were 139.4 ± 0.62 in control group, 139.0 ± 2.0 in control group, P value is 0.20. After 6 weeks mean ± SD values were 139 ±0.76 in control group, 129.8 ± 0.50 in test group, P value is 0.001. After 12 weeks mean ± SD values were 138.06 ± 0.5 in control group, 120.6 ± 1.6 in test group, P_{values} is 0.0001. In Diastolic blood pressure mean ± SD values were 89.6 ±0.8 in control group, 89.7 ±0.7 in test group, P_{value} is 0.29. After 6 weeks mean ± SD values were 86.9±0.2 in control group, 83.8±0.5 in test group, P_{values} 0.001. After 12 weeks mean ± SD values were 86.0 ±0.2 in control group, 80.0±0.2 in test group, P_{values} is 0.0001 (Table 03).

BMI mean ± SD values 39.93 ±0.25 in control group, 39.9 ± 0.25 in test group, P_{value} is 0.5. After 6 weeks mean ± SD values were 39.06±0.25 in control group, 35.06±0.25 in test group, P_{values} 0.01. After 12 weeks mean ± SD values were 2=38.06±0.25 in control group, 25.06±0.25 in test group, P_{Value} is 0.0001 (Table 04). Liver enzymes mean ±SD values were 56.86 ±0.35 in control group, 56.86±0.35 in test group, P values is 0.5 at AST baseline. After 6 weeks mean ± SD values were 56.2 ±0.41 in control group, 45.7 ± 1.9 in test group, P values 0.01. After 12 weeks mean ± SD values were 55.2 ± 0.41 in control group, 37.06 ± 0.88 in test group, P value is 0.0001. ALT base line mean ± SD values were 80.2 ± 0.5 in control group, 80.3 ± 0.7 in test group, P value is 0.38. After 6 weeks mean ± SD values were 78.8 ± 0.4 in control group, 70.8 ±2.3 in test group, P value is 0.01.After 12 weeks mean ± SD values were 77.9 ±0.2 in control group, 63.2 ± 0.56 in test group, P value is 0.0001. In ALKP base line mean ± SD values were 145.86 ± 0.35 in control group, 145.93 ± 0.56 in test group, P_{value} is 0.16. After 6 weeks mean ± SD values were 144.93 ±0.25 in control group, 136.2 ±0.56 in test group, P_{value} is 0.001. After 12 weeks mean ±SD values were 143.93 ± 0.25 in control group, 126. 06 ± 0.25 in test group, P_{value} is 0.0001 (table 05).

DISCUSSION

According to our data and previous research lettuce seed does not have a serious side effect in therapeutic doses. Also, in this study we observed that the serum level of liver enzymes like ALT, AST and ALKP were P value significant in test group.

Some studies showed that green-leaf lettuce contains water-soluble, antioxidant compounds such as phenolic acids, flavonoids, anthocyanins, lactucin, vitamins A and C. Fischedick et al. investigated lipid-lowering activity (in vitro) of sesquiterpene lactones as potential hypolipidemic agents. In a clinical trial by Asgary *et al*., antihyperlipidemic effects of *Achillea wilhelmsii* were attributed to its flavonoids and sesquiterpene lactones. In a study by Hall *et al*., the possible mechanisms of antihyperlipidemic activity of sesquiterpene lactones were also investigated in vitro. Chadchan and Li et al., Some sesquiterpene lactones which can be detected in lettuce are lactucin, desoxylactucin, lactucopicrin, lactucin-15-oxalate, and lactucopicrin-15-oxalate. The above mentioned components may play a role in antihyperlipidemic activity of lettuce seed extract. Also, we hope that this study will help pave the way for other researchers to join in the attempt to bridge the gap between alternative medicine and evidence based medicine.

CONCLUSION

In this pilot study, as evident from mentioned results, the hypolipidemic effect of lettuce seed could be related to lactucin, desoxylactucin, lactucopicrin, lactucin-15-oxalate, and lactucopicrin-15-oxalate as the main antioxidant constituents in lettuce seed extract. However, further researches are required to clarify the mechanism of this effect.

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