ORIGINAL ARTICLE

Antihyperlipidemic Activity of Leaf *Extracts of Leucas* aspera Linn. against Dexamethasone-induced Hyperlipidemia in Rats

G. Vijay Kumar[1], N. Devanna[2]

[1]Department of Pharmaceutical Chemistry, Jangaon Institute of Pharmaceutical Sciences, Warangal, Telangana, India, [2]Department of Chemistry, College of Engineering, Jawaharlal Nehru Technological University, Ananthapur, Andhra Pradesh, India

Abstract

Introduction: Natural products derived from plants kingdom play a vital part in preventing or treating various diseases or disorders in humans. Hyperlipidemia is one of the major pathological factors of cardiovascular diseases and diabetes mellitus. On the other hand, *Leucas aspera* Linn., belonging to the family Lamiaceae, was found to possess many pharmacological activities such as anti-inflammatory, antibacterial, and antiplasmodial activities along with cytotoxic effects. **Materials and Methods:** The present study is an attempt to investigate its antihyperlipidemic activity by *in vivo* animal model. Hyperlipidemia model can be induced by administered with dexamethasone in rats with significant increase in serum cholesterol and triglyceride (TG) levels along with increase in the atherogenic index. **Results:** The ethanolic extract of leaves of *L. aspera* Linn. (200 and 400 mg/kg) treatment has shown significant inhibition against dexamethasone-induced hyperlipidemia in rats by maintaining the serum levels of cholesterol, TGs and near to the normal levels.

Key words: Dexamethasone, hyperlipidemia, *Leucas aspera*

INTRODUCTION

Around 80% of the world population uses the herbal medicines for primary health care mostly in the developing countries.[1] Because of their safety, efficacy, cultural acceptability, better compatibility with the human body and lesser side effects, they stood still. There was mention about the usage of herbal medicines for age-related diseases such as memory loss, diabetic wounds, osteoporosis, and immune and liver disorders in various ancient literatures, for which no modern medicine or only palliative therapy is available. Some of the life-saving and essential drugs discovered from medicinal plants such as digoxin, morphine, emetine, aspirin, and ephedrine were known to modern therapeutics several centuries ago. There was a statement described by Namdeo about secondary metabolites derived from plants; he stated that about a 1/4th of all suggested pharmaceuticals in developed countries containing compounds that are directly or indirectly derived from plants.[2] There is a belief that green medicine is safe and trustworthy.

Today, there is a widespread curiosity in drugs derived from plants. At present, many pharmaceutical companies are concentrating extensive research on plant materials for their potential medicinal value.[3] As per the World Health Organization, 4 billion people (80%) of the world population are using plant-derived products as medicine for some aspect of primary health care. Out of 119 plant-derived medicines, approximately 74% are used nowadays that are directly correlated with their traditional practice as plant medicines by native cultures.

Atherosclerosis is one of the leading causes of death in the world both in developed countries and as well as developing

Address for correspondence:
G. Vijay Kumar, Department of Pharmaceutical Chemistry, Jangaon Institute of Pharmaceutical Sciences, Yeshwanthapur, Janagoan, Waranagal - 506 167, Telangana, India. Ph No: +91-9989734472.
E-mail: vkgugulothu@gmail.com

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countries like India.[4] The elevated levels of low-density lipoprotein (LDL) and very LDLs (VLDLs) associated with cholesterol and triglycerides (TGs) is one of the primary risk factors for atherosclerosis. By targeting the atherogenic process, we can treat hyperlipidemia is one of the palliative treatment approaches for atherosclerosis.[5] A wide number of allopathic antihypolipidemics are available in the market, but they were not popularized due to their side effects and contraindications. To overcome that recently herbal hypolipidemics have gained importance to fill the voids.[6]

On the other hand, *Leucas aspera* was used as a folklore medicine for numerous ailments. The aerial parts of the *Leucas* were used medicinally for reducing pain and swelling. Recent pharmacological studies have shown hepatoprotective, antioxidant activity, and antinociceptive activities.[7,8] The antimicrobial, antimalarial, larvicidal, and antipsoriatic activities of *Leucas* were well established.[9-12] It was also found to possess antiplasmodial and pupicidal activities.[13] Apart from that, the plant extract was found to possess cytotoxic effect, especially against lymphoma cells.[14,15] In addition to that, *Leucas* plant active constituents were found to inhibiting prostaglandin-induced contractions and able to control arthritis.[16] Moreover, it can also be used against thrombolysis and ulcer.[17] The antihyperlipidemic effects of *L. aspera* Linn. were not reported elsewhere. The present investigation was to carry out the antihyperlipidemic effect of ethanolic extract of *L. aspera* Linn. (EELLA) against dexamethasone-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Chemicals

The solvents used for the study (petroleum ether and ethanol) were obtained from SD Fine Chemicals India Ltd. and were of laboratory grade. Gemfibrozil was purchased from Medindia, dexamethasone and all other chemicals used in the present study were purchased from Merck, India and of analytical grade.

Plant materials

L. aspera plant leaves were collected in the month of August 2015 from Warangal, Telangana, India. The plant was then taxonomically identified and authenticated by the botanist in the Department of Botany, Kakatiya University, Warangal.

Experimental animals

Male Wister rats (150-180 g) of approximately the same age, obtained from Teena labs, Hyderabad, India, were used for the study. They were kept in polypropylene animal cages and fed with normal rodent pellet diet (Hindustan Lever Limited, Hyderabad) and water *ad libitum*. All the animals were exposed to an alternate cycle of 12 h of darkness and 12 h of light. Before each test, the animals were withdrawn from food for at least 12 h and the experimental protocols were designed as per the guidelines of the Institutional Animal Ethic Committee (1046/ac/09/CPCSEA, Dated 24-09-2015) and were approved by the same. All the experiments were carried out in the morning as per the current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The standard oral gastric cannula and syringe were used for drug administration in experimental animals.[18]

Methodology

Extraction of L. aspera Linn. leaves

The plant leaves were dried in shade, after confirmation of the moisture content limits, the dried leaves were coarsely powdered using a mechanical grinder. Then, the powder was passed through sieve No. 40 and stored in an airtight container for the extraction.

Preparation of extracts[19]

Petroleum ether extract of L. aspera Linn.

The powder of the dried leaves of *L. aspera* Linn. was extracted with petroleum ether at temperature 60-80°C up to 48 h. After completion of extraction, the solvent was filtered, and the powder was separated. The crude extract was subjected to distillation and solvent was removed. Dark green-colored residue was obtained, and it was stored in desiccators.

Ethanol extract of L. aspera Linn.

The marc left after petroleum ether extraction was dried and then extracted with 95% ethanol at 75-78°C up to 48 h. After completion of extraction, the solvent was distilled off from the crude extract. Dark brown-colored residue was obtained. The residue was concentrated and then stored in desiccators.

Aqueous extract of L. aspera Linn.

The marc left after ethanol extraction was dried and then macerated with distilled water, up to 48 h. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown color residue was obtained, and it was stored in desiccators. The percentage yields of the above extract were shown in Table 1.

Phytochemical evaluation

The residues obtained from the crude extracts were subjected to phytochemical investigation by specific chemical tests for plant secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, carbohydrates, saponins, phenolic

compounds, terpenoids, fixed oils, and steroids.[20,21] The evaluation showed the presence of alkaloids, glycoside, carbohydrate, saponins, phytosterols, and flavonoids [Table 2]. Ethanol extract of *L. aspera* Linn. was found to contain the main phytoconstituents. Hence, it was taken for pharmacological evaluation.

Pharmacological evaluation

Dexamethasone-induced hyperlipidemia model

Glucocorticoid hormonal level elevation induces the plasma lipid concentration but varies from species to species. Few synthesis of triacylglycerol in the liver is stimulated by the injection of glucocorticoid in rats and consequently may lead to the accumulation of fatty liver. The stimulation of the TG production could lead to increased secretion of VLDL. Increasing VLDL secretion has been reported when dexamethasone is injected for several days in rats. The increase in TG level induces imbalance in lipid metabolism leads to hyperlipidemia. Similarly, dexamethasone treatment in newborn rats for 4 days showed widespread increase in serum lipids.[22,23]

Dexamethasone-induced hyperlipidemia in rats[16]

Induction of hyperlipidemia

Hyperlipidemia will be induced using dexamethasone; a glucocorticoid is known to evoke plasma lipid elevation. Dexamethasone (10 mg/kg/day, subcutaneous) was administered to rats for 8 days to induce hyperlipidemia. The animals were divided into five groups each group contains six rats details were shown in Table 3.

Procedure

All the animals in Groups II, III, IV, and V were subjected to subcutaneous injection of dexamethasone (10 mg/kg/day, S.C) for 8 days to induced hyperlipidemia. The animals in normal and hyperlipidemic control groups were received normal saline, whereas Group III animals received gemfibrozil (10 mg/kg/day I.P, suspended in gum acacia in water) and Groups IV and V animals received extract by oral route in doses of 200 mg/kg/day and 400 mg/kg/day, respectively, throughout the 8 days experiment.

Table 1: The percentage yields of the *L. aspera* Linn. leaves extracts

Plant name	Part used	Method of extraction	Yield in percentage %		
			Petroleum ether extract	Ethanol extract	Aqueous extract
L. aspera Linn	Leaves	Continuous hot percolation	3.0	7.0	15.8

L. aspera: *Leucas aspera*

Table 2: Phytochemical investigation of *L. aspera* Linn. leaves extracts

Phytochemicals	Tests	Petroleum ether extract	Ethanol extract	Aqueous extract
Carbohydrates	Fehling's test	+	+	+
Phenolics	Ferric chloride test	-	+	+
Terpenes	Cupperacetate test	-	+	-
Alkaloids	Mayer's test	+	+	-
Flavonoids	Alkaline reagent test	-	+	+
Glycosides	Borntrager's test	-	-	-
Steroids	Liebermann test	+	+	-
Saponins	Foam test	+	-	+

L. aspera: *Leucas aspera*

Table 3: Grouping of animals for pharmacological screening

Details	Group I	Group II	Group III	Group IV	Group V
Group title	Normal control	Hyperlipidemic control	Standard group	Test Group I	Test Group II
Number of animals	6	6	6	6	6
Treatment	Normal saline	Dexamethasone (10 mg/kg/day, S.C) was given to rats for 8 days	Gemfibrozil (10 mg/kg/day, I.P) along with dexamethasone treatment	*L. aspera* Linn. ethanolic extract (200 mg/kg/day, P.O) with dexamethasone treatment	*L. aspera* Linn. ethanolic extract (400 mg/kg/day, P.O) with dexamethasone treatment

L. aspera: *Leucas aspera*

After the experimental period, the overnight fasted experimental rats were sacrificed by decapitation under light ether anesthesia and blood was collected. Serum was separated, and lipid profiles (biochemical parameters) were analyzed. The lipid profiles of dexamethasone-induced hyperlipidemia model and the results of the antihyperlipidemic effect of extract treated groups of dexamethasone-treated groups were shown in Table 4.

Statistical evaluation

All the values were expressed as mean ± standard error of mean. The data were statistically analyzed by one-way ANOVA followed by Dennett's *t*-test, and value $P < 0.05$ was considered to be significant.

RESULTS

The present study was carried out to assess the antihyperlipidemic effect of EELLA against dexamethasone-induced hyperlipidemia in male Wister rats. When EELLA was evaluated for its antihyperlipidemic activity against dexamethasone-induced hyperlipidemia model, it showed a statistically significant activity at doses of 200 and 400 mg/kg by oral administration. After 8 days treatment of dexamethasone, a significant rise in lipid and lipoprotein levels were observed in serum in dexamethasone-induced group when compared to the normal group. The results were depicted in Table 4.

Effect of EELLA in biochemical parameters in serum

Effect on total cholesterol and total TGs

Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 117.83 ± 1.687 mg/dl compared to Group I (normal rat group), in which values lie in the range 65 ± 1.352 mg/dl. This indicates hypercholesterolemia. In the treatment group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values are reduced to 83 ± 2.307 ($P < 0.001$) and 79.0 ± 2.387 mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in EELLA treatment group. On the other hand, gemfibrozil also has significantly reduced serum total cholesterol levels to 71.50 ± 1.352 mg/dl ($P < 0.001$) [Table 4].

The TG levels have reached as 151.83 ± 1.667 mg/dl in dexamethasone-induced group compared to normal rats where the values are 63.83 ± 1.777 mg/dl. This indicates triglyceridemia. In the group treated with EELLA (200 mg/kg) and EELPO (400 mg/kg), the values are significantly reduced to 78.16 ± 1.687 mg/dl ($P < 0.01$) and 74.16 ± 1.687 mg/dl ($P < 0.01$), respectively. In the gemfibrozil treated group, the values are reduced to 67.33 ± 0.764 mg/dl ($P < 0.001$) [Table 4].

Effect on phospholipids

Phospholipids are amphipathic lipid constituents of a membrane. They play an essential role in the synthesis of plasma lipoproteins. They function in transduction of messages from cell-surface receptors to certain messengers that control cellular processes and as surfactants.[19]

Phospholipid levels in the dexamethasone-induced group have significantly increased compared to normal rats. The values have risen to 132.1 ± 2.983 mg/dl compared to normal rat group, in which values lie in the range 92.73 ± 1.166 mg/dl. In the treatment group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values are reduced to 104.65 ± 1.777 mg/dl ($P < 0.01$) and 99.32 ± 1.721 mg/dl ($P < 0.01$), respectively. There is a significant reduction in phospholipid values in EELLA treatment group. On the other hand, gemfibrozil also has significantly reduced serum phospholipid levels to 95.37 ± 1.515 mg/dl ($P < 0.001$) [Table 4].

Table 4: Effect of EELLA against dexamethasone-induced hyperlipidemia in rats

Group	Treatment/dose	Total cholesterol (mg/dl)	Total TG (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
I	Normal-control	65±1.352	63.83±1.777	38.66±1.687	13.66±0.333
II	Dexamethasone (10 mg/kg) S.C	117.83±1.687	151.83±1.667	26.16±0.307	54.33±1.687
III	Dexamethasone (10 mg/kg) S.C+gemfibrozil (10 mg/kg) P.O	71.50±1.352*	67.33±0.764*	34.33±0.421*	21.66±0.33*
IV	Dexamethasone+EELLA-I	83.0±2.307*	78.16±1.687*	24.33±0.33*	31.5±0.223
V	Dexamethasone+EELLA-II	79.0±2.387*	74.16±1.687*	28.33±0.333*	27.5±0.223

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and values $P<0.5$ were considered to the significant. *$P<0.001$; **$P<0.01$ versus control. SEM: Standard error of mean, EELLA: Ethanolic extract of *L. aspera* Linn., TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

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Table 5: Effect of EELLA against dexamethasone-induced hyperlipidemia in rats

Group	Treatment/dose	VLDL-cholesterol (mg/dl)	Atherogenic index	Phospholipids (mg/dl)	Free fatty acid (mg/dl)
I	Normal-control	13.16±0.307	1.65	92.73±1.166	20.37±0.396
II	Dexamethasone (10 mg/kg) S.C	38.33±1.542	4.50	132.1±2.983	35.1±0.152
III	Dexamethasone (10 mg/kg) S.C+gemfibrozil (10 mg/kg) P.O	18.16±0.307**	2.21	95.37±1.515*	22.62±0.223*
IV	Dexamethasone+EELLA-I	29.33±0.333**	3.41	104.65±1.777**	27.73±0.307*
V	Dexamethasone+EELLA-II	25.33±0.333**	2.78	99.32±1.721**	26.37±0.258*

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and values $P<0.5$ were considered to the significant. *$P<0.001$; **$P<0.01$ versus control. SEM: Standard error of mean, EELLA: Ethanolic extract of *L. aspera* Linn., VLDL: Very low-density lipoprotein

Effect on free fatty acid

Free fatty acid levels in dexamethasone-induced group have significantly increased compared to normal rats. The values have risen to 35.1 ± 0.152 mg/dl compared to normal rat group, in values lie in the range 20.37 ± 0.396 mg/dl. In the treatment group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values are reduced to 27.73 ± 0.307 ($P < 0.001$) and 26.37 ± 0.258 mg/dl ($P < 0.001$), respectively. There is a significant reduction in free fatty acid values in EELLA treatment group. On the other hand, gemfibrozil also has significantly reduced serum free fatty acid levels to 22.62 ± 0.223 mg/dl ($P < 0.001$) [Table 4].

Effect on high-density lipoprotein (HDL) cholesterol

HDL-cholesterol in dexamethasone-induced group has significantly decreased compared to normal rats. The values have reduced to 26.16 ± 0.307 mg/dl compared to normal rat group, 38.66 ± 1.687 mg/dl. In the group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values were 24.33 ± 0.3 ($P < 0.01$) and 8.33 ± 0.333 mg/dl ($P < 0.01$), respectively. In gemfibrozil treated group, the values were 34.33 ± 0.421 mg/dl ($P < 0.001$) [Table 4].

Effect on LDL-cholesterol and VLDL-cholesterol

LDL-cholesterol in dexamethasone-induced group has significantly increased to 54.33 ± 1.687 mg/dl compared to normal rat group, 13.66 ± 0.333 mg/dl. In the group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values were reduced to 31.5 ± 0.223 mg/dl ($P < 0.001$) and 27.5 ± 0.223 mg/dl ($P < 0.001$), respectively. There is a significant reduction in LDL-cholesterol values in EELLA treatment group. Gemfibrozil has significantly reduced LDL-cholesterol level to 21.66 ± 0.33 mg/dl ($P < 0.001$) [Table 4].

VLDL-cholesterol in dexamethasone-induced group has significantly increased to 38.33 ± 1.542 mg/dl compared to normal rat group, 13.16 ± 0.307 mg/dl. In the group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values are reduced to 29.33 ± 0.333 ($P < 0.01$) and 25.33 ± 0.333 mg/dl ($P < 0.01$), respectively. There is a significant reduction in EELLA treatment group. Gemfibrozil has significantly reduced VLDL-cholesterol level to 18.16 ± 0.307 mg/dl ($P < 0.001$) [Tables 4 and 5].

Effect on atherogenic index

$$\text{Atherogenic index} = \frac{\text{Total serum cholesterol}}{\text{Total serum HDL-cholesterol}}$$

Atherogenic index in dexamethasone-induced hyperlipidemia control is increased to 4.50 compared to normal rat group, 1.65. In the group treated with EELLA (200 mg/kg) and EELLA (400 mg/kg), the values are significantly reduced to 3.41 and 2.78, respectively. Gemfibrozil has significantly reduced the values to 2.21 [Table 5].

DISCUSSION

Treatment with EELLA produced a significant decrement in the serum level of lipids in the dexamethasone-induced hyperlipidemia in male Wistar rats. Beta-sitosterol a phytosterol is found to be useful in the treatment of hyperlipidemia. Hypolipidemic effect of proteins, gums, saponins, and beta-sitosterol have been reported by several authors. The EELLA contains carbohydrates, glycosides, alkaloids, tannins, saponins, phenolic compounds, phytosterols, and flavonoids. The steroidal phytoconstituents may mimic the cholesterol at the formation of lipoproteins and chylomicrons. Moreover, many of the proven antihyperlipidemic drugs were possessing the similar pharmacophore with the cholesterol. The high amount of phytosterols and alkaloids may be responsible for the hypolipidemic effect. It was found that EELLA was more effective in higher dose as compared to lower dose as an antihyperlipidemic agent against dexamethasone-induced hyperlipidemia model. It also improves that HDL-cholesterol levels and lower atherogenic index. Further experiments are required to prove the mechanism and advantage of EELLA over other drugs.

CONCLUSION

These results suggested that EELLA possesses a significant antihyperlipidemic activity in dexamethasone-induced hyperlipidemia in male Wistar rats. The isolation of the active phytochemical constituents is underway.

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