Research Article

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STHOULYAHARA KARMA OF ELA (*ELETTARIA CARDAMOMUM* MATON.) BEEJA WITH SPECIAL REFERENCE TO DYSLIPIDEMIA: AN EXPERIMENTAL STUDY

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ABSTRACT

Sthoulya (Obesity) is one among the major disorders of Modern era. Introduction of pharmacologically proved better remedy is the need of the hour. Aim of this study is *In-vitro* studies to determine the Anti-dyslipidemic activity was done. Evaluation of Anti-dyslipidemic activity of *Ela* (*Elettaria cardamomum* Maton.) *beeja* by Lipase inhibition, Adipogenesis and Adipolysis assay was adapted. The alcoholic extractive form of *Ela beeja* have shown potent Anti-dyslipidemic activity against Lipase inhibition assay whereas Alcoholic extractive form of *Ela beeja* did not show any positive response against Adipogenesis and Adipolysis assay in tested concentrations.

Keywords: Ela (*Elettaria cardamomum* Maton.), Dyslipidemia, Sthoulya, Lipase inhibition, Adipogenesis, Adipolysis etc.

INTRODUCTION

In the fast paced life of today's world the lifestyles of the people have undergone a major shift. These changes have had a telling impact on the health of the individuals. According to Ayurveda, *Sthoulya* (obesity) is one among *santarpanajanya rogas* (diseases caused due to over nourishment) which is regarded as *ninditha vishesha* (factors to be avoided) and there is mention of eight various complications[1]. Because of luxurious life and sedentary habits bodily fats along with cholesterol are increasing in our body, which invites the disorders like Hypertension, Heart diseases and Hyperlipidaemia. Hyperlipidaemia is a condition in which the levels of lipoproteins i.e. cholesterol, triglycerides or both are raised in plasma to the extent that it may have an adverse effect on health, leading to reduced life expectancy. The complexity and pervasiveness of the Hyperlipidaemia in every aspect of people's lives makes it a challenging and rewarding area for research. Hyperlipidaemia has recently emerged as one of the most important preventable and modifiable risk factors of Coronary Artery Disease and was implicated as one of the four major established conventional risk factors, besides cigarette smoking, Diabetes and Hypertension. Death rate from cardiovascular disease is about 60-70 times greater in people with increased lipid levels in blood. Statins are the approved anti dyslipidemic drugs but are withdrawn because of their potential side effects. According to classics *Ela* (*Elettaria cardamomum* Maton.) is known for its *Sthoulyahara* and *Medohara karma* (Anti Obesity)[2].

Hence with an aim at evaluating the efficacy of the *Ela beeja* (*Elettaria cardamomum* Maton.) as *Sthoulyahara* (Anti obesity activity) with special reference to Dyslipidemia, an experimental study is being attempted.

MATERIALS AND METHODS

Evaluation of Anti-dyslipidemic activity of *Ela* seeds (*Elettaria cardamomum* Maton.) by[3]

a) Lipase inhibition assay
b) Adipogenesis assay -3T3-L1 cell line
c) Adipolysis assay- 3T3-L1 cell line

Lipase inhibition assay

Principle

The assay is based on the conversion of the substrate 4-Methylumbelliferyl oleate to 4- Methylumbelliferone. The product generated is measured at an excitation of 360 nm and emission of 460 nm.[4]

4-Methylumbelliferyl oleate to 4-Methylumbelliferone

Procedure

Lipase inhibition assay was carried out as per the US patent method US2008/0317821 A1[5]. In brief, the total reaction volume of 50 µl contained 11.7 mM Tris buffer /positive control / test sample at various concentrations, 1.160 U/ml of lipase enzyme, 5 µl of De-mineralized water and 0.2 mM of substrate (4-Methylumbelliferyl Oleate). The plate was mixed and the change in fluorescence was determined at 25°C for 20 min at an Excitation of 360 nm and Emission of 460 nm using Fluostar Optima. Positive (Reference) control used in the assay was Orlistat.

Calculations

Mean of the Relative fluorescence unit (RFU) of all samples tested in triplicates were calculated. From the mean values Percentage inhibition (% I) of samples was calculated using the equation:

$$\% I = \frac{(\text{RFU of Control} - \text{RFU of Sample})}{(\text{RFU of Control})} \times 100$$

IC_{50} is that concentration of the inhibitor where the enzyme activity is inhibited by 50%. IC_{50} was calculated by the Finney software.

Adipogenesis assay

The 3T3 L1 cells used in this study was obtained from ATCC were maintained as cryo-preserved stocks in liquid nitrogen container.[6]

Controls

Vehicle control

DMSO at a concentration of 0.1% v/v was used as vehicle control.

Table 1: Reference control

S. No	Chemical	Concentration (µM)	Exposure time (hours)
1	Guggulsterone	20	48 + 48

The sources and grades of positive control drugs are indicated in Table

Table 2: Source of Positive controls

S. No	Chemical	Make	CAS. number	Lot number	Purity
1	Guggulsterone	Natural remedies	39025-24-6	T13D025	≥ 98%

Dose levels

Selection of Solvent

Prior to commencing testing the solubility of the test substance was assessed at 100 mg/mL in DMSO in which it was found to dissolve. Therefore DMSO was chosen as the solvent for the study.

Dose range finding study

The growth inhibitory effect (cytotoxicity) of the test article to the test system was determined in order to allow the selection of appropriate doses to be tested in the adipogenesis assay.

Design

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] test was performed to determine the dose range for the study. Five doses of test article were tested. The test drug was checked for cytotoxicity up to a maximum concentration of 100 µg/mL. The dose levels tested were 3.12, 6.25, 12.5, 25, 50 and 100 µg/mL.

Evaluation of the Dose Range finding Study

Cytotoxicity was determined by a decrease in the optical density compared to the solvent control.

Selection of the Maximum Dose for the Collagen Release Assay

Since no cytotoxicity was observed in the dose range finding study, the highest dose level of test drug, *Ela* (*Elettaria cardamomum* Maton.) Alcoholic extract used in the adipogenesis assay was determined to be 100 µg/mL.

Assay Procedure

Each 48 well plate was labelled with a code system that identified the test substance, test phase, dose levels, and cell line details. Murine 3T3-L1 fibroblasts were adjusted to be 30,000 cells/well in DMEM supplemented with 10% BCS, and 500 µl of the cell suspension were plated into 48-well culture plate and incubated at 37 °C in a humidified 5% CO_2 incubator for 16 h overnight. The medium was changed to DMEM supplemented with 5% FBS, 0.5 mM IBMX, 1 µg/mL Insulin, and 1 µM DEXA, and further incubated for 2 days (initiation of differentiation). The medium was changed to DMEM supplemented with 5% FBS and 5 µg/mL of insulin, and was further incubated for 2 days. Each test sample of desired dilution and positive control were added from the initiation of differentiation (day 0) to day 4. Thereafter, the medium was changed to normal culture medium (DMEM supplemented with 5% FBS), and was freshly replaced every 48 h. On day 8, cells were stained with Oil-red O. For Oil-red O staining, 3T3-L1 adipocytes were washed with PBS and fixed with 10% formalin for 15 min. remove formalin and add 60 % isopropanol and allow it to dry. After 10 min remove the isopropanol and add 40 µl/well of oil red and incubate for 20 min. Wash the plate in distilled water for 4-5 times, remove the traces of water by drying and add 100 % isopropanol and mix well. Oil red is quantified by measuring the absorbance at 500 nm.

Adipolysis assay

Principle

The glycerol present in the test samples react with working reagent to give a coloured product which is calorimetrically measured at 570 nm. The colour intensity is directly proportional to the amount glycerol released from the cells. The 3T3 L1 cells used in this study was obtained from ATCC is maintained as cryopreserved.[7,8]

Controls

Vehicle control

DMSO at a concentration of 0.1% v/v was used as vehicle control.

Table 3: Reference control

S. No	Chemical	Concentration (µM)	Exposure time (hours)
1	Isoproterenol	10	4

The sources and grades of positive control articles are indicated in Table 2

Table 4: Source of Positive controls

S. No	Chemical	Make	CAS. number	Lot number	Purity
1	Isoproterenol	Sigma	51-30-9	055K1021	---

Dose levels

Selection of Solvent

Prior to commencing testing the solubility of the test drug was assessed at 100 mg/mL in DMSO in which it was found to dissolve. Therefore DMSO was chosen as the solvent for the study.

Dose range finding study

The growth inhibitory effect (cytotoxicity) of the test drug to the test system was determined in order to allow the selection of appropriate doses to be tested in the adipolysis assay.

Design

Evaluation of the Dose Range finding Study

Cytotoxicity was determined by a decrease in the optical density compared to the solvent control.

Selection of the Maximum Dose for the Collagen Release Assay

Since no cytotoxicity was observed in the dose range finding study, the highest dose level of test drug, *Ela* (*Elettaria cardamomum* Maton.) Alcoholic extract used in the adipolysis assay is determined to be 100 µg/mL.

Assay Procedure

Each 48 well plate is labelled with a code system that identified the test substance, test phase, dose levels, and cell line details. Murine 3T3-L1 fibroblasts were adjusted to be 4 x 10^4 cells/mL in DMEM supplemented with 10% BCS, and 500 µl of the cell suspension are plated into 48-well culture plate and incubated at 37° C in a humidified 5% CO_2 incubator for 16 h overnight. The medium is changed to DMEM supplemented with 5% FBS, 0.5 mM IBMX, 1 µg/mL Insulin, and 1 µM Dexamethasone, and further incubated for 2 days (initiation of differentiation). The medium is changed to DMEM supplemented with 5% FBS and 5 µg/mL of insulin, and is further incubated for 2 days. Change the media with 5 % FBS for consecutive three days, on day 7 starve the cells overnight with DMEM containing 2% BSA. On day 8, treat the cells with test item and positive control for 4 hours in KRB buffer (pH 7.2). Collect the supernatant and estimate the glycerol.

Glycerol estimation

Collect the supernatant from the tissue culture plate. Add 100 µl of glycerol working reagent and 10 µl of sample/standard in 96 well assay plates. Tap the plate to mix and incubate at room temperature for 20-30 min. Read the colour intensity at 570 nm. The colour intensity should be directly proportional to the amount of glycerol present in the sample.

Observations and Results

Lipase Inhibition Assay

Ela (*Elettaria cardamomum* Maton.) Alcoholic extract were found to inhibit Lipase enzyme activity with an IC_{50} of 16.95 µg/ml .
Ela (*Elettaria cardamomum* Maton.) aqueous extract did not show any appreciable inhibition of lipase activity up to the tested concentration of 200 µg/ml.

Table 5: IC_{50} data of test samples and standard in Lipase inhibition assay

Sample	Concentration (µg/ml)	% Inhibition (Mean ± SEM)	IC_{50} (µg/ml) (95% Confidence Interval)
Orlistat (Reference inhibitor)	5 ng/ml	14.34 ± 1.96	32.94 ng/ml (25.70-43.75)
	10 ng/ml	33.04 ± 1.61	
	25 ng/ml	49.37 ± 1.08	
	50 ng/ml	57.09 ± 1.17	
	100 ng/ml	66.88 ± 0.52	
***Ela* (*Elettaria cardamomum* Maton.) aqueous Ela. AQ**	2.47	10.86 ± 1.95	**
	7.41	16.02 ± 0.76	
	22.22	23.71 ± 7.47	
	66.67	11.06 ± 5.82	
	200	11.91 ± 1.27	
***Ela* (*Elettaria cardamomum* Maton.) alcoholic Ela. Alc**	2.47	14.41 ± 1.32	23.18 (18.69-28.75)
	7.41	27.33 ± 0.80	
	22.22	30.97 ± 5.41	
	66.67	76.23 ± 0.66	
	200	95.07 ± 0.46	
	7.41	12.14 ± 1.16	
	22.22	28.18 ± 3.01	
	66.67	44.61 ± 1.18	
	200	77.30 ± 0.71	

Adipogenesis Assay

Cytotoxicity assay

Table 6: Cytotoxicity assay: Ela

	Dose (µg/mL)	Test system: 3T3-L1 (P/N # 06)	
		OD Mean ± SD	% Cytotoxicity
Cell control	0	111.00 ± 2.93	---
DMSO	0.1 %	100.00 ± 5.89	---
***Ela* (*Elettaria cardamomum* Maton.) Alcoholic**	6.25	85.50 ± 5.28	NS
	12.5	90.80 ± 11.90	NS
	25	92.30 ± 4.75	NS
	50	93.10 ± 3.47	NS
	100	97.40 ± 5.10	NS

NS – Not significant

Adipogenesis assay

Table 7: Adipogenesis assay: Ela Alcoholic extract

	Dose (µg/mL)	Test system: 3T3 L1 (P/N # 06)	
		Mean ± SD	% Inhibition
Undifferentiated	00	27.30 ± 18.90	---
DMSO	0.1 %	100.00 ± 23.90	---
Ela (*Elettaria cardamomum* Maton.) Alcoholic	3.1	145.00 ± 05.76	NS
	6.21	119.00 ± 09.04	NS
	12.5	176.00 ± 13.20	NS
	25	137.00 ± 01.64	NS
	50	149.00 ± 15.60	NS
	100	160.00 ± 13.20	NS
Guggulsterone	20 (µM)	05.10 ± 03.29	95 *

* - P < 0.05, NS – Not significant

Adipolysis Assay

Cytotoxicity assay

Table 8: Cytotoxicity assay: Ela Alcoholic extract

	Dose (µg/mL)	Test system: 3T3-L1 (P/N # 06)	
		OD Mean ± SD	% Cytotoxicity
Cell control	0	111.00 ± 2.93	---
DMSO	0.1 %	100.00 ± 5.89	---
Ela (*Elettaria cardamomum* Maton.) Alcoholic extract	6.25	85.50 ± 5.28	NS
	12.5	90.80 ± 11.90	NS
	25	92.30 ± 4.75	NS
	50	93.10 ± 3.47	NS
	100	97.40 ± 5.10	NS

NS – Not significant

Adipolysis assay

Table 9: Adipolysis assay: Ela Alcoholic extract

	Dose (µg/mL)	Test system: 3T3 L1 (P/N # 06)	
		Mean ± SD	Fold Increase
DMSO	0.1 %	2.26 ± 0.45	---
Ela (*Elettaria cardamomum* Maton.) Alcoholic extract	3.1	2.50 ± 0.25	NS
	6.2	2.56 ± 0.20	NS
	12.5	2.04 ± 0.16	NS
	25	1.88 ± 0.08	NS
	50	2.07 ± 0.48	NS
	100	1.92 ± 0.06	NS
Isoproterenol	10 (µM)	6.37 ± 0.21*	2.81 *

* - P < 0.05, NS – Not significant

DISCUSSION

This study was selected as it is more apt, sensitive and accurate moreover to overcome the laborious task of animal experimentation and clinical study.

Lipase inhibition assay

Dietary fat gets digested, absorbed and gets converted to body fat and stored as triglycerides. These are utilized by the body during energy need. Fat metabolism can be checked at two levels by inhibition of fat digestion, hindrance of fat absorption. Pancreatic lipase is the most important enzyme for the digestion of dietary triacylglycerols. Orlistat, a hydrogenated derivative of lipstatin is a potent inhibitor of gastric, pancreatic and carboxyl ester lipase and proved to be effective for the treatment of human obesity. Lipase inhibition assay prove to be a potent inhibitor of pancreatic lipase. The Aqueous and alcoholic extracts of drug *Ela* (*Elettaria cardamomum* Maton.) are screened by this assay. The study shows positive results in *Ela* (*Elettaria cardamomum* Maton.) Alcoholic extract *Ela* Aqueous extract did not show positive responses against lipase inhibition assay. Triterpenes are potent lipase inhibitors. *Ela* (*Elettaria cardamomum* Maton.) has triterpenes which may be the strong reason for its prompt response in this lipase inhibition assay. *Ela* (*Elettaria cardamomum* Maton.) should show similar lipase inhibition action when given clinically as this drug basically has *katu rasa* (pungent), *deepana* (appetiser) and *pachana* (digestive) and *laghu* (lightness) *guna*. Encouraging response observed in *Ela* group says components responsible for this activity are alcohol soluble.

Adipogenesis and Adipolysis assay

As the *in vitro* study i.e. Adipogenesis and Adipolysis assay, *in vivo* and clinical study already confirmed the strong anti obesity activity only the effective extractive form *Ela* (*Elettaria cardamomum* Maton.) by screening through pancreatic lipase inhibition assay i.e. alcoholic extract was selected for these assays. The formation of adipose tissue is checked in Adipogenesis whereas in Adipolysis destruction of formed adipose tissue takes place. Thus with an intention whether *Ela* (*Elettaria cardamomum* Maton.) has any role at the level of fat generation and destruction this study was taken. But this particular extract of *Ela* (*Elettaria cardamomum* Maton.) did not show any response giving us a clue that either *Ela* (*Elettaria cardamomum* Maton.) has no action on formation or formed adipose tissue or this particular extract has no active ingredient which can control this. Positive extractive form of Lipase inhibition assay was the only extract screened here and did not show any results in the tested concentration. Clinically when given because of its *katu rasa* (pungent), *deepana* (appetiser) and *pachana* (digestive) property may show *medohara* (dyslipidemic) property.

CONCLUSION

Ela (*Elettaria cardamomum* Maton.) have more alcohol soluble constituents. Quantitative rise of most of the components are soluble in alcohol. *In vitro* study revealed that the alcoholic extract of *Ela* (*Elettaria cardamomum* Maton.) has shown significant result against Lipase inhibition assay. As sthoulyahara (Anti obesity activity) property is assigned to *Ela* (*Elettaria cardamomum* Maton.) and there is no clear cut specification for the usage of *suksma* or *sthula Ela*. *Sthula Ela (Ammomum subulatum)* can be taken up for this study to say whether it has anti- adipogenesis and adipolysis activity.

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