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Original Article

INVITRO ANTI-OBESITY EFFECT OF MACROLICHENS *HETERODERMIA LEUCOMELOS* AND *RAMALINA CELASTRI* BY PANCREATIC LIPASE INHIBITORY ASSAY

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ABSTRACT

Objective: Obesity is a chronic disorder caused by an imbalance between energy intake and expenditure in which excessive fat will be deposited in adipose tissue and poses a risk to the health and well-being of humans. Agents which inhibit pancreatic lipase play an important role in the treatment of obesity. The aim of this study was to assess the potential effect of macro lichens *Heterodermia leucomelos* (L.) Poelt a foliose lichen and *Ramalina celastri* (Sprengel) Krog and Swinscow a fruticose lichen in the treatment of obesity.

Methods: *In vitro* anti-obesity inhibitory effect of macro lichens were evaluated by using chicken pancreatic lipase activity. Lipase was extracted from the chicken pancreas. Different concentrations from 5-25 mg/ml of methanol and ethyl acetate extracts of lichens *Heterodermia leucomelos* and *Ramalina celastri* was incubated with pancreas lipase.

Results: With the increase in the concentration of extracts the higher inhibition of the enzyme was observed. Solvent methanol showed good activity compared to ethyl acetate. Percentage of inhibition ranged from 19.7-69.8 and 20.0-86.6 % in the methanol extract of *Heterodermia leucomelos* and *Ramalina celastri* respectively. Comparatively lichen *Ramalina celastri* in methanol extract showed maximum inhibition of 86.6 %, whereas ethyl acetate showed an inhibition of 63.0% at 25 mg/ml against enzyme lipase.

Conclusion: In the present study, the inhibitory activity of lichen indicates its protective role in treating obesity. Molecular sequencing of this lichen helps in future to determine the various metabolic pathways that are responsible for the production of novel compounds.

Keywords: Anti-obesity, Lichen, Lipase, Hyperlipidemia, *Ramalina celastri*

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INTRODUCTION

Obesity is a chronic metabolic disorder caused by an imbalance between energy intake and expenditure. Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health [1]. Obesity is defined in terms of body mass index (BMI), which is calculated as body weight divided by the square of height [2]. According to WHO, high blood cholesterol level leads to approximately 56% of cardiac disease worldwide and about 4.4 million deaths each year [3]. Being overweight or obese should be prevented by constant exercise and dietary routines. Nevertheless, when the latter techniques fail in obtaining 10% weight loss, pharmacological treatment is required. Despite the fact that this alternative is highly recommended, there are controversial results on the use of some pharmacological agents such as sibutramine [4].

Currently available allopathic drugs used in the treatment of hyperlipidaemia are associated with a number of side effects. Drugs like amphetamine, rimonabant and sibutramine licenses have been withdrawn due to an increased risk of psychiatric disorders and non-fatal myocardial infarction or stroke. Even if orlistat is not as effective as other drugs in reducing body weight, orlistat is presently the only available choice for the treatment of obesity because of its safety for cardiovascular events and positive effects on diabetic control [5]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [6]. Recently, newer approaches to the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase as this is the major source of excess calories [7]. More effective and better-tolerated drugs should be developed which much be safe and consumed long-term in the management of obesity.

Pancreatic lipase (PL) is an enzyme, secreted from the pancreas and plays an excellent role in the absorption of triglyceride in the small intestine. Dietary fats are composed of about 95% triacylglycerol's (TG). Pancreatic lipase hydrolyses the water insoluble triacylglycerol's in the intestinal lumen and thereby used for the dietary fat absorption. Pancreatic lipase inhibitors are considered to be a valuable therapeutic agent for treating diet-induced obesity [8]. One of the screening strategies used in the discovery of anti-obesity drugs is to search for potent lipase inhibitors from natural products. Drugs from natural sources are safer and more efficient, even though they are also toxic, but are of less damaging as compared with the pure synthetic ones. The search for novel natural bioactive compounds as a foundation for new drug discovery is receiving attention as previously reliable standard drugs become less effective against the emerging new strains of multiple drug resistant strains [9].

The aim of our study was to evaluate anti-lipase inhibitory drug from lichens. Lichens are symbiotic organisms of fungal and algal and/or cyanobacterial partner. Lichens are ubiquitous organisms occurring in varied climatic conditions ranging from the poles to the tropics on earth and constitute an important component of terrestrial biodiversity. Lichen synthesises numerous metabolites called lichen substances, including aliphatic, cyclo-aliphatic, aromatic and terpenic components [10]. Lichens and their metabolites have manifold biological activity; antiviral, antibiotic, antitumor, allergenic, plant growth inhibitory, antiherbivore, ecological roles and enzyme inhibitory [11]. The work on lipase inhibitory of lichens are very scanty so an attempt has been made to evaluate the anti-obesity activity of some macro lichens using chicken pancreatic lipase.

MATERIALS AND METHODS

Collection and identification of lichens

The lichens selected for this study were collected from Madikeri district, Karnataka. Madikeri lies in the Western Ghats of Karnataka

and is a popular hill station. It features a tropical highland climate as it has an elevation of 1061 meters (3484 feet). Madikeri is located at 12.42 °N 75.73 °E. The collected lichens were identified as *Heterodermia leucomelos* and *Ramalina celastri* based on morphological, anatomical and colour tests.

Heterodermia leucomelos (L.) Poelt; a foliose lichen belongs to the family Physciaceae. Thallus foliose, linear, ribbon-like with black rhizinae along margin, attached to substratum by basal or central part, suberect at the periphery, laciniae up to 1.5 mm wide, lower cortex absent, upper cortex prosoplectenchymatous composed of longitudinally disposed compact hyphae, photobiont a green alga, sorediate or not, apothecia stipitate, lecanorine, hypothecium hyaline, asci 8-spored, spores brown, 2-celled, pachysporia type, polyblastidia always present in spores, spores 35-54 x 18-25 µm.

Ramalina celastri (Sprengel) Krog and Swinscow; a fruticose lichen belongs to the family Ramalinaceae. Thallus shrubby, moderately branched, surface greenish grey to greenish yellow, smooth, shiny, without soredia. Pseudocyphellae linear, laminal, rarely marginal, cortex thin chondroid strands. Apothecia laminal on one side of the blade, disc flat without pruina, margin concolorous with the thallus, asci 8-spored, ascospores hyaline, 1-septate, fusiform 12-16 x 4-6 µm [12].

Chemicals and reagents

Ammonium sulphate, Phenolphthalein, Oxalic acid were purchased from Qualigens Fine Chemicals, Mumbai, India. Sucrose was purchased from HiMedia Laboratories, Mumbai, India. Phosphate buffer [Potassium chloride and Potassium dihydrogen phosphate], Sodium hydroxide, Potassium hydrogen phthalate were purchased from E. Merck, Mumbai, India. Methanol, ethyl acetate, acetone, ethanol, olive oil used were of analytical grade.

Preparation of extracts

Collected lichens were washed with distilled water and kept to dry at room temperature. The dried lichen materials were ground to fine powder and extracted by soxhlet apparatus using methanol and ethyl acetate as solvents. The extracts were filtered using whatman filter paper no. 1. Filtered extracts were concentrated by air-drying for 4–5 d or until the extracts crystallized, and preserved at 5 °C in airtight bottles until further use.

Anti-lipase activity of lichens

Extraction of lipase from chicken (*Gallus domesticus*) pancreas

The pancreas of freshly slaughtered chicken was collected, washed thoroughly and placed in ice-cold sucrose solution (0.01 M). The pancreas was homogenized in 0.01 M sucrose and centrifuged. The supernatant solution was separated and subjected to ammonium sulphate precipitation (50% saturation). The obtained white pellets after centrifugation were dissolved in sucrose solution and again saturated with 50% ammonium sulphate and centrifuged. Finally, pellets were used as enzyme source by dissolving in phosphate buffer (pH 7) [13].

Determination of chicken pancreatic lipase activity

The chicken pancreatic lipase activity was determined by incubating an emulsion containing 8 ml of olive oil (dietary fat), 0.4 ml of phosphate buffer and 1 ml of chicken pancreatic lipase for an hour. The reaction was stopped by addition of 1.5 ml of a mixture containing acetone and 95% ethanol (1:1). The amount of liberated fatty acid was determined by titrating the emulsion against 0.02 M sodium hydroxide (standardized by potassium hydrogen phthalate) using phenolphthalein as an indicator. The end point is the appearance of pink colour [14].

Pancreatic lipase inhibitory activity

Lichen extracts were prepared in different concentrations such as 5, 10, 15, 20, 25 mg/ml. A 100 µl of each concentration of the sample was mixed with 8 ml of olive oil, 0.4 ml phosphate buffer and 1 ml of chicken pancreatic lipase and it were incubated for 60 min. The reaction was stopped by the addition of 1.5 ml of a mixture containing acetone and 95 % ethanol (1:1).

The appearance of pink colour from yellow colour shows the liberated fatty acids, which was determined by titrating the solution against 0.02 M sodium hydroxide (standardized by 0.01 M oxalic acid) using phenolphthalein as an indicator and the percentage inhibition of lipase activity was calculated using the following formula:

Lipase inhibition percentage

$$= 1 - \left\{\frac{\text{Lipase activity before treatment}}{\text{Lipase activity after treatment}}\right\} \times 100$$

RESULTS

Pancreatic lipase inhibition is one of the most widely studied mechanisms used to determine the potential efficacy of natural products as anti-obesity agents. Hence, in the present study lipase was isolated from the chicken pancreas and determined the inhibitory activity of pancreatic lipase when incubated with different concentrations from 5, 10, 15, 20, 25 mg/ml of methanol and ethyl acetate extracts of lichens *Heterodermia leucomelos* (L.) poelt and *Ramalina celastri* (Sprengel) krog and swiss cow.

With the increase in the concentration of extracts, the higher inhibition of the enzyme was observed. Comparatively lichen *Ramalina celastri* showed maximum inhibition against enzyme lipase. Solvent methanol extract showed good activity compared to ethyl acetate extract. Lipase activity ranged from 6.23±0.05 to 7.30±0.05 in methanol extract and 1.36±0.50 to 3.50±0.10 respectively in methanol and ethyl acetate extracts of *Heterodermia leucomelos* (table 1).

Whereas *Ramalina celastri* showed inhibitory activity at concentrations ranging from 6.00±0.50 to 8.50±0.50 and 4.05±0.05 to 6.50±0.10 of methanol and ethyl acetate extracts respectively (table 2). Percentage of inhibition ranged from 19.7-69.8 and 20.0-86.6 % in the methanol extract of *Heterodermia leucomelos* and *Ramalina celastri* respectively (fig. 1 and 2).

The maximum inhibition observed in *Ramalina celastri* in methanol extract showed an inhibition of 86.6% with lipase activity of 6.00±0.50 at 25 mg/ml, whereas ethyl acetate showed an inhibition of 62.0% with lipase activity of 4.05±0.05 at 25 mg/ml. The inhibition percentage observed in *Heterodermia leucomelos* in methanol extract showed an inhibition of 68.8%, whereas ethyl acetate showed an inhibition of 56.2% with lipase activity of 6.23±0.05 and 1.36±0.05 at 25 mg/ml respectively.

Table 1: Lipase activity of lichen *Heterodermia leucomelos* (L.) poelt

Concentration of extract (mg/ml)	Lipase activity	
	Methanol extract	Ethyl acetate extract
5	7.30±0.50	3.50±0.10
10	7.16±0.50	3.13±0.05
15	7.00±0.10	2.53±0.10
20	6.53±0.05	1.90±0.10
25	6.23±0.05	1.36±0.05

*Values are in mean±standard deviation, n = 3.

Table 2: Lipase activity of lichen *Ramalina celastri* (sprengel) krog and swinscow

Concentration of extract (mg/ml)	Lipase activity	
	Methanol extract	Ethyl acetate extract
5	8.50±0.50	6.50±0.10
10	7.12±0.10	5.53±0.05
15	6.66±0.10	4.80±0.10
20	6.10±0.05	4.26±0.50
25	6.00±0.50	4.05±0.05

*Values are in mean±standard deviation, n = 3.

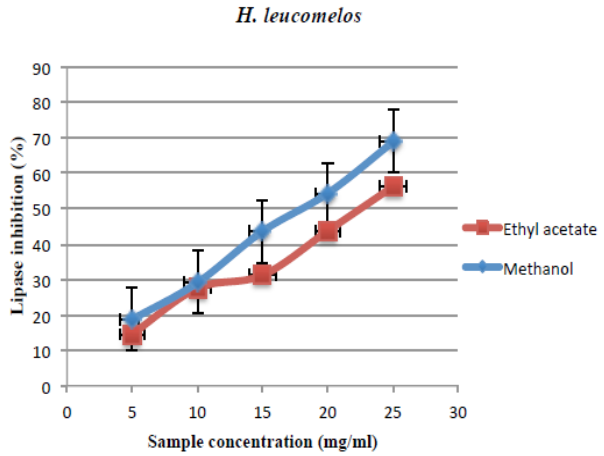

Fig. 1: Lipase inhibition of lichen *Heterodermia leucomelos* with different concentrations

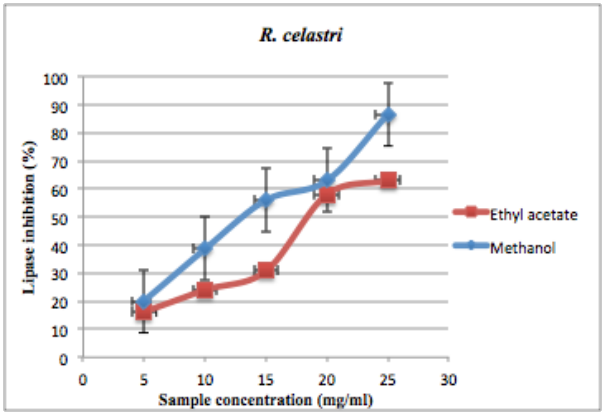

Fig. 2: Lipase inhibition of lichen *Ramalina celastri* with different concentrations

DISCUSSION

Pancreatic lipase or triacylglycerol acyl hydrolase, the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. Pancreatic lipase is responsible for the hydrolysis of 50–70% of the total dietary fats. It removes fatty acids from the α′ apdsitions of dietary triglycerides, yielding β-monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products [15]. Naturally occurring compounds present an exciting opportunity for the discovery of newer anti-obesity agents. Medicinal plants have played a vital role in inhibiting pancreatic lipase in order to reduce obesity like *Eleusine indica, Myristica fragrans*, *Melastoma candidum* and *Phyla nodiflora* [16], *Punica granatum* [17], *Citrullus lanatus* [18], *Abroma augusta* [19] etc. Lichen *Everniastrum cirrhatum* had shown a marked inhibition of 64% at a concentration of 20 mg/ml [20]. Ramalin was isolated from Antarctic lichen *Ramalina terebrata* showed 25% reduced body weight when administered orally with 50-100 mg/kg to high-fat-diet-induced obese mice [21]. *Ramalina celastri* revealed the presence of oleic, palmitic and stearic acids as the main triacylglyceride constituents [22]; glycolipid O-alpha-D-galactopyranosyl-(1-->6)-O-beta-D-galactopyranosyl-(1-->1)-D-glyceritol [23]; a glycosphingolipid *O*-β-D-galactopyranosyl-(1→1)-ceramide [24]; antitumor activity of α-D-glucan polysaccharides with (1→3) (1→4) bonds [25]. From this study, the lichen *Ramalina celastri* inhibits the activity of pancreatic lipase, which indicates its protective role in treating obesity.

CONCLUSION

Obesity is characterized as abnormal or excessive fat deposition in adipose tissue and other internal organs such as liver, heart and skeletal muscle. It is a chronic disorder of carbohydrate and fat metabolism and poses a risk to the health and well-being of humans. Natural herbal products for weight reduction may be effective in the treatment of obesity and associated disorders. Consistent and safe herbal product for weight reduction is a need of developed and developing countries. Hence, in the study, an attempt has been made to test the inhibition of lipase by lichens.

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CONFLICT OF INTERESTS

Declared none

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