INVITRO ANTI-OBESEITY EFFECT OF MACROLICHENS HETERODERMA LEUCOMELOS AND RAMALINA CELASTRI BY PANCREATIC LIPASE INHIBITORY ASSAY

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INTRODUCTION

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 our study was to assess the potential effect of macro lichens Heteroderma leucomesos (L.) Poelt 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 Heteroderma leucomesos 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 Heteroderma leucomesos 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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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 *Heteroderma leucomelos* and *Ramalina celastri* based on morphological, anatomical and colour tests.

*Heteroderma leucomelos* (L.) Poelt; a foliose lichen belongs to the family Physciaceae. Thallus foliose, linear, ribbon-like with black rhizineae 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, photochlorophylline green alga, sorediate or not, apothecia stipitate, lecanorine, hypothecium hyaline, asci 8-spored, spores brown, 2-celled, pachyspora type, polyblastidia always present in spores.

*Ramalina celastri* (Sprengel) Krog and Swinscow; a fruticose lichen belongs to the family Ramalinaeae. 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.

### 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, aceton, 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 were used as enzyme source by dissolving in phosphate buffer (pH 7) [13].

**Determinantion 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 aceton 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 aceton 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. A 0.02 M sodium hydroxide (standardized by 0.01 M oxalic acid) using phospholphthalein as an indicator and the percentage inhibition of lipase activity was calculated using the following formula:

\[
\text{Lipase inhibition percentage} = 1 - \frac{\text{Lipase activity before treatment}}{\text{Lipase activity after treatment}} \times 100
\]

### 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 *Heteroderma 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±0.05 to 3.50±0.10 respectively in methanol and ethyl acetate extracts of *Heteroderma 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 *Heteroderma 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 *Heteroderma 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>
<thead>
<tr>
<th>Concentration of extract (mg/ml)</th>
<th>Methanol extract</th>
<th>Ethyl acetate extract</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>7.3±0.05</td>
<td>3.50±0.10</td>
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<tr>
<td>10</td>
<td>7.1±0.50</td>
<td>3.13±0.05</td>
</tr>
<tr>
<td>15</td>
<td>7.0±0.10</td>
<td>2.53±0.10</td>
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<tr>
<td>20</td>
<td>6.5±0.05</td>
<td>1.9±0.05</td>
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<tr>
<td>25</td>
<td>6.2±0.05</td>
<td>1.36±0.05</td>
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*Values are in mean±standard deviation, n = 3.*

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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 and α′ positions of dietary triglycerides, yielding β-monoglycerides and long chain saturated 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 key role in inhibiting pancreatic lipase in order to reduce obesity like Eleusine indica, Myristica fragrans [16], Abroma augusta [17], Everniastrum cirrhatum [18], Phylla nodiflora [19], and polyunsaturated fatty acids as the lipolytic products [15].

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

REFERENCES

DISCUSSION

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

<table>
<thead>
<tr>
<th>Concentration of extract (mg/ml)</th>
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<tr>
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<tr>
<td>25</td>
<td>6.0±0.50</td>
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</table>

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

![Fig. 1: Lipase inhibition of lichen Heterodermia leucomelos with different concentrations](image1)

![Fig. 2: Lipase inhibition of lichen Ramalina celastri with different concentrations](image2)
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