In vitro pancreatic lipase inhibition potential of commonly used Indian spices

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Abstract: Obesity is the excessive accumulation of fat that may have deleterious health effects. One of the most approached methods of treating obesity is inhibition of pancreatic lipase activity. Pancreatic lipase breaks down triglycerides into fatty acids which then get absorbed through the duodenal mucosa. A pancreatic lipase inhibitor prevents the formation of fatty acids and hence prevents any accumulation of fats in the body. The aim of this study is to evaluate the 3 Indian spices and their mixture for inhibitory activity against pancreatic lipase.

In the present study, three Indian spices namely, Nigella sativa, Trigonella foenum-graecum, Trachyspermum ammi and their mixture (1:1:1 combination) were evaluated for their inhibitory effect on porcine pancreatic lipase enzyme using in vitro assay. The inhibitory activity was demonstrated by all the 3 Indian spices and their mixtures which can play a beneficial role in the treatment of obesity.

Keywords – Nigella sativa, obesity, pancreatic lipase inhibition, Trachyspermum ammi, Trigonella foenum-graecum.

I. INTRODUCTION

Obesity is an epidemic which leads to diseases and social biases all around the globe. WHO defines obesity as excessive accumulation of fat that may have deleterious health effects. [1] These health effects include high blood pressure, diabetes, heart diseases like atherosclerosis, joint problems like osteoarthritis and psychological problems. [2] According to the statistical reports of the World Obesity Federation in 2014, nearly 20 billion adults are overweight and 600 million adults worldwide are obese. The World Obesity Federation estimates a rise in the number of obese and overweight adults by 2025. The figures show that 2.7 billion adults worldwide will be overweight and 177 million adults will be obese by 2025. [3] Therefore, current research is focused on developing drugs or identifying and formulating herbal products into medicines that could reduce body fat. Obesity can be attributed to several factors such as lack of access to healthy food, oversized food portions, family history, health conditions like Cushing’s syndrome or hypothyroidism, medicines like corticosteroids or antidepressants, emotional factors, smoking or age. [4]

There are several pathways in human body that directly or indirectly cause obesity; these can be targeted to combat obesity. There are many anti-obesity medications, namely, orlistat, lorcaserin, sibutramine, and rimonabant. Lorcaserin is a serotoninergic agent officially approved by FDA for its use in the treatment of obesity for adults, however, with certain restrictions and patient monitoring as it produces certain side-effects. [5] Sibutramine is a centrally-acting serotonin-norepinephrine reuptake inhibitor. It was prescribed as an anti-obesity drug but was soon withdrawn from the market due to cardiovascular effects. Rimonabant mainly acts by reducing appetite. It was withdrawn from the market because it had many psychiatric side effects. Orlistat is a saturated form of lipstatin, obtained from actinobacterium. [6] It is a reversible inhibitor of the pancreatic triglyceride lipase which in turn causes inhibition of fat absorption. This function not only reduces obesity risks but also is useful in cases like high blood pressure and type 2 diabetes. Since orlistat structure is simpler and more stable than lipstatin, the former is the drug of choice for obesity. However, the utility of orlistat is limited due to its toxicity to several internal organs, including, kidney and liver. Therefore, the use of synthetic anti-obesity drugs is limited by fatal adverse reactions, resulting in poor tolerability and patient compliance. This prompts an urgent need to search agents that are less toxic and thereby provide better safety and efficacy even on long term usage. Apart from the usual ways of dieting, exercising and yoga, people include Ayurveda products into their daily meal courses and regularly intake powders or juices of herbal plants as a remedy for treatment of obesity. Many extracts from plants, fungi, bacteria or algae are tested for their inhibition of pancreatic lipase activity. Many such extracts or powders are reported in literatures that show high levels of inhibitory activity. Polyphenols and saponins are known to show a promising inhibiting effect. Since lipids from the ingesta represent the prime source of unwanted calories, inhibition of fat digestion is a preferable approach for reducing absorption of fats. [7, 8] Decrease in intestinal fat digestion is related to a reduction in intra-abdominal fat content. [7, 9]
Ayurveda has been tested for over 50 centuries and now its effectiveness is proven. Ayurvedic medicines may offer less toxicity and side effects than the synthetic medicines. This can be attributed to the fact that they do not target a single disease but act by treating the body as a whole. This paper focuses to elaborate a study carried out to test the activity of *Nigella sativa*, *Trigonella foenum-graecum*, *Trachyspermum ammi* and their mixture (1:1:1 combination) in the inhibition of pancreatic lipase activity. *Nigella sativa*, a member of Ranunculaceae family, is an annual flowering plant. The seed of this plant is rich in conjugated linoleic (18:2) acid, thymoquinone, nigellone (dithymoquinone), melanthin, nigiline, damascenine, and trans-anethole. [10] *Trigonella foenum-graecum* is an annual plant and the member of the Fabaceae family. Its seed contains a C-steroidal sapogenin peptide ester, fenugreekine, diosgenin, trigonelline and trigonellic acid, lecithin, a natural emollient. [11] *Trachyspermum ammi* is an annual herb of the Apiaceae family. The fruit contains thymol as the major constituent (35% to 60%). The nonthynol fraction (thyme) contains para-cymene, γ-terpinene, α- and β-pinenes, dipentene, α-terpinene, and carvacrol. *Trachyspermum ammi* is also mentioned in dravyaguna vigyan. [12] These herbs are commonly used spices in every Indian household and have been used widely in folklore for treatment of obesity and hyperlipidemia. Thus aim of the present study was to determine the *in vitro* pancreatic inhibitory potential of *Nigella sativa*, *Trigonella foenum-graecum*, *Trachyspermum ammi* and their mixture (1:1:1 combination).

**II. MATERIALS AND METHODS**

2.1. Plant material

*Nigella sativa* seed, *Trigonella foenum-graecum* seed, *Trachyspermum ammi* fruit were purchased from local market. They were ground and passed through a 0.8 mm mesh sieve. The powders were allowed to macerate in Phosphate buffered saline (1:1) for 4 hours at 37˚C followed by centrifugation at 4000 g for 10 minutes. The supernatant thus obtained (1mg/ml) was used as stock solution and was serially diluted with Phosphate buffered saline (PBS) to obtain various test concentrations.

1.2. Reagents

PNPB (para-nitrophenylbutyrate), porcine pancreatic lipase (Advanced Enzymes, Mumbai), Sodium dihydrogen phosphate (SD Fine Chemicals, Mumbai), Disodium hydrogen phosphate (SD Fine Chemicals), Sodium Chloride (SD Fine Chemicals), Triton-X-100 (Sigma Aldrich, USA), acetonitrile (Sigma Aldrich, USA), Orlistat (Bico, Bangalore, India).

1.3. Buffer preparation

100 mM PBS with 150mM Sodium Chloride and 0.5% (v/v) Triton-X-100, pH 7.2.

1.4. Enzyme preparation (Concentration of enzyme)

Porcine pancreatic lipase enzyme solution was prepared by dissolving 6mg of the enzyme in 10ml of buffer solution by gentle vortexing. It was prepared immediately before use.

1.5. *In vitro* pancreatic lipase inhibition assay

Total assay volume was 200µl. Substrate used was p-Nitrophenylbutyrate (PNPB). PNPB working solution was prepared with 8.403µl of PNPB stock solution in a vial and volume was made up to 10ml by acetonitrile. Solution of the standard drug was prepared by dissolving one capsule content of Orlistat in 12ml of DMSO (dimethylsulphoxide). Test solutions were prepared as mentioned previously. Test solution or Standard (25µl) was incubated with 50µl of enzyme solution, 100µl of buffer solution and 25µl of PNPB solution for 30 minutes at 37˚C. Lipase activity was determined by measuring the hydrolysis of PNPB to p-nitrophenol at 400 nm using an ELISA plate reader (Bieteck).

% Inhibitory activity was calculated using the following formula:

\[
\text{% Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100
\]

**III. RESULTS**

Orlistat is the only commercial drug in the market effective against pancreatic lipase. The aim of this study was to evaluate the pancreatic lipase inhibition potential of commonly used Indian spices. The inhibitory activity of 3 plant powders and their mixture was experimentally determined and the results of the same are tabulated in Table1 which describes % inhibition values for 1µg/ml, 3µg/ml, 10µg/ml, 30µg/ml concentration of drug. The concentration related inhibitory activity is represented in a graphical form in Figure.1.

**Table1: Percentage inhibition of pancreatic lipase by commonly used Indian spices and its combination**

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trigonella foenum-graecum</em></td>
<td>Fenugreek</td>
<td>1</td>
<td>64.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>62.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>66.0</td>
</tr>
</tbody>
</table>
In vitro pancreatic lipase inhibition potential of commonly used Indian spices

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trachyspermum ammi</em></td>
<td>61.0</td>
<td>55.6</td>
<td>52.3</td>
<td>6.1</td>
</tr>
<tr>
<td><em>Nigella sativa</em></td>
<td>80.4</td>
<td>72.2</td>
<td>79.2</td>
<td>68.5</td>
</tr>
<tr>
<td><strong>Mixture (1:1:1)</strong></td>
<td>66.3</td>
<td>64.5</td>
<td>64.8</td>
<td>59.6</td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Orlistat</strong></td>
<td>38.2</td>
<td>40.1</td>
<td>57.8</td>
<td>66.0</td>
</tr>
</tbody>
</table>

Figure 1: Percentage inhibition of pancreatic lipase by commonly used Indian spices and its combination

IV. DISCUSSION

Hydrolysis of triglyceride into free fatty acids and monoglyceride is accomplished majorly by pancreatic lipase, a water soluble enzyme. It is a lipolytic enzyme which catalyzes the hydrolysis of the ester bonds present in the triacylglycerols. The enzyme works to remove the fatty acids located at position 1 and position 3 of the triglyceride, hence leaving a 2-monoglyceride and two free fatty acids. [13] The enzyme is present in the gastrointestinal tract inside the adipocytes. Pancreatic lipase on entering the pancreatic duct mixes with bile salts and liquids following which it reaches the duodenal lumen in order to complete fat digestion. The enzyme is substrate specific. It gives preference to triglycerides over phospholipids. It does not act on water soluble substrates and prefers water insoluble substrates. Pancreatic lipase activity increases when it encounters a water-oil interface. This property is called interfacial activation. Even though lipase is secreted into the duodenum, it is inhibited by bile salts and hence requires a pancreatic protein called colipase for its activity. The property of hydrolyzing water-insoluble substrates and interfacial activation distinguishes pancreatic triglyceride lipase from the rest of the lipases. [14] After the action of lipase, the fatty acids and monoglycerides hence formed are still associated with the bile salts and they complex with other lipids to form structures termed as micelles. Micelles reach the microvilli and the lipids along with fatty acids and the monoglycerides are absorbed into the epithelial cells. This is the mechanism by which the fats are broken down and then absorbed into the cells of small intestine, eventually, entering the blood stream. [13] When an anti-obesity agent which inhibits pancreatic lipase is ingested, it reaches the intestine via the gastrointestinal tract. As soon as it reached the pancreas, it comes in contact with pancreatic triglyceride lipase enzyme. It acts on the active site of the enzyme and causes inhibition of the enzyme hence preventing the binding of lipids on to the active site of the enzyme. This in turn prevents the hydrolysis of the dietary lipids hence hindering their absorption through the intestinal membrane. These lipid molecules are hence excreted via the large intestine. This mechanism is targeted to reduce the amount of body fat and hence the body weight. The table 1 depicts the %inhibitory activity of the
powders and their mixture. Of all the concentrations tested, *Trigonella foenum-graecum* shows maximum activity at 30µg/ml, *Trachyspermum ammi* shows at 1 µg/ml, *Nigella sativa* shows at 1µg/ml and their mixture shows at 1µg/ml. The plot indicates that *Nigella sativa* shows the most promising pancreatic lipase inhibitory activity. *Trachyspermum ammi* shows decrease in activity with increase in its concentration. Mixture shows activity less than *Nigella sativa*. This may be due to antagonistic activity of *Trachyspermum ammi* at higher concentration. The inhibitory activity of the powders and their mixture is comparable to orlistat activity.

V. CONCLUSION

Thus the results of this study indicate that the commonly used Indian spices, *Nigella sativa* seed, *Trigonella foenum-graecum* seed, *Trachyspermum ammi* fruit and their mixture significantly inhibit the activity of pancreatic lipase which can be attributed to the presence of saponins, phenols, flavonoids or alkaloids which is comparable to orlistat. This rationalizes its effectiveness in treatment of obesity and related disorders such as hyperlipidemia. Being a nutraceutical, these Indian spices will achieve the therapeutic result with minimal side effects. Further, *in vivo* studies are warranted to determine the usefulness of these spices in treatment of obesity and hyperlipidemia.

REFERENCES


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