Inhibitory potential of chebulagic acid on digestive enzymes *in vitro*

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**INTRODUCTION**

Obesity is an epidemic which leads to diseases and social biases globally. The WHO defines obesity as excessive accumulation of fat that may have deleterious health effects.[1] These health effects include high blood pressure, diabetes, heart disease such as atherosclerosis, joint problem such as 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.[3] There are many antiobesity medications, namely orlistat, lorcaserin, sibutramine, and rimonabant which produce certain side-effects. Therefore, the current research is focused on developing drugs or identifying and formulating herbal products for the treatment of obesity and associated complications, reinforcing the need for the search of new sources of amylase, glycosidase, and lipase inhibitors. Therefore, digestive inhibitors who assist in reducing fat and carbohydrate absorption in the small intestine may be useful helpers in the treatment of obesity.

Chebulagic acid is one of the main bioactive constituents of *Terminalia chebula* fruit powder. Chebulagic acid has been shown to inhibit α-glucosidase activity,[4] reactive oxygen species generation from PMA (phorbol 12-myristate 13-acetate)-stimulated leukocytes,[5] and CTL-mediated cytotoxicity.[6] In addition, it has been reported to suppress arthritis in mice[7] and lipopolysaccharide (LPS)-induced nitric oxide generation in RAW 264.7 mouse macrophage cells.[8] Moreover, chebulagic acid inhibits NF-κB activity and phosphorylation of mitogen-activated protein kinases in LPS-stimulated RAW 264.7 macrophages. Recently, chebulagic acid has reported to have gastroprotective effect on ethanol-induced gastric injury in rats.[9] However, there are no previous reports of any *in vitro* α-glucosidase, α-amylase, and

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lipase inhibitory activity of chebulagic acid. Therefore, the present study was aimed to investigate the effect of chebulagic acid on the α-glucosidase, α-amylase, and lipase inhibitory activities in vitro, and those inhibitory assays were compared with acarbose and orlistat - synthetic antidiabetic and anti-obesity drugs used as reference drug. *Terminalia chebula* fruits and structure of chebulagic acid are given in Figure 1.

**MATERIALS AND METHODS**

**Chemicals**
Chebulagic acid was procured from Chem Faces, China. Acarbose, porcine pancreatic α-amylase and lipase, rat intestinal α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG), p-nitrophenyl laurate, and triton were products of Sigma-Aldrich Co., St Louis, Missouri, USA, while soluble starch and dinitrosalicylic acid were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Water used was glass distilled, and other chemicals and reagents were of analytical grade.

**In vitro α-amylase Inhibitory Assay**
The α-amylase inhibitory activity of chebulagic acid was estimated by the method of Bernfeld using acarbose as the standard. Briefly, a series of concentrations of chebulagic acid (10, 20, 40, 80, and 100 μg/ml) was prepared and allowed to react with α-amylase and 2 mM of phosphate buffer (pH - 6.9). After incubation for 20 min, to the reaction mixture, 0.1 ml of 1% starch solution was added. The same procedure was followed for control samples without the enzyme. Finally, 0.5 ml of dinitrosalicylic acid reagent was added to both control and test and kept in a boiling water bath for 5 min. Acarbose was prepared in distilled water at the same concentrations as chebulagic acid. Then, the absorbance was measured at 540 nm using spectrophotometer, and the percentage inhibition was calculated using the formula.

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\text{% of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

**Alpha-Glucosidase Inhibitory Assay**
The α-glucosidase inhibition was determined by the following modified methods. The α-glucosidase reaction mixture contained 2.9 mM pNPG, varying concentrations (10, 20, 40, 80, and 100 μg/ml) of chebulagic acid and 1.0 U/mL α-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only buffer, enzyme, and substrate while in positive controls, acarbose was replaced the chebulagic acid. Mixtures without enzyme, sample extract, and acarbose served as blanks. The reaction mixtures were incubated at 25°C for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting p-nitro phenol was determined at 405 nm using spectrophotometer and was considered directly proportional to the activity of the enzyme. Acarbose was used as the reference drug for α-glucosidase inhibition assay. All the tests were performed by triplicates. The percentage inhibition was calculated using the formula.

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\text{% of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

**Pancreatic Lipase Inhibition Assay**
Lipase activity was determined according to Souza et al. (2011), using 8 mmol 1⁻¹ p-nitrophenyl palmitate in Tris-HCl 0.05 mmol 1⁻¹, pH 8.0 buffer containing 0.5% Triton-X100 as substrate. In the assay, chebulagic acid (10, 20, 40, 80, and 100 μg/ml) and lipase enzymes

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![Figure 1: The *Terminalia chebula* tree bearing fruits and its main chemical constituents - Chebulagic acid](image-url)
were incubated in water bath at 37°C, for four periods of time after addition of the substrate. The reaction was stopped, transferring the tubes to an ice bath and adding Tris-HCl 0.05 mmol l⁻¹, pH 8.0 buffer. The same procedure was followed for control samples without the enzyme. Orlistat was prepared in distilled water at the same concentrations as chebulagic acid. The p-nitrophenol of yellow coloration, a product of the lipase action on p-nitrophenylpalmitate, was read at 410 nm. All the tests were performed by triplicates. The percentage inhibition was calculated using the formula:

\[
\text{% of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
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Statistical Analysis
Data were analyzed using SPSS statistical software (SPSS, Chicago, IL, USA). Values were expressed as mean ± standard deviation of three replications of the experiment. The differences in the mean values were analyzed using the Fisher’s least significant difference procedure and were considered statistically significant at \( P < 0.05 \).

RESULTS
Different concentrations (10, 20, 40, 80, and 100 μg/mL) of chebulagic acid and acarbose were separately subjected to α-amylase and α-glucosidase inhibitory assays [Figures 2 and 3]. Results showed that chebulagic acid and orlistat effectively inhibited the actions of pancreatic lipase enzyme by a dose-dependent manner. The maximum percentage of inhibition was observed 68.85 ± 0.28 in α-amylase and 63.35 ± 0.17 in α-glucosidase enzymes at a concentration of 100 μg/ml of chebulagic acid. The standard drug acarbose was shown maximum percentage of inhibition 71.46 ± 0.61% in α-amylase and 69.82 ± 0.66 in α-glucosidase at a concentration of 100 μg/ml. The results produced chebulagic acid were comparable to that of acarbose - an antidiabetic drug.

The inhibitory activities of chebulagic acid against porcine pancreatic lipase are shown in Figure 4. Results showed that chebulagic acid and orlistat effectively inhibited the actions of lipase enzyme by a dose-dependent manner. The maximum percentage of inhibition was observed 86.16 ± 0.87 at a concentration of 100 μg/ml of chebulagic acid. The standard drug orlistat was shown maximum percentage of inhibition (91.61 ± 0.47) at a concentration of 100 μg/ml. The results produced by chebulagic acids were comparable to that of orlistat - an antiobesity drug.

DISCUSSION
The enzyme α-amylase and α-glucosidase may be responsible for the breakdown of carbohydrates into glucose. Alpha-amylase is responsible for hydrolyzing the starch which breaks down into glucose before absorption. Alpha-glucosidase is an enzyme present in the small intestine used for the cleavage of disaccharides into glucose. Inhibition of α-amylase and α-glucosidase can lead reduction in post-prandial hyperglycemia.[12] 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. In the present study, chebulagic acid has inhibited actions of both α-amylase and α-glucosidase, and those inhibitory activities were comparable to that of acarbose - an antidiabetic drug, used as reference drug. In addition, chebulagic acid inhibited the lipase activity and that activity was comparable to that of orlistat - a reference drug.

CONCLUSION

Chebulagic acid was able to inhibit digestive enzymes such as α-amylase, α-glucosidase, and lipase in vitro. These results clearly indicate that chebulagic acid can be used as an auxiliary in the treatment of obese associated comorbidities and in the control of type 2 diabetes.

REFERENCES


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