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Research Article

Comparative Evaluation of BerberineQA™ and Standard Berberine Extract for Digestive Enzyme Inhibition: An *In-vitro* Study of Antiobesity and Antidiabetic Potential

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ABSTRACT

This study investigated the comparative *in-vitro* antidiabetic and antiobesity properties of BerberineQA™ and standard berberine by evaluating digestive enzyme inhibitory activities. The antiobesity potential of BerberineQA™ (97%) versus standard berberine hydrochloride (97%) was evaluated using the porcine pancreatic lipase inhibition assay with 4-nitrophenyl butyrate as the substrate. To evaluate the antidiabetic activity, the samples were screened for their inhibitory effects on carbohydrate-digesting enzymes such as α-amylase and α-glucosidase using standard methods and were also compared to acarbose (standard reference). For the pancreatic lipase inhibition assay, BerberineQA™ exhibited detectable anti-lipase activity, while standard berberine showed no measurable inhibition under the experimental conditions. Both BerberineQA™ and standard berberine revealed dose-dependent enzyme inhibition of α-amylase and α-glucosidase. However, BerberineQA™ demonstrated significantly greater inhibitory activity than standard berberine. In the α-amylase inhibition assay, BerberineQA™ had a lower IC_{50} value of 344.42 µg/mL than that of standard berberine (385.92 µg/mL). In the α-glucosidase inhibition assay, BerberineQA™ also showed improved activity (IC_{50} value: 336.11 µg/mL) compared to 370.34 µg/mL for standard berberine. The findings suggest that BerberineQA™ has comparatively greater antidiabetic and antiobesity potential than standard berberine. The improved enzyme inhibitory potential of BerberineQA™ indicates its promising use for managing metabolic diseases.

Introduction

Diabetes mellitus and obesity are among the most common chronic metabolic disorders across the globe. They pose major public health challenges due to their links to serious complications like cardiovascular diseases, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, hypertension, and dyslipidemia.[1,2] Diabetes mellitus is considered to be the most common type of diabetes, resulting in chronic high blood sugar levels due to insulin resistance, poor insulin secretion, or both.[1,3] One key treatment approach for managing high blood sugar after meals involves delaying the digestion and absorption of carbohydrates through diet by blocking enzymes such as α-amylase and α-glucosidase.[4] In addition, blocking pancreatic lipase has become a useful method for managing obesity by reducing the absorption of dietary fat.[5,6]

α-Amylase is a crucial digestive enzyme mainly produced by the salivary glands and pancreas. It breaks down complex polysaccharides, such as starch, into smaller oligosaccharides and maltose. These intermediate products are further broken down by α-glucosidase enzymes in the intestinal brush border into glucose, which is quickly absorbed into the bloodstream.[4] Fast digestion and absorption of carbohydrates greatly contribute to high sugar levels after meals, which is considered a significant risk factor for complications in diabetes. Thus, inhibiting carbohydrate-digesting enzymes can effectively slow

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down the rate of glucose release and absorption, improving blood sugar control in people with diabetes.[4]

Pancreatic lipase is the main enzyme that digests dietary triglycerides in the gastrointestinal tract. It breaks triglycerides into free fatty acids and monoglycerides, which are then absorbed by intestinal cells.[5,6] Common enzyme inhibitors like acarbose and orlistat are widely used to manage diabetes and obesity, but long-term use of these drugs often leads to gastrointestinal adverse effects like diarrhea, discomfort in the abdomen, oily stools, and liver toxicity.[5] As a result, there has been a focus on finding naturally occurring bioactive compounds that have strong enzyme-blocking abilities and better safety profiles.

Berberine is a plant-derived isoquinoline alkaloid that is widely distributed among various medicinal plant species, including *Berberis aristata*, *Hydrastis canadensis*, and *Tinospora cordifolia*.[2, 7] Traditionally, plants containing berberine have been used in Ayurvedic and Chinese systems of medicine to treat infections, gastrointestinal issues, inflammatory conditions, and metabolic diseases. Recently, berberine, a nutraceutical, has attracted significant interest as it exerts a diverse range of pharmacological effects such as antimicrobial, antidiabetic, antiobesity, antioxidant, cardioprotective, anti-inflammatory, and hepatoprotective properties.[2,7] Multiple studies have shown that berberine can influence glucose and fat metabolism through various mechanisms. Because of berberine's multifunctional properties, it is seen as a promising natural treatment option for managing metabolic syndrome and related disorders. Although many studies have shown berberine's enzyme-inhibitory properties, there is still a lack of comparative investigations between a proprietary processed berberine and conventional berberine under the same conditions. Thus, this study evaluates the *in-vitro* antidiabetic and antiobesity activities of BerberineQA™ and standard berberine using α-amylase inhibition, α-glucosidase inhibition, and pancreatic lipase inhibition assays.

Materials and Methods

Test Products

Two samples of berberine (each 500 g) were evaluated in this study. The first sample was BerberineQA™ [An Energized Active Supplement Ingredient (EASI)™, Greenspace, Bengaluru, India] capsules, and the second sample was a standard berberine hydrochloride (97%) capsule that had not undergone the processing procedure. The EASI™ sample (BerberineQA™) was prepared using a proprietary method developed by Greenspace, Bengaluru, India. Both samples were derived from the same botanical source, contained 97% berberine hydrochloride, and were maintained under identical storage conditions throughout the study.

Proprietary processing method

Berberine was exposed to a proprietary processing method using controlled exposure to low-intensity pulsed electromagnetic and acoustic fields (Radiation Frequency Range: 80 MHz to 1 GHz at a field strength of 10 V/m; Pulsed Electromagnetic Field: 60 A/m [75 μT] at 50 Hz).

Pancreatic Lipase Inhibition Assay

The pancreatic lipase inhibitory activity of BerberineQA™ and standard berberine at concentrations between 50 and 250 μg/mL was evaluated using standard protocols.[8] For this study, 4-nitrophenyl butyrate was used as the substrate. The final reaction volume for performing the assay was 250 μL, which consisted of 100 μL of porcine pancreatic lipase (1-mg/mL), 50 μL of 0.2 M sodium phosphate buffer, and an appropriate concentration of test samples. The reaction mixture was then pre-incubated for 30 minutes to help the enzyme interact with the test samples. After pre-incubation, 50 μL of 10 mM 4-nitrophenyl butyrate was added to start the enzymatic reaction. Then the reaction mixture was incubated for 20 minutes. After incubation, the absorbance was measured at 415 nm using a microplate reader. The release of 4-nitrophenol from the substrate results in the formation of a yellow-colored product, which is proportional to lipase inhibitory activity. The decrease in absorbance relative to the control was used to calculate the percentage inhibition. The following formula was used in the calculation:

$$\text{Lipase Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the reaction containing the enzyme without the test samples, and A_{sample} represents the absorbance of the reaction containing the test samples.

α-Amylase Inhibition Assay

The α-amylase inhibitory activity of BerberineQA™ and standard berberine was investigated using standard procedures.[9] Different concentrations ranging from 100 to 500 μg/mL of the test samples (BerberineQA™ and standard berberine) were mixed with 500 μL of sodium phosphate buffer (0.02 M; pH 6.9) containing 0.006 M NaCl and 0.5 mg/mL of porcine pancreatic α-amylase enzyme. The reaction mixture was then incubated at 25°C for 10 minutes to allow interaction between the enzyme and the test samples. After incubating the reaction mixture, 500 μL of a 1% starch solution (prepared in the same phosphate buffer as the substrate) was added. The reaction mixture was then incubated at 25°C for another 10 mins. Then the reaction was stopped by the addition of 1.0 mL of dinitrosalicylic acid (DNSA), followed by heating the tubes in boiling water for 5 minutes and then cooling to room temperature. After cooling to room temperature, the mixture was diluted with 10 mL of

distilled water, and the absorbance was measured at 540 nm using a spectrophotometer. Each experiment included a control containing all reagents except the test sample. The α-amylase inhibitory activity was expressed as percentage inhibition, and it was calculated using the following formula.

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the reaction containing the enzyme without the test samples, and A_{sample} represents the absorbance of the reaction containing the test samples.

α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was conducted according to the methods given by Apostolidis *et al.* (2007) with minor modifications.[4] Various concentrations of samples (100–500 µg/mL) were taken and mixed with 100 µL of α-glucosidase solution. The α-glucosidase solution was prepared by mixing 0.5 mg/mL α-glucosidase in 0.1 M phosphate buffer (pH 6.9). Then, the reaction mixture was incubated at 25°C for 10 mins. After incubation, the enzymatic reaction was initiated by adding 50 µL of 5 M p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9). The reaction mixtures were incubated for 5 minutes at 25°C, after which the absorbance was recorded at 405 nm using a spectrophotometer. Each experiment included a control containing all reagents except the test sample. The inhibitory activity was expressed in terms of percentage inhibition of α-glucosidase and was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the reaction containing the enzyme without the test samples, and A_{sample} represents the absorbance of the reaction containing the test samples.

Statistical Analysis

All the experiments were performed in triplicate, and the results are expressed as Mean ± Standard Deviation (SD). IC_{50} values were estimated from concentration-response curves generated from percentage inhibition data. Statistical analyses were performed using one-way Analysis of Variance (ANOVA), followed by Tukey-Kramer multiple comparison tests. GraphPad InStat software (Version 3.06 Demo, GraphPad Software, San Diego, CA, USA) was used for analyses. A $p < 0.05$ was considered statistically significant.

Results

Pancreatic Lipase Inhibition Assay

The results of the assay showed a clear difference in the inhibitory effects of BerberineQA™ and standard berberine. BerberineQA™ exhibited noticeable anti-lipase activity, while standard berberine did not show any measurable inhibition under the experimental conditions (Fig. 1). The results also revealed that BerberineQA™ exhibited a dose-dependent increase in pancreatic lipase inhibition, whereas standard berberine showed no detectable inhibitory activity throughout the tested concentration range.

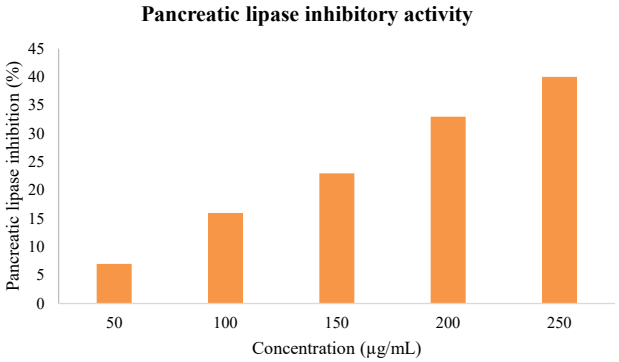

Fig. 1: Effect of BerberineQA™ on pancreatic lipase inhibition. Standard berberine did not show any inhibitory effects under experimental conditions and is therefore not shown

α-Amylase Inhibition Assay

Both the berberine samples showed concentration-dependent inhibition of α-amylase *in-vitro*, indicating their ability to reduce carbohydrate breakdown and glucose release (Fig. 2). Statistical comparison between the two berberine samples (BerberineQA™ and standard berberine) showed that BerberineQA™ produced significantly greater inhibition than standard berberine across all tested concentrations ($p < 0.001$). At 100 µg/mL, BerberineQA™ had significantly ($p < 0.001$) greater inhibition (14.37 ± 0.15%) when compared with the standard berberine (12.33 ± 0.15%) group. As the concentration increased to 200 µg/mL, BerberineQA™'s inhibition increased to 28.63 ± 0.23%, while standard berberine exhibited 24.37 ± 0.21% inhibition; therefore, BerberineQA™ showed significantly ($p < 0.001$) greater inhibition when compared with the standard berberine. At intermediate concentrations of 300 µg/mL and 400 µg/mL, BerberineQA™ showed 42.43 ± 0.06% and 59.57 ± 0.20% inhibition, respectively, which were significantly ($p < 0.001$) higher than the standard berberine group's 35.60 ± 0.20% and 52.33 ± 0.11%, respectively. At the highest concentration of 500 µg/mL, BerberineQA™ achieved 72.33 ± 0.11% inhibition, while standard berberine had lower inhibition at 65.40 ± 0.10%; therefore, BerberineQA™ showed significantly greater inhibition when compared with the standard berberine group ($p < 0.001$). The IC_{50} value for BerberineQA™ was lower than that of standard berberine; therefore, BerberineQA™ is showing improved inhibitory potency against the α-amylase enzyme. Both samples had lower activity than

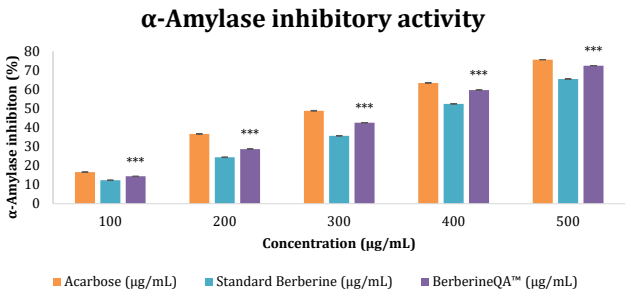

Fig. 2: α-Amylase inhibitory effect of BerberineQA™ and standard berberine. Values are given as mean ± SD (n = 3). *** $p < 0.001$ compared with the standard berberine group

the standard drug acarbose, but BerberineQA™'s activity was closer to the standard acarbose (Table 1).

α-Glucosidase Inhibition Assay

The effects of BerberineQA™ and standard berberine on the α-glucosidase inhibition assay were evaluated to assess their ability to reduce carbohydrate digestion. Both samples showed dose-dependent inhibition, with BerberineQA™ consistently demonstrating better inhibition than standard berberine at all concentrations tested (Fig. 3). Between-group comparison showed that BerberineQA™ produced significantly greater α-glucosidase inhibition than standard berberine across the entire concentration range ($p < 0.001$). At 100 µg/mL, BerberineQA™ had an inhibitory activity of 14.63 ± 0.15%, exceeding that of standard berberine at 13.40 ± 0.20%. The difference remained significant with increasing concentrations, with BerberineQA™ demonstrating 32.50 ± 0.10% versus 27.07 ± 0.42% inhibition at 200 µg/mL, 45.40 ± 0.15% versus 39.17 ± 0.25% at 300 µg/mL, and 58.60 ± 0.30% versus 54.53 ± 0.30% at 400 µg/mL. At 500 µg/mL, BerberineQA™ showed 72.40 ± 0.20% inhibition, significantly exceeding the 66.50 ± 0.30% recorded for standard berberine ($p < 0.001$). The IC_{50} value of BerberineQA™ against α-glucosidase was lower than that of the standard berberine, which confirms BerberineQA™'s

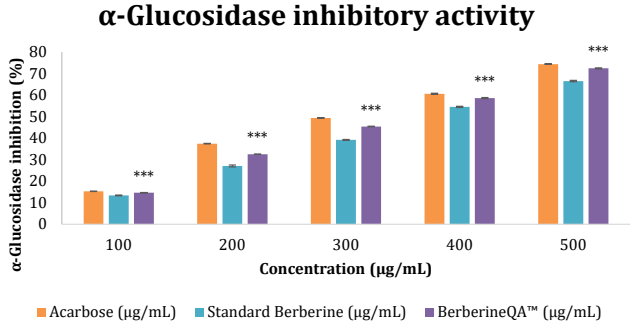

Fig. 3: α-Glucosidase inhibitory effect of BerberineQA™ and standard berberine. Values are given as mean ± SD (n = 3). *** $p < 0.001$ compared with the standard berberine group

Table 1: Comparison of IC_{50} values for α-amylase and α-glucosidase inhibition by the test samples

Antidiabetic models	*IC_{50} Values*		
	Standard Berberine (µg/mL)	*BerberineQA™ (µg/mL)*	*Acarbose (µg/mL)*
α-Amylase inhibition assay	385.92	344.42	309.90
α-Glucosidase inhibition assay	370.34	336.11	306.29

Lower IC_{50} values indicate greater inhibitory activity

enhanced inhibitory potency. BerberineQA™'s inhibitory activity was also closer to that of the standard acarbose (Table 1).

Comparative Insight

A comparison of results revealed that BerberineQA™ showed better inhibition of metabolic enzymes compared to standard berberine (Table 1). Both samples of berberine had antidiabetic activity, but only BerberineQA™ showed anti-lipase potential. The statistically significant improvement in both α-amylase and α-glucosidase inhibitory activity ($p < 0.001$), together with the increased pancreatic lipase inhibitory activity observed for BerberineQA™, indicates an enhanced inhibitory profile against enzymes involved in carbohydrate and lipid metabolism. The result clearly shows that BerberineQA™ has significantly better enzyme inhibition compared to standard berberine, indicating its superior potential for managing diabetes and obesity-related metabolic disorders.

Discussion

Lipid metabolism is a multistep process that starts in the gut and ends with energy production or fat storage. Pancreatic lipase plays an important part in the digestion of dietary fats by hydrolyzing them primarily into free fatty acids and 2-monoglycerides. Pancreatic lipase acts in the intestines and controls how much fat enters the body. Strong inhibition of this enzyme would mean less fat gets absorbed into the bloodstream. Orlistat remains the most widely used pancreatic lipase inhibitor for obesity management. Though effective, it has its own set of adverse effects, such as flatulence, abdominal pain and cramping, diarrhea, and gastrointestinal discomfort. As a result, the current research is dedicated to developing newer pancreatic lipase inhibitors, especially from natural and plant-based sources.[10,11]

Several berberine formulations or ingredients are available in the market. They are either generic or branded ingredients with complex formulations aimed at improved bioavailability. On the contrary, EASI™ is an innovation based on Ayurvedic concepts integrated with

quantum physics. The ingredients are energized with low-intensity electromagnetic radiation to observe the energy shifts in the functional groups (measured by Raman spectroscopy or FTIR); however, without changing their chemical structure. Such an energized state is maintained with proprietary technology until delivered to the human system.

It becomes essential to establish the efficacy of the novel product and evaluate its enzyme-inhibitory activity by comparing it with a standard reference. The pancreatic lipase inhibition assay showed noticeable differences between the two samples. BerberineQA™ demonstrated detectable pancreatic lipase inhibition, while standard berberine showed no measurable effects, suggesting a negligible response on the pancreatic lipase inhibitory activity. In a previous study[12], it was shown that berberine inhibits pancreatic lipase by binding to essential residues in the active site. The study indicated that berberine interferes with triglyceride breakdown, contributing to antiobesity effects. The improved anti-lipase activity in BerberineQA™ suggests the energization process may have contributed to the inhibitory activity.

The known inhibitors of pancreatic α-amylase and α-glucosidase are acarbose. Acarbose has a similar structure to carbohydrates and acts as a competitive inhibitor to these enzymes. Acarbose is very effective, but a lot of patients report discontinuing it due to its gastrointestinal side effects.[13, 14] Therefore, developing a better inhibitor that has a multi-target effect and better tolerability becomes important.

In the α-amylase inhibition assay, BerberineQA™ achieved a lower IC_{50} value compared to standard berberine, indicating greater inhibition. In the α-glucosidase inhibition assay, BerberineQA™ also showed better inhibition than that of standard berberine. Previous reports indicated that berberine exhibits strong inhibitory activity against α-amylase and α-glucosidase by interacting with enzyme active sites and inducing conformational changes that lower catalytic activity.[15,16] The research also showed that berberine inhibits carbohydrate digestion and reduces glucose release by binding to catalytic site residues for substrate breakdown. The better α-glucosidase inhibition by BerberineQA™ is important since inhibiting this enzyme is an effective way to lower post-meal blood glucose. Past studies have indicated that berberine enhances glucose metabolism through several pathways, including the activation of AMPK, increased insulin sensitivity, modification of gut bacteria, and reduced glucose absorption in the gut.[17,18] These findings further validate berberine's antidiabetic potential and show that BerberineQA™ has improved enzyme inhibition compared to standard berberine.

Diabetes mellitus and obesity are closely related metabolic disorders with high worldwide prevalence. Inhibiting both carbohydrate and lipid-digesting enzymes may provide greater therapeutic outcomes in managing metabolic syndrome.[4,5] Imenshahidi and Hosseinzadeh (2019) detailed berberine's pharmacological effects, such as lowering glucose, reducing lipids, and its antioxidant and anti-inflammatory properties.[2] In another study, it was reported that berberine enhances insulin sensitivity, lowers liver glucose production, boosts glucose uptake, and regulates lipid metabolism.[19]

Overall, these findings suggest that BerberineQA™ offers better antidiabetic and antiobesity potential compared to standard berberine. The lower IC_{50} values against α-amylase and α-glucosidase, along with detectable pancreatic lipase inhibitory effects, show improved multifunctional metabolic enzyme inhibition. The increased enzyme inhibitory activity seen for BerberineQA™ may be linked to differences in its processing. However, this study did not explore physicochemical characteristics or molecular mechanisms.

Limitations

The pancreatic lipase inhibition assay was done as an exploratory screening experiment with a single absorbance measurement at each concentration. Therefore, measures of variability, such as standard deviation or standard error, and corresponding error are not represented.

The objective of the current study was the comparison of standard extract and EASI™, thus orlistat, as a positive control for the pancreatic lipase inhibition assay, was not included.

Conclusion

The current study showed that both BerberineQA™ and standard berberine exhibit antidiabetic activity by inhibiting α-amylase and α-glucosidase enzymes. However, BerberineQA™ consistently showed greater inhibitory effects than standard berberine. Additionally, BerberineQA™ showed some ability to inhibit pancreatic lipase, while standard berberine did not demonstrate any measurable anti-lipase effect under the test conditions. These results suggest that BerberineQA™ may have better enzyme inhibitory activity affecting both carbohydrate and lipid metabolism. It may be a more effective option for managing diabetes and obesity than conventional berberine. Further studies on its mechanisms, pharmacological properties, and effects in living organisms are needed to strengthen its therapeutic potential.

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