α-Glucosidase Inhibitory Activity of Curcumin and Its Comparison With Combinatorial Extract Consisting of Curcumin with Piperine and Quercetin.

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Summary

The present investigation compares the glucosidase inhibitory characteristics of curcumin with the combinatorial extract consisting of "Curcumin with piperine and quercetin".α-glucosidase is one among the number of glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion. α-glucosidase inhibitors block the actions of the enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption. Postprandial glucose peaks may be attenuated by delayed glucose absorption. Combinatorial extract consisting of "Curcumin with piperine and quercetin" were evaluated *in vitro* for the α-glucosidase inhibitory activity via UVspectroscopy.The results indicated that the combinatorial extact is a potent inhibitor of enzyme α-glucosidase (with IC50 value of 16.91 mg/ml) when compared to curcumin alone (whose IC50 value is 25.86 mg/ml.) thus providing more insight into the mechanisms of the antidiabetic action of combinatorial extract of curcumin and also provided a scientific basis for its usage in the traditional systems of medicine, for the management of diabetes.

Keywords: α-glucosidase, curcumin, piperine, quercetin, diabetes

Introduction

Diabetes and its complications are major health problem in modern society[1]. More than 171 million people worldwide are currently believed to be afflicted with type 2 diabetes and it is estimated that the number will rise to 366 million by 2030[2,3]. α-Glucosidase is the enzymes found in epithelium membrane of the small intestine, and it is the key enzyme for the digestion of carbohydrates[4]. It specifically hydrolyzes the α-glucopyranoside bond, there by releasing a α-D-glucose from the non-reducing end of the sugar at optimum temperature of 37ºC and a pH range of 6.0 to 6.5[5]. The availability of α-glucosidase inhibitors is useful in controlling the blood glucose level for hyperglycemic person[6]. Recently there had been widespread interest in these enzymes, partly because of their potential as therapeutic targets, especially, the inhibition of α-glucosidase had been found to help control postprandial blood glucose levels in diabetic patients[7,8.] Therefore, the search for effective and safe α-glucosidase inhibitors from natural materials, for antidiabetic agents is necessary.

Curcumin is one such medicine that has enormous potential for a variety of diseases, higher safety margin than the synthetic drugs and is cost effectiveness. Despite having wide spectrum of pharmacological actions, the medicinal properties of curcumin cannot be utilized due to its low *in vivo* bioavailability[9]. Therefore in view of the foregoing, there is an extensive need for combinatorial extracts "Curcumin with piperine and quercetin" which may enhance bioavailability of oral curcumin by inhibiting the enzymes responsible for the metabolism of curcumin. Piperine and quercetin are the two other components which also has individual effect on the inhibitory activity of α-Glucosidase enzyme. The interesting discovery of the α-glucosidase inhibitory activity of phenolic compounds prompted us to study an effect of curcumin in comparison with the combinatorial extract consisting of curcumin with piperine and quercetin which may be the possible mechanism for the antidiabetic activity of this extract and can be considered as a potential candidate in the treatment of diabetes.

Materials and methods

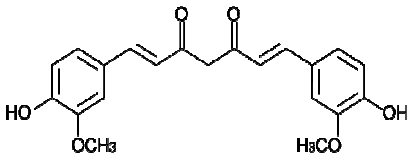
Glacial acetic acid, Maltose and Sodium hydroxide was purchased from fisher chemicals ltd. Mumbai.
Yeast was purchased from the commercial bakery shop. Glucose Oxidase kit was purchased from Transasia-Biomedicals Limited, Mumbai. All other chemicals were of AR grade.

Plant material

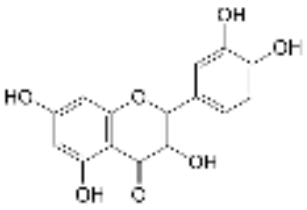
Dried rhizomes of *Curcuma longa* Linn. (Zingiberaceae), and dried seeds of *Piper nigrum* (Piperaceae) and the red onion of *Allium cepa* (Alliaceae) were collected from the from the local market of Mumbai, India and authenticated by Department of Raw and Crude drug material, National Institute of Science Communication and Information Resource (NISCAIR), New Delhi. The voucher specimens were deposited in department for future reference.

Extraction and isolation of active constituents

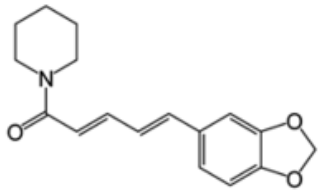
Extraction and isolation of curcuminoid, piperine and quercetin from *Curcuma longa Linn, Piper nigrum and Allium cepa* was done using petroleum ether, chloroform and ethanol as a solvent. The % yield obtained was found to be about 6.18 %, 9 % and 0.1 % (w/w) for curcumin, piperine and quercetin respectively.



Curcumin



Quercetin



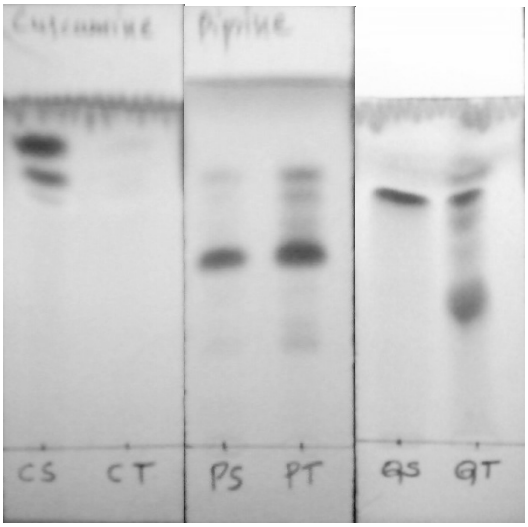
Piperine

Fig 1: Structure of main constituents from *Curcuma longa, Allium cepa,* and *Piper nigrum.*

TLC was developed by using mobile phase, as Chloroform: Methanol (9:1) for curcumin, Toluene: Ethyl acetate (7:3) for piperine and Chloroform: Methanol: Toluene (7:3:1) for quercetin. The R_f value was found to be similar with that of the standard (Fig. 2) & (Table 1).

Table 1: Solvent system for the thin layer chromatography of curcumin, piperine and quercetin.

	Mobile Phase	Std. R_f value	Isolated R_f value
Curcumin	Chloroform : Methanol (9:1)	0.79	0.78
Piperine	Toluene: Ethyl acetate (7:3)	0.51	0.52
Quercetin	Chloroform: Methanol :Toluene(7:3:1)	0.69	0.69



Curcumin Piperine Quercetin

Fig 2: TLC plates of curcumin, piperine and quercetin with their standards.

Procedure

The method is based on principle that, α-Glucosidase is the enzyme present in the intestinal lining, which hydrolyses terminal non-reducing α-1, 4-linked glucose residues in different substrates, releasing α-D-Glucose. The enzyme has optimum temperature of 37ºC and a pH range of 6.0 to 6.5. The tubes were divided into three groups named control, blank, and test. 100 µL Acetate buffer (pH 6.3) was added in the test tubes of all groups., 50 µL Enzyme (as a 4% yeast suspension) was added in "control" and "test", and 50 µL of curcumin and combinatorial extract at the concentration of 1, 12.5, 25, 37.5, and 50 (mg/mL), was added in "test" and "blank" tubes followed by the addition of 50 µL of distilled water in "control" and "blank" to equalize volume. At the end of 10 mins, 50 µL of 20 % maltose solution was added to the "control" and "test" tubes after 10 mins of mixing above substances. Then all the tubes were kept in water bath at 37 ºC for 30 mins for reaction to be carried out. At the end of 30 mins, the tubes were transferred to boiling water bath for 5 mins to stop the reaction. The dilution was carried out according to the following table (Table 2)

Table 2: Study design to check inhibitory activity on α-Glucosidase.

Substances	Control	Blank	Test
Acetate Buffer	100 µL	100 µL	100 µL
Enzyme	50 µL		50 µL
Distilled water	50 µL	50 µL	
Plant extracts		50 µL	50 µL

Glucose estimation:

The glucose estimation was done using the glucose oxidase kit, spectrophotometrically. The test solutions from the above section were taken and dilution was made according to following table (Table 3).

Table 3: Estimation of glucose

	Main blank	Standard	Unknown
Distilled water	20 µl		
Glucose standard		20 µl	
Test solutions			20 µl
Glucose reagent	3 ml	3 ml	3 ml

The tubes were mixed thoroughly and kept at 37 ºC for 15 mins, after incubation the color produced is stable for at least 2 hours if not exposed to direct sunlight. Above solutions were analyzed spectrophotometrically and the absorbance was taken, and the % inhibition was calculated from the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of actual test}}{\text{Absorbance of control}} \times 100$$

Where;

Absorbance of actual test = Absorbance of test – absorbance of blank

Since all the plant samples used have their own glucose content, it becomes necessary to consider it while performing the enzyme assay. To eliminate the effect of this glucose, an enzyme blank was prepared. As it contains only plant extract and buffer, the colorimetric reading obtained after the GOD test corresponds to the glucose content of the plant samples itself. This value was subtracted from the colorimetric reading of the test to give the amount of glucose obtained by the actual conversion of maltose because of the enzyme. The % inhibition was calculated from the above equation and the graph of concentration vs. percent inhibition was plotted for both the preparation curcumin as well as combinatorial extract. And from the graph IC 50 value was calculated. The IC 50 value is the value of concentration at which the plant extract shows the 50 % inhibitory activity.

Results and Discussion

The present investigation compares the glucosidase inhibitory characteristics of curcumin with the combinatorial extract of curcumin. With a constant rise in the incidence of type II diabetes around the world it appears that more anti-diabetic drugs with complementary mechanisms of action should be developed, in order to achieve durable glycemic control by inhibiting, in a reversible way, the hydrolysis of disaccharides and the ultimate steps of the digestion of dietary polysaccharides, to reduce the rise of postprandial blood glucose in diabetics [5]. α-glucosidase is one among the number of glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion[10].α-glucosidase inhibitors block the actions of the enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption. Postprandial glucose peaks may be attenuated by delayed glucose absorption. The results showed that combinatorial extract of curcumin can cause glucosidase enzyme inhibition in lower concentration range than curcumin extract alone (Table 4). The inhibition of α-glucosidase by combinatorial extract of curcumin can be attributed to the presence of flavonoids and steroids[11], which are reported to act as strong antioxidants and anti-inflammatory agents thus have a significant α-glucosidase inhibitory activity.

Table 4: α-glucosidase inhibitory activity of CPQ consisting of curcumin with piperine and quercetin.

Concentrations of Drug (mg/mL)		Blank		Test		Actual test		% Inhibition	
	Control	C	CPQ	C	CPQ	C	CPQ	C	CPQ
1.00	0.24	0.05	0.05	0.25	0.25	0.20	0.19	14.98	20.72
12.50	0.24	0.1	0.17	0.33	0.32	0.18	0.15	22.33	37.68
25.00	0.24	0.29	0.35	0.41	0.43	0.12	0.08	48.87	67.15
37.50	0.24	0.42	0.51	0.50	0.54	0.07	0.02	69.63	88.70
50.00	0.24	0.55	0.69	0.58	0.64	0.02	0.04	88.97	119.99

Where C: Curcumin, and
CPQ: Combinatorial extract of curcumin with piperine and quercetin.

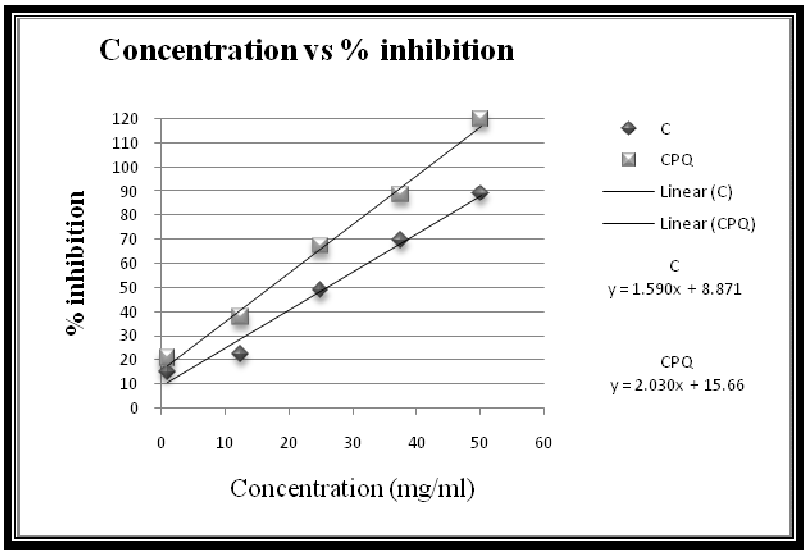


Fig 3: Comparison of the α-Glucosidase inhibitory activity of the curcumin and combinatorial extract

The following tables (Table 4) and figure 3 shows the %inhibition of α-glucosidase along with IC_{50} of curcumin and CPQ. An increased IC_{50} was observed (25.86 mg/ml) for the curcumin extract when compared with CPQ (16.91 mg/ml). All determinations were done in triplicate and the mean values were determined.

Conclusion

In conclusion, the results of the present study indicates that the combinatorial extract is more effective in inhibiting the enzyme α-glucosidase than curcumin extract alone thus providing more insight into the mechanisms of the antidiabetic action of CPQ and also provide a scientific basis for its usage in the traditional systems of medicine, for the management of diabetes.

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