Inhibitory activities of *Ficus benghalensis* bark against carbohydrate hydrolyzing enzymes - An *in vitro* study

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ABSTRACT

The present study evaluated the effect of *Ficus benghalensis* Linn. (Moraceae) stem bark on porcine pancreatic α-amylase, rat intestinal α-glucosidase and sucrase. Further, the effect of heat treatment was also studied. Both untreated and heat-treated samples inhibited α-amylase to a significant extent. Further, the aqueous extracts prepared form untreated and heat treated samples exhibited significant inhibition ($p \leq 0.05$) of α-glucosidase and sucrase in a dose dependent manner. Heat treatment of the sample increased α-glucosidase and sucrase inhibitory activities at lower concentrations, however no statistical differences were observed at higher concentrations. Consequently, the untreated extracts showed IC_{50} values of 158 and 193 µg mL^{-1} for α-glucosidase and sucrase respectively while, the heat-treated extracts showed the IC_{50} values 77 and 141 µg mL^{-1} respectively. Further, a significant correlation ($p \leq 0.05$; $r = 0.698$) was observed between α-glucosidase and sucrase inhibitory activities of both untreated and heat-treated extracts. The results clearly demonstrate that inhibition of carbohydrate hydrolyzing enzymes is one of the mechanism through which *F. benghalensis* bark exerts its hypoglycemic effect *in vivo*.

Key words: *Ficus bengalensis,* α-amylase, α-glucosidase, sucrase, diabetes

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1] Control of postprandial plasma glucose rise is critical in the early treatment of diabetes mellitus[2] as studies indicate that postprandial hyperglycemia induces non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications such as micro and macro vascular diseases.[3] Postprandial glucose rise can be controlled by reducing/delaying the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes such as α-amylase, α-glucosidase, β-glucosidase and sucrase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial hyperglycemia.[4] A number of alpha glucosidase inhibitors have been recently developed from the natural sources,[5-6] of which acarbose, miglitol and voglibose are in clinical use.[7]

Ficus benghalensis Linn (Moraceae) commonly known as banyan tree in English and bargad in Hindi is named after the Hindu traders, called Banyans, who favored the tree.[8] Different parts of *F. benghalensis* tree have been found to possess medicinal properties; leaves are good for ulcers, aerial roots are useful in gonorrhea, seeds and fruits are cooling and tonic.[9] The bark is astringent and is useful in the treatment of dysentery, diarrhea and diabetes. Stem bark is used as antihelminthic.[10] *F. benghalensis* is one of the most widely explored medicinal plants for the antidiabetic activity wherein, the antidiabetic potential of various parts of *F. benghalensis,* particularly of the bark has been evaluated in experimental diabetes using alloxan/ streptozotocin as diabetogenic compounds in animal models. It is one medicinal plant whose active components such as perlargonidin derivatives, leucopelargonin derivatives and α-amyrin acetate have been extensively evaluated for the antidiabetic activity *in vivo*.

In the present investigation *Ficus benghalensis* stem bark, a proven hypoglycemic agent,[11-12] being used by various cultures across India for the treatment of diabetes was studied for its ability to inhibit the carbohydrate hydrolyzing

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enzymes using *in vitro* model systems. Further, the effect of heat treatment on the enzyme inhibitory activities was also studied.

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic α-amylase (23 u/mg solid) was purchased from Sigma Aldrich, India. p-nitrophenyl-α-D-glucopyranoside and 3,5-dinitrosalysilic acid were purchased from Sisco Research Laboratory, India. Glucose oxidase peroxidase assay kit was purchased from Agappe Diagnostics, India. All the chemicals and reagents used in the study were of extra pure analytical grade.

Collection of plant material

Ficus bengalensis stem bark was collected form a mature tree in the campus of University of Mysore, India and was identified by Dr. Niranjan, Department of Botany, University of Mysore, India. The bark was cut into small pieces, dried at 50°C overnight, powdered and passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

Heat treatment

The bark powder was subjected to heat treatment in a vacuum oven at 100°C for 60 minutes, cooled in a desiccator and used for the preparation of the heat-treated extract (FBH).

Preparation of extracts

Aqueous extracts (Untreated and heat-treated) were prepared by extracting the untreated and heat-treated bark powders with distilled water (1:8 w/v) on a mechanical shaker, for 24 hours, at room temperature.

Assay of α-amylase inhibitory activity

The effect of *F. benghalensis* on α-amylase activity was studied using an enzyme-starch system.[13] *F. benghalensis* powder (1%) was mixed by stirring with 25 mL of 4% potato starch in a beaker; 100 mg of α-amylase was added to the starch solution, stirred vigorously, and incubated at 37°C for 60 minutes. After the incubation period 0.1 M NaOH was added, to terminate enzyme activity. The mixture was centrifuged (3000 xg; 15 minutes) and the glucose content in the supernatant was determined.

Assay of α-glucosidase inhibitory activity

A crude enzyme solution of rat intestinal α-glucosidase and sucrase, prepared according to the method of Dahlqvist,[14] was used to assay the α-glucosidase and sucrase inhibitory activities, according to the method of Honda and Hara.[15] Ten milliliters of enzyme solution and varying concentrations of the aqueous extract (100-500 µg) were incubated together for 10 minutes, at 37°C, and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 µl of 2 mM p-nitrophenyl-α-D-glucopyranoside solution and further incubated at 37°C for 30 minutes. The reaction was terminated by treating the mixture in a boiling water bath for five minutes. After the addition of 1.0 ml of 0.1 M disodium hydrogenphosphate solution, the absorption of liberated p-nitrophenol was read at 400 nm.

Assay of sucrase inhibitory activity

The effect of *F. benghalensis* on sucrase activity was assayed according to the method of Honda and Hara.[15] The enzyme solution (10 µL) and varying concentrations of the aqueous extract (100-500 µg) were incubated together for 10 minutes at 37°C, and the volume was made up to 200 µL with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 µl sucrose solution (60 mM). After 30 minutes, the reaction was terminated by adding 200 µL of 3,5- dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for five minutes. The absorbance of the solution was read at 540 nm.

The percent inhibitory activities were calculated using the following formula:

$$\%\ \textit{inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where, *Abs control* is the absorbance of the control reaction (containing all reagents except the test sample), and the *Abs sample* is the absorbance of the test sample. An untreated enzyme solution was used as the control. All the experiments were carried out in triplicate.

Statistical analysis

The data was analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences, using SPSS 14.0 computer software. The values were considered significant when $p \leq 0.05$. IC_{50} values were calculated by Boltzmann's dose response analysis using Origin 6.1 software.

RESULTS

Effect of *Ficus benghalensis* on α-amylase activity

The α-amylase inhibitory activity of *F. benghalensis* powder (FBP) was studied using α-amylase-starch model system and the results indicate that untreated FBP inhibited α-amylase to an extent of 50% at the concentration of 1%, while heat-treated FBP inhibited α-amylase by 47% at the same concentration. No statistical difference was observed

between the α-amylase inhibitory activity of untreated and heat-treated FBP.

Effect of *Ficus benghalensis* on α-glucosidase and sucrase activity

The effect of *F. benghalensis* extracts on α-glucosidase activity is shown in Figure 1. Both the untreated and heat treated extracts significantly inhibited ($p \leq 0.01$) α-glucosidase in a dose dependent manner. The inhibitory activities ranged between 35-97% and 55-98% respectively for untreated and heat-treated extracts. The IC_{50} value of heat-treated extract was significantly lower ($p \leq 0.05$) than that of untreated extract. An IC_{50} value of 77 μg mL^{-1} was observed for heat-treated extract while, the IC_{50} value of untreated extract was found to be 158 μg mL^{-1}.

Effect of *Ficus benghalensis extracts* on sucrase activity

The sucrase inhibitory activity ranged between 40-71% and 45-73% for untreated and heat-treated *F. benghalensis* extracts respectively (Figure 2). A dose dependent inhibition of rat intestinal sucrase was observed in both untreated and heat treated *F. benghalensis* extracts and heat treatment did not result in any significant change ($p \leq 0.05$) in the sucrase inhibitory activity of the sample. Consequently, no significant difference ($p \leq 0.05$) was observed between the IC_{50} values of both the extracts. The IC_{50} value for heat-treated extract was 141 ± 22.1 while, the IC_{50} value for untreated extract was 193 ± 21.6.

A significant correlation ($p \leq 0.05$; $r = 0.698$) was observed between α-glucosidase and sucrase inhibitory activities of both untreated and heat treated *F. benghalensis* extracts and the enzyme inhibitory activities of *F. benghalensis* extracts were directly proportional to the sample concentration.

Table 1: IC_{50} values for α-glucosidase, and sucrase activities (μg mL^{-1})

Enzyme	FBU	FBH
α-glucosidase	158[b] ± 4.1	77.1[a] ± 8.7
Sucrase	193[a] ± 21.6	141[a] ± 22.1

*FBU: untreated *Ficus benghalensis* extract, FBH: heat-treated *Ficus benghalensis* extract
**Mean values carrying different superscript letters a & b in rows, differ significantly ($p \leq 0.05$)

DISCUSSION

The present study evaluated the effect of *F. benghalensis* bark on carbohydrate hydrolyzing enzymes viz. α-amylase, α-glucosidase and sucrose since, the development of antidiabetic drugs with complementary mechanisms of action appears essential to achieve good glycemic control by inhibiting in a reversible way the hydrolysis of carbohydrates, to reduce postprandial blood glucose rise in type 2 diabetics.[16]

Several mechanisms have been proposed for the hypoglycemic effect of phytochemicals, such as inhibition of carbohydrate

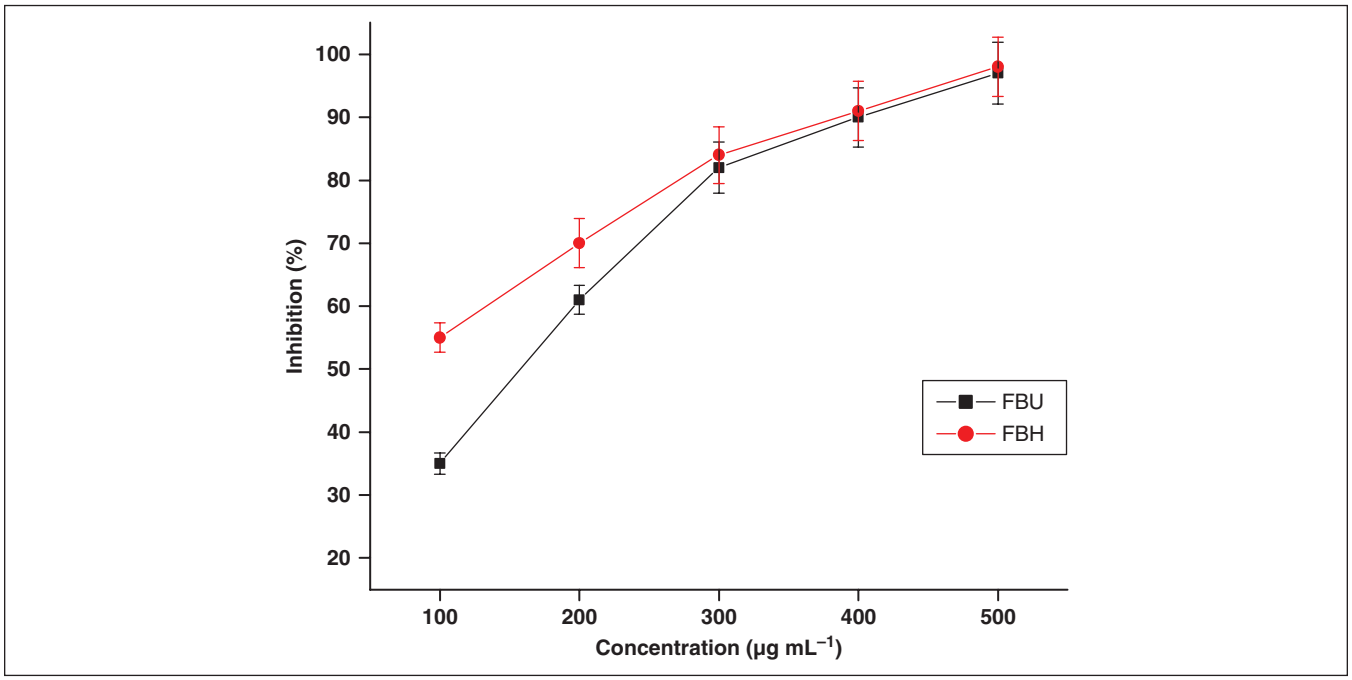

Figure 1: Effect of *Ficus benghalensis* bark extracts on α-glucosidase activity.
FBU: untreated Ficus benghalensis *extract, FBH: heat-treated* Ficus benghalensis *extract*

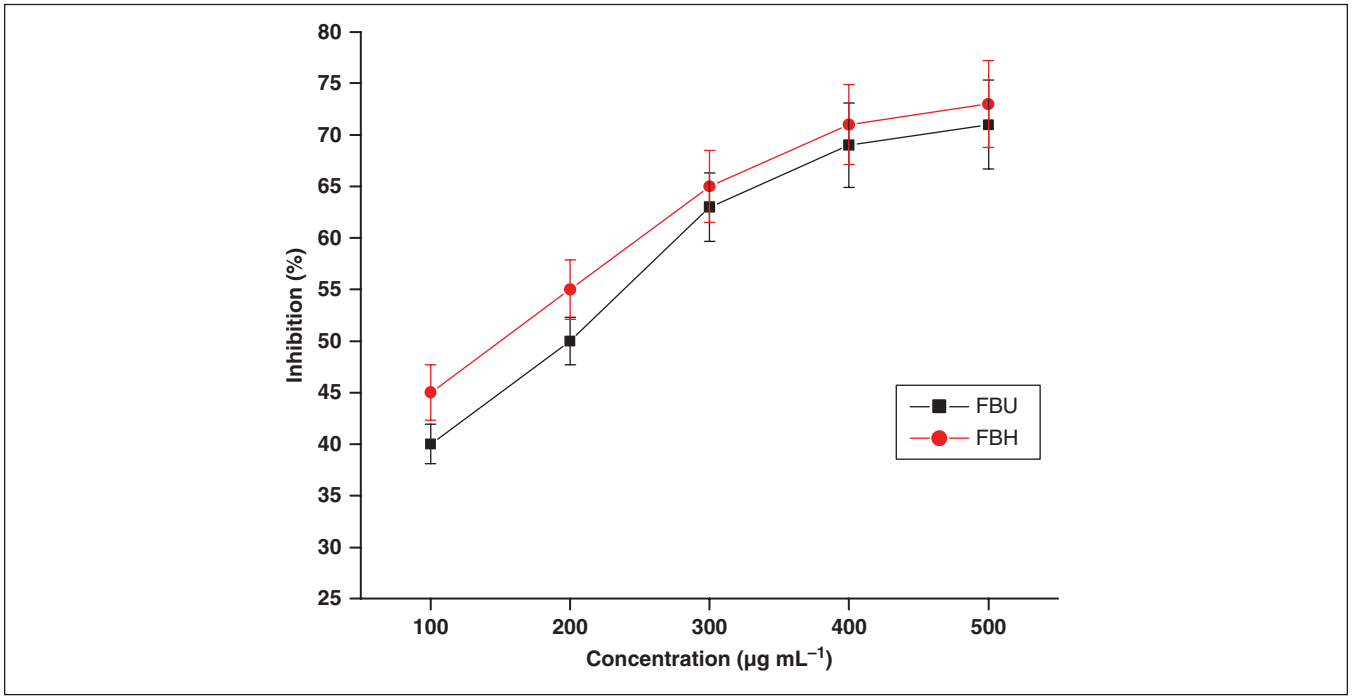

Figure 2: Effect of *Ficus benghalensis* bark extracts on sucrase activity.
FBU: untreated Ficus benghalensis *extract, FBH: heat-treated* Ficus benghalensis *extract*

metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration and enhancing insulin releasing activity and sensitivity.[17] In the present investigation, both untreated and heat treated samples effectively inhibited porcine pancreatic α-amylase which can possibly be attributed to several factors such as fiber concentration, presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample thereby reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers leading to decreased amylase activity.[13]

Glucosidases are crucial in many biological processes, including breakdown of edible carbohydrates.[18] 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.[19] The inhibition of α-glucosidase by *F. benghalensis* bark can be attributed to the presence of flavonoids and phenolic glycosides[20] having potential antioxidant activity, as reports indicate that phenolic enriched extracts of *Solanum melongena* with moderate free radical scavenging linked antioxidant activity had high α-glucosidase inhibitory activity. Inhibition of this enzyme provides a strong biochemical basis for management of type 2 diabetes by controlling glucose absorption.

The increase in the α-glucosidase by heat treatment may be due possible inactivation of the phytoconstituents that hinder/decrease the inhibition of glucosidases by the heat treatment. The main benefits attributable to α-glucosidase inhibitors are reductions in both postprandial glycemic levels and in the total range of postprandial glucose levels.[21]

Rat intestinal sucrase occurs as a complex of sucrase and isomaltase which converts sucrose into glucose.[22] The inhibition of sucrase by *F. benghalensis* bark extracts may also be due to its phenolic compounds. Further, the correlation observed between α-glucosidase and sucrase inhibitory activities of both untreated and heat-treated extracts represents a parallel and effective inhibition of the brush border enzymes in the digestive tract.

CONCLUSION

The findings of the present study emphasizes that, inhibition of carbohydrate hydrolyzing enzymes such as α-amylase, α-glucosidase and sucrase is one of the mechanisms through which *F. benghalensis* bark exerts its hypoglycemic effect *in vivo*. The study also supports the traditional usage of *F. benghalensis* bark as an antidiabetic agent.

REFERENCES

1. WHO. *Definition, diagnosis and classification of diabetes mellitus and its complications*, Part 1: diagnosis and classification of diabetes mellitus. Report of WHO Consultation, Geneva; 1999.
2. Shim YJ, Doo HK, Ahn SY, Kim YS, Seong JK, Park IS, *et al*. Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. J Etnhopharmacol. 2003; 85:283-7.

3. Baron AD. Postprandial hyperglycemia and α-glucosidase inhibitors. Diabetes Res Clin Pract. 1998; 40:S51-5.
4. Rhabasa-Lhoret R, Chiasson JL. International textbook of diabetes mellitus. Vol 1, 3rd ed. UK: John Wiley and Sons Ltd; 2004.
5. Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, *et al*. Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). J Agric Food Chem. 2001; 49:4208-4213.
6. Hiroyuki F, Tomohide Y, Kazunori O. Efficacy and safety of *Touchi* extract, an alpha-glucosidase inhibitor derived from fermented soybeans, in non-insulin-dependent diabetic mellitus. J Nutr Biochem.12001; 2:351-356.
7. Bailey CJ. Textbook of diabetes. Vol 2, 3rd ed. UK: Blackwell Science Ltd; 2003.
8. Neal MC. Gardens of Hawai'i. Bernice P. Bishop Museum Special Publication 40, Honolulu, Hawaii; 1965.
9. Satyavati GV, Raina MK, Sharma M. Medicinal plants of India. Vol. 1, Indian Council of Medical Research, New Delhi; 1976.
10. Anonymous. The Wealth of India: Raw Materials. Vol. 4, Council of Scientific and Industrial Research, New Delhi; 1956.
11. Augusti KT, Daniel RS, Cherian S. Effect of leucopelargonin derivative from *Ficus benghalensis* Linn: On diabetic dogs. Indian J Med Res. 1994; 99:82-86.
12. Singh AB, Yadav DK, Maurya R, Srivastava AK. Antihyperglycaemic activity of alpha-amyrin acetate in rats and db/db mice. Nat Prod Res. 2009; 23:876-882.
13. Ou S, Kwok K, Li Y, Fu L. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. J Agric Food Chem. 2001; 49:1026-9.
14. Dahlqvist A. Method for assay of intestinal disaccharides. Anal Biochem. 1964; 7:18-25.
15. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Biosci Biotechnol Biochem. 1993; 57:123-4.
16. Blickle JF, Andres E, Brogard JM. Current status of the treatment of type 2 diabetes mellitus: Alpha-glucosidase inhibitors. Food Chem. 2008; 106:247-52.
17. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci. 2002; 23:30-3.
18. Marshall J. Application of enzymic methods to the structural analysis of polysaccharides. Adv Carb Chem Biochem. 1974; 30:257-370.
19. Caspary WF. Sucrose malabsorption in man after ingestion of alpha-glucosidehydrolase inhibitor. Lancet. 1978; 1:1231-3.
20. Daniel RS, Mathew BC, Devi KS, Augusti KT. Antioxidant effect of two flavonoids from the bark of *Ficus bengalensis* Linn. in hyperlipidemic rats. Indian J Exp Biol. 1998; 36:902-906.
21. Lebovitz HE. Alpha-glucosidase inhibitors. Endocrinol Metab Clin North Am 1997; 26:539-51.
22. Hauri HP, Wacker H, Rickli EE, Bigler-Meier B, Quaroni A, Semenza G. Biosynthesis of sucrase-isomaltase: Purification and NH2-terminal amino acid sequence of the rat sucrase-isomaltase precursor (pro-sucrase-isomaltase) from fetal intestinal transplants. J Biol Chem. 1982; 257:4522-8.