Alpha Glucosidase inhibitory activity of *Morus Alba*

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Summary

Alpha-glucosidase inhibitors (AGIs; acarbose, voglibose) are widely used in the treatment of patients with type 2 diabetes. Alpha-glucosidase inhibitors are oral antidiabetic drugs. AGIs delay the absorption of carbohydrates from the small intestine and thus have a lowering effect on postprandial blood glucose and insulin levels. The present study showed on water extract of *Morus Alba* leaves were tested (in-vitro) for alpha glucosidase inhibitory activity. *Morus Alba* showed potent activity with an IC_{50} value of 28.11µg/ml.

Keywords: Alpha glucosidase inhibition, *Morus Alba, diabetes, insulin, acarbose*

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Introduction

Diabetes mellitus is a metabolic disorder characterized by elevation of fasting blood sugar (glucose). While the cause of the elevated blood glucose may be associated with either too little or too much insulin, the complications of chronically high serum glucose are devastating to the individual. Complications of uncontrolled blood sugar include increased risk of heart disease, stroke, kidney disease, blindness, and loss of nerve function. Regulating blood sugar for diabetics is therefore crucial to both the immediate as well as long-term care of diabetic patients (1).

A new class of antidiabetic drugs, Alpha-glucosidase inhibitors has been known as an approach for treating diabetes type II, and was introduced with the marketing of acarbose (Bayer Germany). Alpha-glucosidase inhibitors delay the digestion of oligosaccharide and disaccharide to monosaccharide by inhibiting alpha-glucosidases, maltase, isomaltase (disaccharidases) on the small intestinal brush-border and reduce the rate of glucose absorption. As a result, they decrease the postprandial rise in blood glucose concentration (2, 3). This more stable blood glucose concentration is important for diabetic patients, because it prevents hyperglycemia and the complications associated with diabetes. Therefore, the alpha-glucosidase inhibitor acarbose is a first-line drug for treating type-2 diabetes mellitus that is insufficiently controlled through diet alone (2).

Plant. *Morus Alba* L. (Moraceae) leaves were collected from Karnataka, India in 2008. The samples were authenticated by our Botany department, where the voucher specimens are preserved [Reference no.MI/PRO/04/09].

The *Morus Alba* is a short-lived, fast-growing and small to medium sized mulberry tree, which grows to 10–20 m tall. In traditional medicine, the plant has been extensively studied for its Hypoglycemic, hypolipidemic, neuroprotective, hepatoprotective, hypouricemic, antioxidant and cardio protective actions. The plant is reported to contain the phytoconstituent tannins, phytosterols, sitosterols, phenols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as the main active principles (4, 5&6). The present study is carried out to confirm this ethanopharmacological claim of the plant

Tested material: Water extract of *Morus Alba* dried leaves (yield: 14.30% on dry basis).

Materials and Methods:

Chemicals used: Acarbose from Bayer's Pharma, Glucose kit from Mercodia. Lowry's reagent & Sucrose were procured from Himedia Laboratory Ltd, Mumbai, India.

Preparation of Enzyme:

The rats were sacrificed and the intestine was removed, chilled with ice cold 80 mM phosphate buffers (pH-7.0). The intestine was then cut open & the mucosa was scraped off with a piece of glass rod and homogenized in homogenizer with four parts (v/w) of cold 80 mM buffer (pH-7.0). The tube was chilled with crushed ice during homogenization. Nuclei and large cell debris were removed by centrifugation at 4000 rpm for 10 minutes and supernatant was stored at – 20^0C. Adjusted protein content approximately 0.5 g/dl. by Lowry's method (7).

Assay procedure:
This was performed as per Matsuo *et al.*, (8). A pre-incubation volume of 50µl enzyme with 250µl of various concentrations of test samples was incubated at 37^0C for 30 minutes. Added 500µl of sucrose solution and incubated at 37^0C for 20 minutes, heat on boiling water bath for 2 minutes to arrest the reaction and cooled. The glucose concentration was measured by glucose oxidase method.

Glucose estimation (Glucose oxidase method)***:*** Mixed 10µl of sample with 500µl of glucose reagent (Glucose reagent kit) then incubated at room temperature for 10 minutes. The absorbance was measured at 510 nm in a Hidex micro plate reader. Acarbose was used as reference standard.

Statistical Analysis — All data are expressed as the mean ± SEM. The Statistical data were evaluated by using Graph pad Prism4 software. The % inhibition was calculated using the formula, control – test/control x 100. The IC_{50} value was determined by nonlinear regression curve fit using Graph pad Prism4.

Results

Table 1. : **Alpha glucosidase inhibitory activity of *Morus Alba* leaves water extract**

Sl. No.	Tested material	Concentration µg/ml	% inhibition ± S.E.M	IC_{50} (µg/ml) (95% C.I)[a]
1	*Morus Alba* (n=3)	0	2.98 ± 1.20	31.04 (19.35-80.61)
		1	5.68 ± 1.40	
		5	8.99 ± 1.60	
		10	14.99 ± 0.46	
		20	28.77 ± 0.90	
		40	39.87 ± 1.50	
		80	51.22 ± 1.84	
		160	60.28 ± 1.24	
2	Acarbose[b] (n=3)	0	2.1 ± 1.35	6.79 (5.81-26.79)
		0.5	4.68 ± 2.0	
		1	8.07 ± 1.9	
		2.5	13.21 ± 1.67	
		5	26.44 ± 2.04	
		10	42.55 ± 2.08	
		20	50.22 ± 1.94	
		40	59.22 ± 1.9	

a – 95 % confidence interval
b – Reference inhibitor, Sigma, St Louis, USA

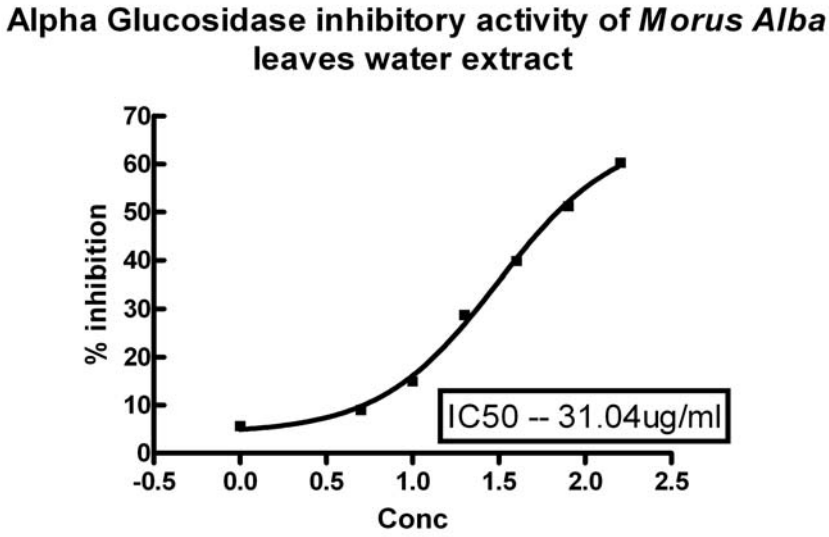

Fig 1. Alpha glucosidase inhibitory activity of *Morus Alba* leaves water extract

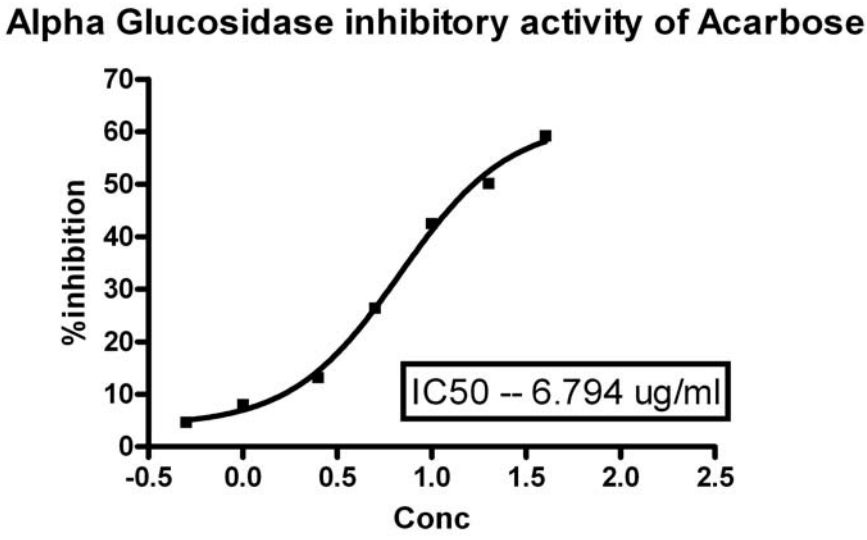

Fig 2. Alpha glucosidase inhibitory activity of Acarbose, Positive control

Discussion

Alpha-glucosidases are a series of enzymes located on the intestinal brush-border. The most important carbohydrates in food, such as starch and sucrose, are hydrolyzed to monosaccharide, such as glucose and fructose, by an [alpha]-glucosidase, and then absorbed into the blood, thereby increasing blood glucose value. Usually, these processes take place in the upper portion of the small intestine and greatly increase blood glucose concentration, especially in diabetic patients. Alpha-glucosidase inhibitors can prolong the processes along the entire intestine, lengthen the duration of carbohydrate absorption, and flatten the blood glucose concentrations over time curve (Bischoff, 1993). Because the alpha-glucosidase inhibitor acarbose prevents an abnormally high rise in postprandial blood glucose concentrations, it is a first-line drug in treatment of type-2 diabetes that is not controlled through diet alone (Hanefeld et al., 1991). In our experiments, *Morus Alba* inhibited brush border enzymes alpha-glucosidase significantly and it may delay absorption carbohydrates that lead to flatten blood glucose concentrations. This may explain the antidiabetic effect of *Morus Alba*, since alpha-glucosidase inhibitory activity is involved in the carbohydrate blockers for delaying the absorption of glucose from the small intestine. These effects are similar to those for the alpha-glucosidase inhibitor, acarbose. *Morus Alba* water extract exhibited competitive type of enzyme inhibition.

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References:

1. Muller G. The molecular mechanism of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug Amaryl. Mol Med 2000; 6(11): 907–933.
2. Bischoff H. Pharmacology of [alpha]-glucosidase inhibition. Eur J Clin Invest 1995; 24 (3): 165-168
3. Hanefeld M. Fischer S, Schulze J, et al. Therapeutic potentials of acarbose as first-line drug in NIDDM insufficiently treated with diet alone. Diabetes Care 1991; 14: 732-738
4. Nomura T. Fukai T, Hano Y, Nemato K, Terada S, Kuramochi T, et al. Isolation of two new natural Diels-Alder adducts from Root Bark of *Morus alba*. Planta Med 1983; 47: 151-156
5. Kusano G. Orihara S, Tsukamato D, Shibano M, Coskun M, Guvenc A, et al. Five new nortropane alkaloids and six new amino acids from the fruit of *Morus alba* L growing in Turkey. Chem Pharm Bull (Tokyo) 2002; 50: 185-192
6. Chen C. Liu L, Huang H, Yang M, Wang C, et al. Mulberry extract inhibits the development of atherosclerosis in cholesterol fed rabbits. Food Chem 2005; 91:601-617
7. Dahlquist A. Method for assay of intestinal disaccharidases. Anal Biochem 1964; 7: 18-25
8. Matsuo T. Odaka H, Ikeda H, et al. Effect of an intestinal disaccharidase inhibitor. Am J Clin Nutr 1992; 55(1): 314S - 317S