

IN-VITRO EVALUATION OF GLUCOSE DIFFUSION AND AMYLOLYSIS KINETICS OF BETA-GLUCOGALLIN

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ABSTRACT

The standardized Beta-glucogallin was studied for its effects on glucose diffusion and amylolysis kinetics using in-vitro models. The results verified the antidiabetic potential of the standardized Beta-glucogallin.

KEYWORDS: Beta-glucogallin; glucose diffusion; amylolysis kinetics.

INTRODUCTION

Beta-glucogallin is an important tannin precursor naturally found in a variety of plants such as gooseberry (fruits of *Embellica officinalis*), raspberry, amla fruit extracts, and date palms (β -D-glucogallin present in fruits of *Phoenix dactylifera L.var.*), etc.^[1-3] Beta-glucogallin was reported to be a potential therapeutic agent in the management of a variety of diseases including diabetic complications such as diabetic cataract, prevention of cataract development and progression, retinal degradation in diabetic eyes, hyperglycemia, and inflammatory diseases and associated stress.^[1-6] It was found to possess hepatoprotective, anti-hyperlipidemic, nephroprotective, cardioprotective, and significant photoprotective efficacy against UV-induced cytotoxicity and enhanced melanogenesis. β -D-glucogallin was reported to possess antioxidant, anticancer, antibacterial, antimutagenic, and antiprotozoal activities.^[1-6] The present study was undertaken to verify the antidiabetic potential of Beta-glucogallin using various in vitro techniques and also as an attempt to predict its mechanism of action.

MATERIALS AND METHODS

Plant marker- The Beta-glucogallin (BGG) [(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3,4,5-trihydroxybenzoate (Product code: G012; Lot. no.: T18D239) was purchased from Natural Remedies Pvt. Ltd., Bangalore. Purity of BGG was determined by the manufacturer by HPLC area normalization and was certified as 95.4%.

Chemicals

Glucose oxidase peroxidase kit was procured from Pathozyme Diagnostics, Kagal, India. Dialysis bags (12 000 MW cutoff; Himedia laboratories, India) were used. All the chemicals used in the study were of extra pure analytical grade.

Evaluation of antidiabetic activity of Beta-glucogallin using various in vitro methods.

1. Effect of Beta-glucogallin on in-vitro glucose diffusion

It was performed according to the method stated by Ahmed *et al.*^[7] A total of 25 mL of glucose solution (20 mmol/ L) and the samples of plant extracts (1%) were dialyzed in dialysis bags against 200 mL of distilled water at 37 °C in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min using glucose oxidase peroxidase diagnostic kit. A control test was carried out without sample.

2. Effect of Beta-glucogallin on in-vitro amylolysis kinetics^[8]

A total of 40 g of potato starch was added to about 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution after stirring at 65 °C for 30 min was made up to a final volume of 1 000 mL to give a 4% (w/v) starch solution. And 25 mL of the above starch solution, α -amylase (0.4%), and the plant extracts (1%) were dialyzed in a dialysis bags against 200 mL of distilled water at 37 °C (pH 7.0) in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min. A control test was carried out without sample.

Glucose dialysis retardation index (GDRI) was calculated using the following formula.

$$\text{GDRI} = 100 - \frac{[\text{Glucose content with addition of sample (mg/dl)}]}{[\text{Glucose content of the control (mg/dl)}]} \times 100$$

Statistical analysis- All the determinations were carried out in triplicates and data were analyzed by ANOVA followed by students T test. Values were considered at $P < 0.05$.

RESULTS

Effect of Beta-glucogallin on in vitro glucose diffusion

The effect of Beta-glucogallin on retarding glucose diffusion across the dialysis membrane is shown in figures 1 and 2. The rate of glucose diffusion was found to increase with time from 30 onwards till 180 minutes. In this study, movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, Beta-glucogallin demonstrates significant inhibitory effects on movement of glucose into external solution across dialysis membrane as compared to the control.

Effect of Beta-glucogallin on in vitro amylolysis kinetics

The effects of Beta-glucogallin on the amylolysis kinetics are shown in the figures 3 & 4. The GDRI was found to be 62.77 % at sixty min time interval which gradually got reduced to 21.55 % at 120 minutes.

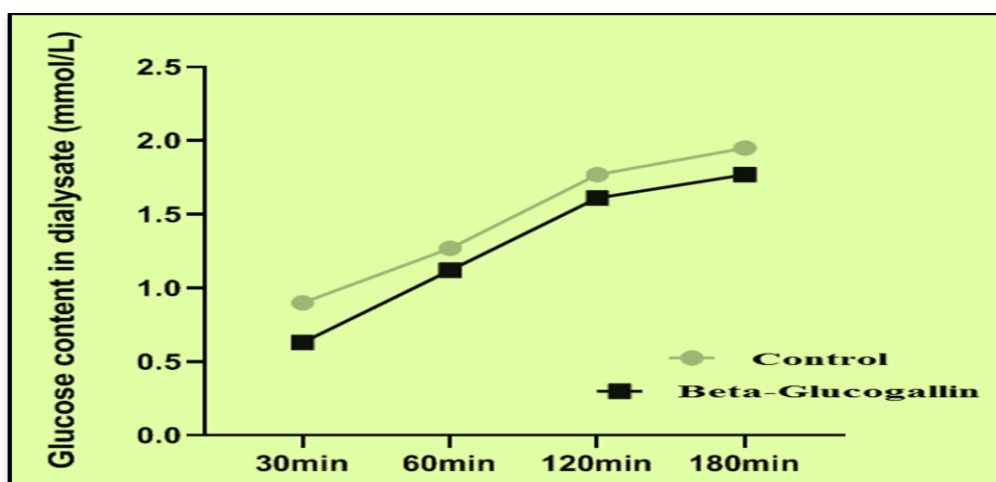


Figure 1: Effect of Beta-glucogallin on glucose diffusion.

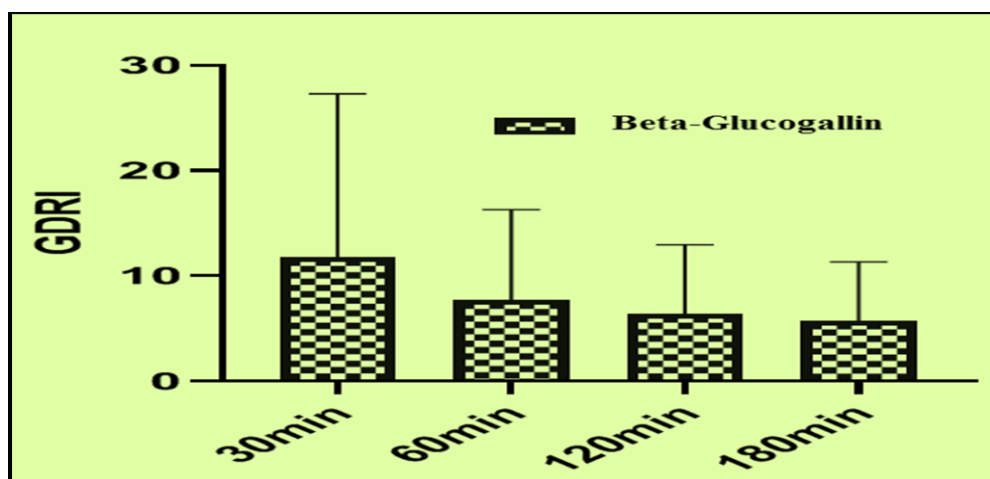


Figure 2: Effect of Beta-glucogallin on glucose GDRI.

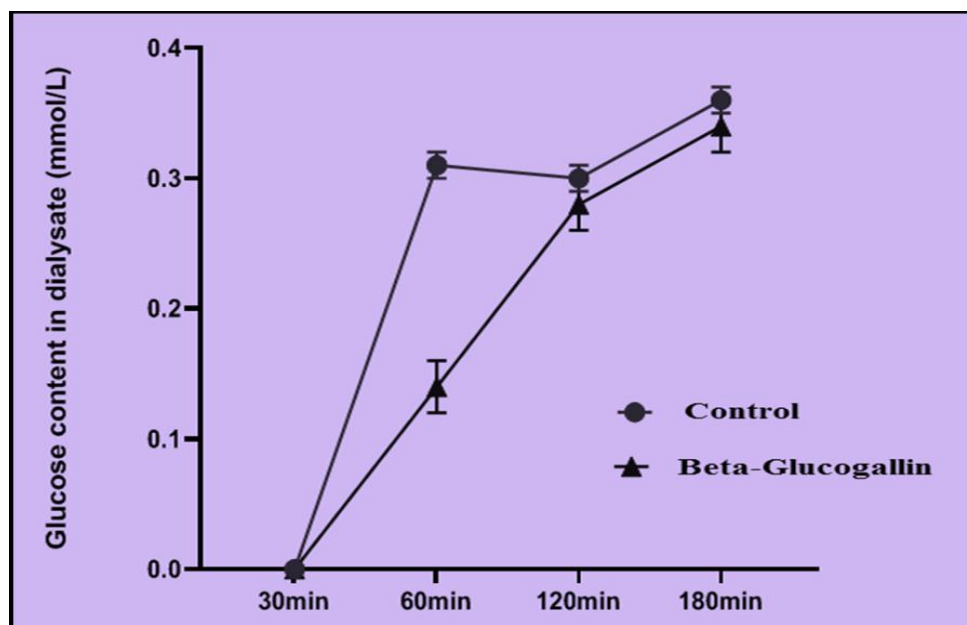


Figure 3: Effect of Beta-glucogallin on starch digestibility.

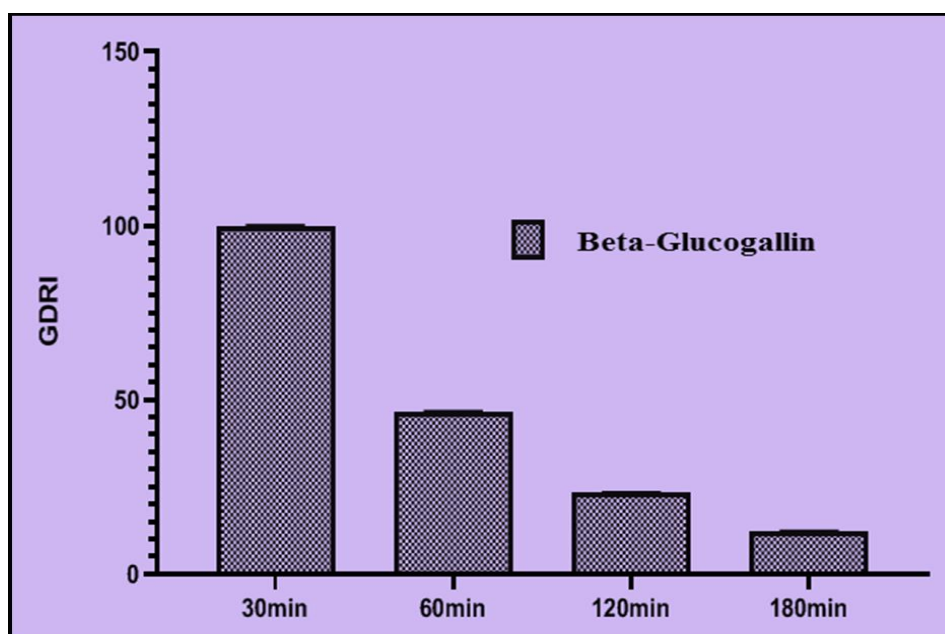


Figure 4: Effect of Beta-glucogallin on starch GDRI.

DISCUSSION

The retardation of glucose diffusion may also be due to the inhibition of α -amylase by the Beta-glucogallin thereby limiting the release of glucose from the starch. Ou et al. have mentioned several possible factors that may be responsible for α -amylase inhibition such as fiber concentration, the 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.^[9] GDRI

is a useful in vitro index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract. A higher GDRI indicates a higher retardation index of glucose by the sample.^[7,8] The GDRI was found to be 32.22 % at 30 minutes.

Amylolysis kinetic experimental model the rate of glucose diffusion was found to increase with the time from 30 to 180 minutes and both the extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane as compared to control.

To conclude, the results of the present study suggest hypoglycemic effect of Beta-glucogallin might be mediated by decreasing glucose diffusion rate.

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