Original Article

Hypoglycemic Potential of *Basella alba* Linn. - An *In Vitro* Study

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Abstract

Basella alba L., often referred as Malabar spinach, is a nutritionally valuable leafy vegetable grown in Asian nations such as India and Bangladesh. Apart from being an excellent source of vitamins and minerals, the vegetable also contains significant amount of polysaccharides, which have been proven to have effective antidiabetic action in experimental diabetes. As a reason, the current study was conducted to determine the exact mechanism of hypoglycemic action of *B. alba* aerial parts. The effect of *B. alba* powder (BAP) on starch digestibility diffusion, and adsorption of glucose, was investigated *in vitro*. *B. alba* extract (BAE) was also investigated for its influence on glucose transport in yeast cells. The results showed that BAP, at both 2% and 4% concentrations, bound more glucose than wheat bran (2%) and also retarded glucose diffusion across the dialysis membrane. In a starch digestibility experiment, BAP at both doses (2- and 4%) demonstrated full retardation of glucose diffusion until 120 minutes, similar to acarbose, which showed no glucose diffusion until 240 minutes. The effect of BAP at 4% was the more pronounced followed by BAP at 2% and wheat bran (2%). Furthermore, in a dose-dependent manner, BME enhanced glucose uptake by yeast cells. However, the percentage increase in glucose uptake was inversely related to the concentration of glucose. It is concluded that *B. alba* has potential hypoglycemic effect, which is mediated by creating a physical barrier to glucose absorption in the GI tract and improving peripheral glucose utilization.

Keywords: Malabar spinach, Glucose binding, Starch digestion, Hypoglycemic

Introduction

Diabetes mellitus is a chronic metabolic disease caused by a multitude of etiology that results in high blood glucose levels. Because of the associated health complications, this illness imposes a massive economic burden on the world [1-3]. Although a variety of treatments, including oral hypoglycemic medications and insulin, are available for diabetes management, indigestible soluble polysaccharides have also been linked to improved blood glucose. Different polysaccharides isolated from mushrooms, beans, oats, pumpkin, as well as cucumber have been shown to have considerable antidiabetic properties [4-7]. A water-soluble polysaccharide comprising repeating units of rhamnose, arabinose, mannose, and galactose exhibited excellent blood glucose reduction in streptozotocin-induced diabetic rats [7]. A similar polysaccharide comprising repeating units of mannose, rhamnose, glucose, galactose, and xylose inhibited glucose absorption *in vitro* [8] and demonstrated strong antidiabetic efficacy in alloxan-induced diabetic rats [9]. In light of these findings, the quest for dietary sources high in soluble polysaccharides is intensifying in order to produce inexpensive, effective, and safe supplements for diabetes therapy [1].

Basella alba L., popularly known as Malabar/Indian spinach, is a neglected mucilaginous leafy vegetable grown in Asian countries like India and Bangladesh for its nutritional value [10-14]. In addition to carbohydrates, lipids, soluble polysaccharides, and amino acids (leucine, isoleucine, lysine, arginine, threonine, and tryptophan), it is high in micronutrients such as vitamin A, vitamin C, vitamin K, thiamine, riboflavin, niacin, biotin, calcium, magnesium, and iron [14-17].

Basellasaponins A, B, C, D, β-sitosterol, stigmasterol glucoside, β-vulgaroside I, syringic acid, lupeol, kaempferol, ferulic acid, rutin, betacyanin and acacetin are some of the bioactive compounds reportedly isolated from *B. alba* aerial parts [14, 18-21]. Furthermore, an acidic polysaccharide (pH 5.3-5.4) with arabinose, rhamnose, galactose, galacturonic acid and glucose as major repeating exhibited slow swelling

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capacity, strong suspending ability and high viscosity resulting in considerable glucose entrapment ability and α-glucosidase inhibitory activity *in vitro* [22-24]. Apart from their prospective applications in the nutraceutical industry as thickening, suspending, binding, and gelling agents, these complex polysaccharides have shown significant antidiabetic efficacy in animal studies [25-27]. However, the mechanism of its antidiabetic action has not been studied. Therefore, the current study was designed to investigate the mechanism of antidiabetic effect of *B. alba* aerial parts utilizing *in vitro* protocols of glucose adsorption, diffusion and glucose transport studies.

Materials and Methods

Materials

B. alba leaves with stem (aerial parts) were purchased from a local vegetable shop, later on recognized by Dr. Sharanappa and the voucher specimen (RU-002/1441-42) was retained in the laboratory for reference. Wheat bran and dried baker's yeast were purchased from Nesto Hypermarket in Ar Rass, Saudi Arabia. Dialysis membrane (12 KD, Sigma Aldrich), glucose assay kit (GOD-POD, Randox) and α-amylase (Sigma Aldrich) were used. All of the other reagents and chemicals utilized in the analysis had the finest analytical purity.

Processing of the Sample

The sample was rinsed under running water to remove dirt before being spread out on big trays and dried in a hot air oven at 60°C for 24 hours. *B. alba* powder (BAP) was made by powdering the dried sample in a cyclonic laboratory blender until it flowed through a 60-mesh screen. BAP was stored in the refrigerator in an airtight receptacle for subsequent use.

Determination BAP's Glucose Binding Ability

BAP's ability to adsorb/bind glucose was measured using the *in vitro* method defined by Ahmed and Urooj [28]. In 50 mL centrifuge tubes, BAP (500 and 1000 mg) was transferred to 25 mL glucose dilution with raising concentrations (5, 10, 20, 50, and 100 mM) and vortexed for 10 seconds. After that, the tubes were incubated at 37°C in an automatic shaking water incubator. The tubes were centrifuged at 4000 ×g for 20 minutes after 6 hours of incubation to measure the glucose content in the supernatant using GOD-POD assay package. The glucose bound was measured by the formula below and expressed in milli moles (mM). As a reference, 2% wheat bran (500 mg) was used.

$$\text{Glucose Bound} = \frac{G_{0h} - G_{6h}}{Weight\ of\ the\ sample} \times Vol\ of\ solution \quad (1)$$

Wherein, G_{0h} is the initial glucose concentration of the solution and G_{6h} is the glucose concentration of the solution after 6 hours.

Impact of BAP on Retardation of Glucose Dispersion

The effect of BAP on retardation of glucose dispersion was studied based on the method of Ahmed and Urooj [28]. Twenty-five mL of glucose dilution (20 mM) and BAP (500 and 1000 mg) were added into dialysis bag (12 KD MW cut off) and dialyzed against 200 mL of distilled water taken in 250 mL tall form beakers. Then at the temperature of 37°C the beakers were placed in a shaker water bath and glucose level in the dialysate was specified at 60, 120, 180- and 240-minutes by glucose oxidase peroxidase assay kit. In the lack of BAP, a control test was run. Acarbose 0.2% (50 mg) was utilized as reference. Using the below formula the Glucose Dialysis Retardation Index (GDRI) was computed.

$$\text{GDRI} = 100 - \frac{Glucose\ content\ with\ sample}{Glucose\ content\ of\ control} \times 100 \quad (2)$$

Effect of BAP on Starch Digestibility

The impact of BAP on *in vitro* starch digestibility BAP was specified based on the technique of Ahmed and Urooj [28]. Potato starch (40 g) was added to 900 mL of 0.05 M phosphate buffer (pH 6.5). The solution was heated for 30 min at 65°C on a magnetic stirrer with continuous stirring. The volume was made up to a terminal volume of 1000 mL to provide a 4% (w/v) starch dilution. 25 mL of starch dilution, 0.4% α-amylase (100 mg) and test samples (500 and 1000 mg) were added into a dialysis bag and sealed dialysis bags were placed into a beaker with 200 mL of distilled water. The beaker was put in a shaker water bath at 37°C and at 60, 120, 180 and 240 minutes the glucose value in the dialysate was specified. In the lack of BAP, a control test was run. Acarbose 0.2% (50 mg) was utilized as reference. Glucose Dialysis Retardation Index (GDRI) was computed by the following formula.

$$\text{GDRI} = 100 - \frac{Glucose\ content\ with\ sample}{Glucose\ content\ of\ control} \times 100 \quad (3)$$

Effect of B. alba on Glucose Transport in Yeast Cells

To study the effect of *B. alba* on glucose transport in yeast cells, *B. alba* water extract (BAE) was obtained by extracting B. alba powder (10g) with hot distilled water (150 mL; 70°C) in a mechanical shaker. After 24 hours, the extract was filtered and freeze dried to yield *B. alba* extract (BAE) which was preserved in a deep freezer for subsequent use. Working stock containing 1 mg/mL BAE was prepared before starting the experiment. Yeast cells were prepared as previously described by Harish *et al.* [29]. Briefly, 10 grams of baker's yeast was added to 50 mL of distilled water in a stoppered centrifuge tube and vortexed for 30 seconds. The tube was centrifuged at 3000 g for 5 minutes. After that, the supernatant was discarded and the yeast pellet was redissolved in 50 mL of distilled water and vortexed for 30

seconds followed by centrifugation. The process was repeated until the supernatant fluid was clear. Yeast suspension (10% w/v) was freshly prepared with distilled water before starting the experiment. Various concentrations of BAE (1–5 mL) taken in 15 mL polycarbonate tubes were mixed with 1 mL of glucose dilution (5–25 mM). The volume was made up to 6 mL with distilled water and incubated together at 37 °C. After 10 min, 100 µL of yeast suspension was added, vortexed and incubated further for 60 min at 37°C. The tubes were then centrifuged at 2500 ×g for 5 min and the glucose content in the supernatant was determined by GOD-POD assay. For calculating the percentage of enhancement in glucose uptake by yeast cells, the following formula was used:

$$\text{Increase in glucose uptake (\%)} = \frac{Abs(C) - Abs(S)}{Abs(C)} \times 100 \quad (4)$$

In which, *Abs*(*C*) is the absorption of the control reaction that contained all reagents except the test sample and *Abs*(*S*) is the absorption of the test sample.

Statistical Analysis

All *in vitro* experiments were performed in three replicates (n = 6). Using SPSS 20.0 software, data were subjected to Analysis of Variance (ANOVA) followed by post-hoc test (Tukey's multiple comparisons) to determine significant differences between groups at 95% confidence level. All of the graphs were plotted by OriginPro software (OriginLab Corporation, MA, USA). Double axis layered graphs were plotted in case of glucose diffusion and starch digestibility experiments having line chart corresponding to GDRI values in Y2 axis.

Results and Discussion

Glucose Binding Ability of BAP

The glucose binding ability of BAP and wheat bran are presented in **Figure 1**. Glucose-binding potential of both BAP and wheat bran were found to be straightly proportionate to glucose concentration and highest amount of glucose was adsorbed at 100 milli moles concentration. Similar observations were reported with respect to oats, barley and psyllium husk, wherein glucose adsorption capacity of these fiber sources increased with increasing molar concentration of glucose [30]. BAP (4%) was able to bind significantly more ($p<0.05$) amount of glucose than BAP at 2%, however glucose adsorption capacity of BAP at both 2% and 4% level was significantly more ($p<0.05$) than that of wheat bran at all glucose concentrations. This could be because wheat bran primarily contains insoluble fibers whose water holding capacity is lower than that of soluble gel forming polysaccharides that are abundantly present in *B. alba* aerial parts [23, 30]. It is noteworthy that *B. alba* polysaccharides are reported to form a stable gel which resists phase separation, precipitation, change in color, odor or pH during heating/cooling operations [23]. Reports have indicated that dietary fibers vary in their ability to control hyperglycemia depending on their source, composition and the type of dietary formulation they are used in [31, 32].

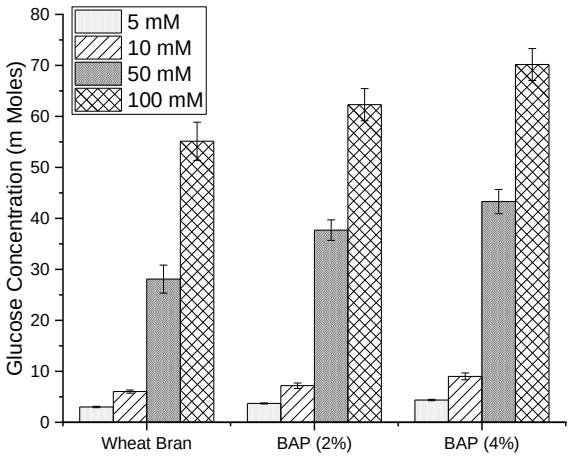

Figure 1. Glucose Binding Ability of *Basella alba* Powder

Soluble and mucilaginous polysaccharides including gums and pectin are known to increase viscosity of intestinal contents and bind glucose, cholesterol and triglycerides in GI tract ultimately resulting in the attenuation of post-prandial glucose response and serum lipid levels [33]. The ability of BAP to adsorb substantial amounts of glucose in a dose dependent manner therefore, create an opportunity to use *B. alba* vegetable as a functional ingredient in food products for effectively reducing the rate of glucose absorption from the GI tract and consequently decreasing post-meal plasma glucose rise in diabetics.

Effect of BAP on Retardation of Glucose Diffusion

Studying the dynamics of glucose diffusion across dialysis membrane is a suitable *in vitro* technique helpful to anticipate the ability of dietary components to delay glucose absorption from the gastrointestinal lumen into circulation [33]. In this research, the amount of glucose dispersion through dialysis membrane of 12 KD cutoff was controlled once in 60 minutes for 240 minutes using BAP at two levels (2% & 4%) and compared with wheat bran (2%) as a reference fiber source. The findings of the experiments are presented in **Figure 2**. It was found that, BAP at 2- and 4% resulted in a substantial inhibition of glucose diffusion into external solution through dialysis membrane in comparison to control experiment without the presence of any sample. It was also observed that though, BAP at 4% indicated significantly higher ($p<0.05$) prohibition of glucose dispersion in comparison to BAP at 2% as indicated by Glucose Dialysis Retardation Index (GDRI) values, the glucose dispersion inhibitory effect of BAP at both 2- and 4% was higher ($p<0.05$) than that of wheat bran at all time intervals.

The dialysis procedures are intended to replicate activities that occur in the gastrointestinal tract. In this procedure,

movement is aided by the convective motion of the intestinal contraction *in vivo* by shaking, which more accurately simulates the biological system than an unstirred system. This study was conducted to determine the effect of BAP in inhibiting glucose movement and to use the findings to predict how these polysaccharides would lower postprandial serum glucose levels.

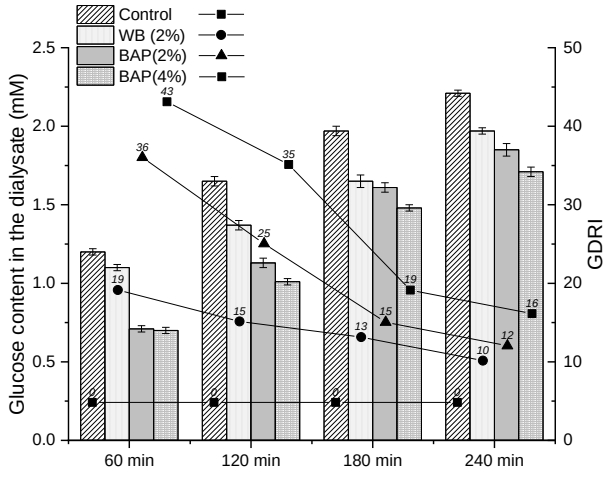

Figure 2. Effect of BAP on Retardation of Glucose Diffusion

The GDRI is an *in vitro* index that predicts the influence of fiber on glucose absorbance from GI tract [34]. The current study showed that GDRI values for all samples decreased from 60 to 240 minutes, which is consistent with an earlier study that found that the maximum GDRI values were found at 30 minutes and then decreased by 180 minutes for soluble fiber dense foods like oats and psyllium [30]. The occurrence of viscous polysaccharides in *B. alba* leaves [23] are clearly responsible for the observed pattern of declining GDRI values over time, as fiber molecules act as physical barrier to the movement of glucose by entrapping them within the fiber matrix [33-35].

Effect of BAP on in vitro Starch Digestibility

In a typical Asian diet, starch is the major dietary component providing glucose as a source of energy consequently resulting in higher blood glucose levels in circulation post consumption of starch-rich meal. Thus, use of dietary components which delay the digestion of dietary carbohydrates and absorption of glucose from GI tract is a viable therapeutic strategy to manage postprandial hyperglycemia in diabetics [36]. In the current study, the ability of BAP to delay starch digestion and glucose absorption was studied using starch-amylase-BAP system *in vitro*. The findings are shown in **Figure 3**. In comparison to control, the dispersion amount of glucose in the systems including samples (BAP 2% and 4%) was substantially ($p<0.05$) lower at each period of time. For acarbose, which results in completing the inhibition of α-amylase, no glucose diffusion was observed at all time intervals, while in a system containing BAP (2% and 4%) no glucose diffusion was observed at 120 minutes.

GDRI is a measure of the ability of the sample to inhibit glucose diffusion through dialysis membrane. Acarbose exhibited the highest GDRI of 100 at all time and prevented diffusion of glucose completely as it is known inhibiter of α-amylase thereby preventing digestion of starch [37, 38]. On the other hand, both BAP 2% and 4% showed a GDRI of 100 till 120 min and subsequently allowed diffusion of glucose at 180 min and onwards, while wheat bran showed complete inhibition of glucose diffusion only at 60 min.

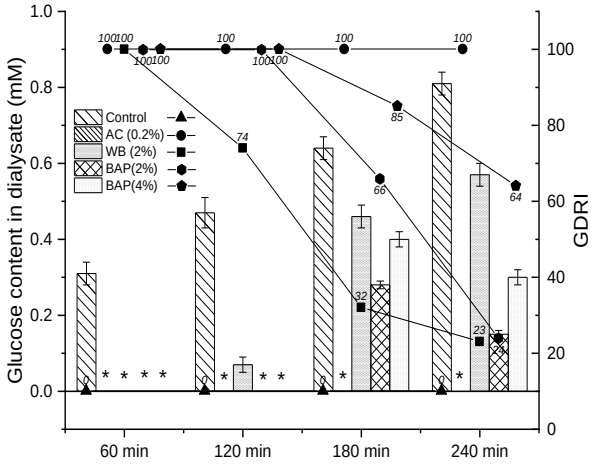

Figure 3. Effect of BAP on Starch Digestibility

The presence of water-soluble gel forming polysaccharides composed of galactose, arabinose, glucose, galacturonic acid, and rhamnose repeating units in the ratio of 41:24:16:13:5 and starch type glucan composed of L-arabinose, D-galactose, uronic acid, and L-rhamnose can be clearly attributed to *B. alba's* glucose diffusion inhibitory effect [23]. Although *B. alba* leaves contain some flavonoids and phenolics, including kaempferol, a potent inhibitor of α-amylase, the observed activity cannot be attributed to them because various extracts and juice prepared from the aerial parts of *B. alba* have shown no α-amylase inhibitory activity *in vitro* [39, 40]. As a consequence, it is reasonable to believe that the polysaccharides molecules interfere with glucose movement by physically adsorbing or entrapping glucose molecules within the fiber matrix [30], with the effect being directly proportional to the level of viscosity given by the gel-forming polysaccharides of *B. alba*. These findings are consistent with a previous study in which mucilage extracted from *B. alba* demonstrated significant glucose entrapment ability *in vitro* [24]. These results are also consistent with an earlier study in which juice produced from *B. abla* leaves decreased both starch and glucose-induced post-prandial glycemic load in normoglycemic rats [40].

Effect of BAE on Glucose Transport in Yeast Cells

The pathways of glucose transport through the yeast cell membrane have been explored as an *in vitro* approach for

evaluating the antidiabetic impact of different dietary components. A late study about transportation of nonmetabolizable sugars and specific metabolizable glycosides shows that, while stereospecific membrane carriers are involved in sugar transport through the yeast cell membrane, it is mostly a facilitated diffusion process influenced by concentration gradients [41].

The amount of glucose transport through the cell membrane in yeast cells has been shown in **Figure 4**. The content of glucose left in the medium after a determined time period acts as a measure of yeast cell glucose uptake. After being added to the medium, BME enhanced yeast cells' glucose uptake in a dose-dependent way. In all five glucose concentrations, the content of glucose absorption into yeast cells was linear. Nevertheless, the percentage of enhancement in yeast cells' glucose uptake was shown to be oppositely related to the glucose concentration and reduced as the concentration of the glucose increased. This could be due to the fact that glucose transport in yeast cells is quite complicated, and that glucose is carried in yeast by a facilitated dispersion mechanism wherein, facilitated carriers move solutes along a concentration gradient in a specified way. This implies that effective transport requires the removal of intracellular glucose [41, 42]. These findings are consistent with previous research, which found that various plant extracts elicited a dose-dependent enhancement in yeast cells' glucose uptake that was oppositely proportionate to the molar concentration of the glucose [29, 43, 44].

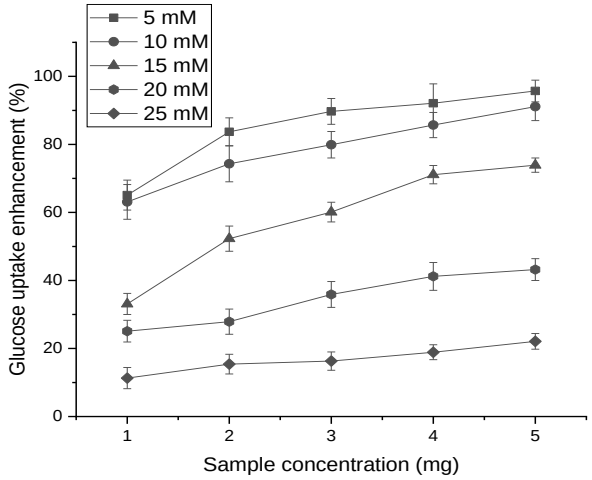

Figure 4. Impact of BAE on Glucose Transport in Yeast Cells

Conclusion

Based on the findings of this study, it is concluded that *B. alba* aerial parts rich in soluble polysaccharides have a potential hypoglycemic effect *in vitro*. Furthermore, the hypoglycemic effect is mediated by creating a physical barrier to glucose absorption in the GI tract, which aids in the extended release of glucose into the bloodstream and curtails rapid blood glucose surges.

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