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Short Communication

In Vitro Antidiabetic Activity of Ethanolic Leaf Extract of *Bruguiera Cylindrica* L. – Glucose Uptake by Yeast Cells Method

Revathi Pitchaipillai [1*], Thirumalaikolundusubramanian Ponniah[2]

1. Postgraduate and Research Department of Pharmacology, Chennai Medical College Hospital & Research Centre (SRM Group), Tamilnadu Dr. MGR Medical University, Tiruchirapalli, India.

2. Department of Medicine, Chennai Medical College Hospital & Research Centre (SRM Group), Tamilnadu Dr. MGR Medical University, Tiruchirapalli, India.

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The current epidemic of diabetes indicates the need of proper and effective medications that are limited in their potency to have many side effects. Thus, introduction of alternative and complementary medicine is now in picture. The main objective of this study was to analyze the rate of glucose transport across cell membrane in yeast cells system in the presence of the ethanolic extract of *Bruguiera cylindrical* leaves. After the treatment of the yeast cells with the ethanolic extract of *B. cylindrica*, the glucose uptake did not increase in a dose dependent manner. The increase of percentage of glucose uptake by the yeast cells was found in the glucose concentrations varying from 5 to 25 mM in the presence of 1 and 5 mg/ml of *B. cylindrical* ethanolic extract (BCEE). The BCEE can serve as a therapeutic agent and can be used as potential source of novel bioactive compounds for treating type 2 diabetes mellitus.

Keywords: *Bruguiera cylindrica*, ethanolic extract, glucose uptake, antidiabetic activity

Diabetes Mellitus is an established non-communicable disease and often described as fourth or fifth leading cause of mortality in high income countries (1). According to World Health Organization, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries. India presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world (2).

Sulfonylureas, biguanide, thiazolidinedione, and glycosidase inhibitors are widely used to control the hyperglycemia, hyperlipidemia and insulin resistance of type 2 diabetes, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects and high rates of secondary failure. Moreover, they are not safe for use during pregnancy (3). Thus, the management of diabetes without any side effects is still a challenge. There is continuous search for alternative drugs (4).

As a result of the global epidemic of diabetes, the limited potency and many side effects of medications currently in use, the need for new diabetes therapies is expected to grow dramatically during the next decade. An intense research has been

*Correspondence: Postgraduate and Research Department of Pharmacology, Chennai Medical College Hospital & Research Centre (SRM Group), Tamilnadu Dr. MGR Medical University, Tiruchirapalli, India. E-mail: reva1923@gmail.com

conducted to identify new therapeutic targets and pharmacologic compounds that might correct the impaired glucose tolerance. During the recent years many investigators have shown that natural products are a potential source for new drug candidates for many diseases in general, and diabetes in particular (5). Some recent studies showed the medicinal value of mangroves, and associated plants persist to provide invaluable treatment modalities, both in modern and traditional systems of medicine (6, 7). The medicinal properties of mangrove trees provide a wide domain for medical uses including diabetes, cancer, etc…. Recently, there has been a growing interest in the identification of biomolecules from plant sources to reduce the hyperglycemic conditions (8).

Bruguiera cylindrica (L.) (Family: Rhizophoraceae) is a rare tree mangrove found along the western coast of India. Traditionally, *B. cylindrica* is used to treat hepatitis. All parts of this plant (leaves, fruits, bark, stem and root) are medicinally important in the traditional system of medicine in India and have been used extensively in hepatic disorders, jaundice, diabetes, blood pressure, ulcers, infections, and as an anti-inflammatory agent. *B. cylindrica* was tested against antibiotic resistant pathogens (ARB) viz. *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae, Pseudomonas aeruginosa* and eye pathogens viz. *E. coli, Proteus*, *Acinetobacter* and *Staphylococcus epidermidis* (9). Leaf and bark of *B. cylindrica* were studied for antimicrobial effect and antiviral activity against new castle disease virus, vaccinia virus, encephalomyocarditis virus, simian foamy virus, and hepatitis B virus. The extracts of the seeds of *B. cylindrica* showed antiviral activity against tobacco mosaic virus. Leaves were used for treating blood pressure and as fodder for livestock. A study mentioned that the leaf juice of *B. cylindrica* was used for digestion and inducing appetite in diabetic patients (10). The leaves have also been in use for the treatment of diabetes by tribal people (information from local tribes). Pentacyclic triterpenoid esters from the fruits and brugine alkaloids (tropane alkaloids present in *Bruguiera*) have been isolated from stem and bark (11).

Several mechanisms have been proposed for the hypoglycaemic effect of primary and secondary phytochemicals, such as manipulation of glucose transporters, *β*-cell regeneration and enhancing insulin-releasing activity (11, 12), glucose adsorption, retarded diffusion and inhibition of carbohydrate metabolizing enzymes at gut level, stimulating effect on glucose utilization, food adjuvants for diabetic patients, and uptake of glucose by cells through facilitated diffusion in yeast cell model system (11, 13, 14). The recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase) have led to the development of newer pharmacological agents (15).

The phytochemical screening of n-Hexane, chloroform, ethanol and aqueous extracts of leaves of *B. cylindrica* showed the presence of carbohydrate, protein, amino acids, lipids, fatty acids, fiber, alkaloids, flavonoids, tannin, tri terpenoids, saponins, phenols/gallic acid equivalent, glycosides, cardiac glycosides, and lignin (11). The present investigation was undertaken to evaluate the possible mechanism of action for antidiabetic potential of ethanolic leaf extract of *B. cylindrica* using a suitable *in vitro* technique.

Materials and methods

Plant collection

The leaves of the *B. cylindrica* were collected from Pichavaram mangrove forest near Chidambaram, Cuddalore district, Tamilnadu, India, after obtaining permission from the forest office_during post monsoon period. The plant was identified and authenticated by a botanist.

Extract preparation

Leaves were cleaned, dried in a hot air oven (50 °C) and then grinded into a fine powder in a grinder. The powdered plant material was subjected to sequential extraction using soxhlet apparatus. The sequential extraction involved four different solvent

systems n-Hexane, chloroform, ethanol and aqueous, from low to high polarity. Then, on complete analysis of quantitative phytochemical and *in vitro* antioxidant analysis, ethanolic extract exhibited better anti-diabetic properties. Further, the ethanolic extract of leaves of *B. cylindrica* was used for *in vitro* analysis.

***In vitro* evaluation of glucose uptake by yeast cells**

Commercial baker's yeast was washed by repeated centrifugation (3,000×g, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1 – 5 mg/ml) were added to 1 ml of glucose solution (5, 10 and 25 mM) and further incubated for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant (16). Metformin was taken as standard antidiabetic drug used. The percentage of increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Activity \%} = \frac{[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100}{(\text{Increase in glucose uptake})}$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. Absorbance was measured at 540 nm and all experiments were carried out in triplicates.

Results

The rate of glucose transport across cell membrane in yeast cells system is presented in Figure 1. After the treatment of the yeast cells with the ethanolic extract of *B. cylindrica*, no dose dependent glucose uptake was observed. The percentage of glucose uptake by the yeast cells increased with glucose concentrations (5, 10, and 25 mM) in the presence of 1 mg/ml and 5 mg/ml of *B. cylindrica* ethanolic extract (BCEE), while it was not enhanced at 2, 3 and 4 mg/ml of BCEE, except that 66.675% glucose uptake was found in the

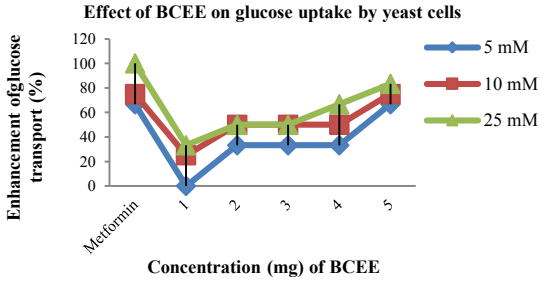

Figure 1. The effect of *B. cylindrical* ethanolic extract on glucose uptake by the yeast cell at different glucose concentrations. The percentage of glucose uptake by the yeast cells was measured in the presence of various concentrations of BCEE and glucose. BCEE: *B. cylindrica* ethanolic extract.

presence of 25 mM glucose. The highest concentration of BCEE sample (5 mg/ml BCEE) exhibited maximum activity at all glucose concentrations and showed the maximum increase (83.33%) in the presence of at 25 mM glucose.

Discussion

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycaemic effect of various compounds/ medicinal plants (17). The results of the present study revealed that BCEE increased glucose uptake in yeast cells (0–90%) at various glucose concentrations. The amount of glucose remaining in the medium after a specific time serves as an indicator of glucose uptake by the yeast cells (14). Recent studies on the transport of non metabolizable sugars and glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast by a facilitated diffusion process.

Facilitated carriers are specific carriers that transport solutes down the concentration gradient highlighting that the effective transport is only attained if there is removal of intracellular glucose (14, 18). Hence glucose transport occurs only if the intracellular glucose is effectively reduced(utilized). The present data, suggests that the plant extract

is capable of enhancing glucose uptake effectively, which in turn suggests that it is capable of enhancing effective glucose utilization at 5 mg/ml concentration, thereby controlling blood glucose level as also suggested by other reports (2, 19).

Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels. Three new pentacyclic triterpenoid esters 1–3 together with six known lupane-type triterpenoids were isolated from *B. cylindrica*. The pentacyclic triterpenoids were reported to exhibit significantly higher glucose uptake activity at all concentrations studied (20). Comparatively, in the present study, the increased ability of the samples to adsorb glucose may also be attributed to the dietary fiber (insoluble and soluble fibers) present in the sample. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species (21). The beneficial effect of plant fibers for blood glucose concentrations control have been proven (22).

Along with the presence of fiber, studies suggest the potential of phytochemicals in combating diabetic disorders through different possible mechanisms such as inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration, and enhancing insulin releasing activity (12, 13). Similar observations were reported for insoluble fiber rich fractions isolated from *Averrhoa carambola* (23). It was hence speculated that the samples might help retain the glucose in the intestinal lumen even in the presence of low glucose concentration. In addition to glucose adsorption, the retardation in glucose diffusion might also be attributed to the physical obstacle presented by fiber particles toward glucose molecules and the entrapment of glucose within the network formed by fibers (23, 24).

In conclusion, this report suggests that the increase of glucose transport across the cell membrane might be the anti-hyperglycaemic mechanism of action of *B. cylindrica* leaves. The plant extract under study can serve as a therapeutic agent and can be used as a potential source of novel bioactive compounds for treating type 2 diabetes mellitus.

Conflict of interest

The authors declared no conflict of interest.

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