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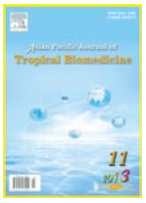

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In vitro hypoglycemic effects of *Albizzia lebbeck* and *Mucuna pruriens*

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the hypoglycemic activity of *A. lebbeck* and *M. pruriens* using various *in vitro* methods. The activity was assessed by studying the effects of plant extracts on glucose adsorption, diffusion amylolysis kinetics and glucose transport across yeast cells. It was found that the hypoglycemic effect exhibited by the plant extracts is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells.
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ABSTRACT

Objective: To verify the antidiabetic potential of stem bark of *Albizzia lebbeck* (*A. lebbeck*) and seeds of *Mucuna pruriens* (*M. pruriens*) using various *in vitro* techniques.
Methods: The plant extracts were studied for their effects on glucose adsorption, diffusion amylolysis kinetics and glucose transport across yeast cells.
Results: Both the plant extracts adsorbed glucose and the adsorption of glucose increased remarkably with an increase in glucose concentration. No significant ($P \leq 0.05$) differences were observed between the adsorption capacities of *A. lebbeck* and *M. pruriens*. In amylolysis kinetic experimental model the rate of glucose diffusion was found to increase with time from 30 to 180 min, and both the plant extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane as compared to control. The retardation of glucose diffusion by *A. lebbeck* extract was significantly higher ($P \leq 0.05$) than *M. pruriens*. These effects were reflected with higher glucose dialysis retardation index values for *A. lebbeck* than *M. pruriens*. The plant extracts also promoted glucose uptake by yeast cells. The rate of uptake of glucose into yeast cells was linear in all the 5 glucose concentrations used in the study. *M. pruriens* extract exhibited significantly higher ($P \leq 0.05$) activity than the extract of *A. lebbeck* at all concentrations.
Conclusions: The results verified the antidiabetic potential of *A. lebbeck* and *M. pruriens*. The hypoglycemic effect exhibited by the extracts is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells.

KEYWORDS
Hypoglycemic, *In vitro*, Glucose diffusion

1. Introduction

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease[1]. It is characterized by group of metabolic disorders. The deficiency or insensitivity of insulin causes glucose to accumulate in the blood, leading to various complications[2]. Currently available pharmacotherapies for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal glucose homeostasis and they are not free from side effects[3]. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored[4].

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Albizzia lebbeck Benth (Family: Leguminosae) (*A. lebbeck*) is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds, and grows wild. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent, and are given in piles and diarrhea. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties[5]. *Mucuna pruriens* Linn. (Family: Fabaceae) (*M. pruriens*) is one of the popular drug in Ayurvedic system of medicine[6]. Various preparations from the seeds of this plant are used for the management of several free radical mediated diseases such as ageing, rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders[7]. The present study was undertaken to verify the antidiabetic potential of seeds of *M. pruriens* and bark of *A. lebbeck* using various *in vitro* techniques as an attempt to explore their mechanism of action.

2. Materials and methods

2.1. Plant material

The plant material was collected from local areas of Karad and was further identified and authenticated by the Department of Botany, Science College, Karad. The bark of *A. lebbeck* and the seeds of *M. pruriens* were cleaned, dried in a hot air oven (50 °C), powdered, passed through 60 mesh sieve (BS) and stored in an airtight container at 4 °C till further use.

2.2. 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.

2.3. Preparation of plant extracts

Aqueous extracts were prepared by extracting the powders of bark of *A. lebbeck* and the seeds of *M. pruriens* with hot water (70 °C) in a mechanical shaker (24 h), filtered and freeze dried.

2.4. Evaluation of antidiabetic activity of plant extracts using various in vitro methods

2.4.1. Determination of glucose adsorption capacity

Glucose adsorption capacity of the samples was determined by the method of Ou *et al*[8]. Briefly, the samples of plant extracts (1%) were added to 25 mL of glucose solution of increasing concentration (5, 10, 20, 50 and 100 mmol/L). The mixture was stirred well, incubated in a shaker water bath at 37 °C for 6 h, centrifuged at 4 800 r/min for 20 min and the glucose content in the supernatant was determined. The concentration of bound glucose was calculated using the following formula:

$$\text{Glucose bound} = \frac{G_1 - G_6}{\text{Weight of the sample}} \times \text{Volume of solution}$$

G_1 is the glucose concentration of original solution.
G_6 is the glucose concentration after 6 h.

2.4.2. Effect of plant extracts on in-vitro glucose diffusion

It was performed according to the method stated by Ahmed *et al*[9]. 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. Glucose dialysis retardation index (GDRI) was calculated by using the following formula:

$$\text{GDRI}_{\%} = 100 - \frac{\text{Glucose content with addition of sample (mg/dL)}}{\text{Glucose content of the control (mg/dL)}} \times 100$$

2.4.3. Effect of plant extracts on in-vitro amylolysis kinetics

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[8].

2.4.4. Glucose uptake by yeast cells

Yeast cells were prepared according to the method of Cirillo[10]. Commercial baker's yeast was washed by repeated centrifugation (4 200 r/min, 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) were added to 1 mL of glucose solution (5-25 mmol/L) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 μL of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3 800 r/min, 5 min) and

glucose was estimated in the supernatant. The percent increase in glucose uptake by yeast cells was calculated using the following formula:

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

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.

2.4.5. Statistical analysis

All the determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences. Values were considered at $P<0.05$. Graphs were plotted using Graph Pad Prism 6 software.

3. Results

3.1. Glucose adsorption capacity of A. lebbeck and M. pruriens extracts

Glucose adsorption capacity of the selected plant extracts is depicted in Figure 1. The adsorption capacities of the samples were found to be directly proportional to the molar concentration of glucose and higher amounts of glucose was bound with increased glucose concentration. No significant ($P\leq0.05$) differences were observed between the adsorption capacities of *A. lebbeck* and *M. pruriens*.

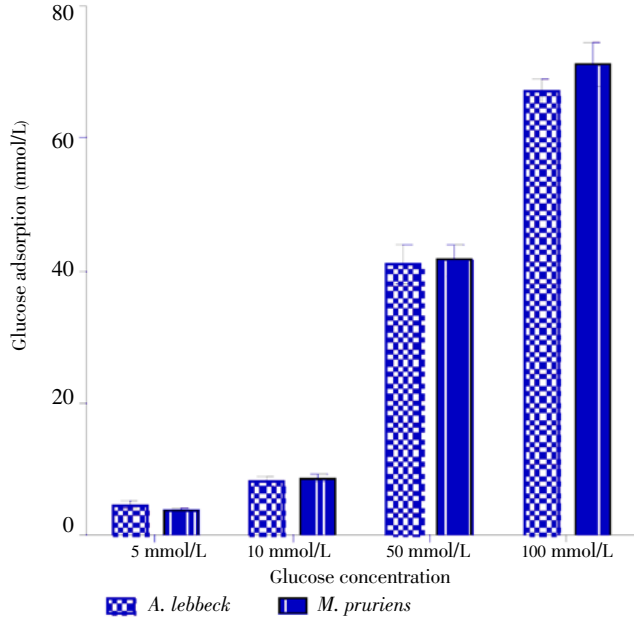

Figure 1. Glucose binding capacity of *A. lebbeck* and *M. pruriens* at different concentrations of glucose.
Values are mean±SD of triplicate determinations.

3.2. Effect of A. lebbeck and M. pruriens extracts on in vitro glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 1. The rate of glucose diffusion was found to increase with time from 30 to 180 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, both the samples of plant extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control. The retardation of glucose diffusion by *A. lebbeck* extracts was significantly higher ($P\leq0.05$) than *M. pruriens*. These effects were reflected with higher GDRI values for *A. lebbeck* than *M. pruriens*.

Table 1
Effect of selected samples on glucose diffusion and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	$0.90^{c}\pm0.01$	$1.27^{c}\pm0.01$	$1.77^{c}\pm0.01$	$1.95^{c}\pm0.01$
A. lebbeck	$0.61^{a}\pm0.01$ (32.22)	$1.06^{a}\pm0.01$ (18.11)	$1.55^{a}\pm0.01$ (12.99)	$1.70^{a}\pm0.01$ (12.82)
M. pruriens	$0.65^{b}\pm0.01$ (27.77)	$1.08^{b}\pm0.01$ (14.96)	$1.59^{b}\pm0.01$ (10.16)	$1.79^{b}\pm0.01$ (8.20)

Values in parenthesis indicate GDRI.
Mean values ($n=3$) with different superscript letters in columns differ significantly from each other ($P\leq0.05$)

3.3. Effect of A. lebbeck and M. pruriens extracts on in vitro amylolysis kinetics

The effects of *A. lebbeck* and *M. pruriens* on the amylolysis kinetics are shown in the Table 2. The GDRI was found to be 42.85% and 33.33% in *A. lebbeck* and *M. pruriens* respectively at 60 min which gradually got reduced to 20.68% and 13.79% respectively at 120 min.

Table 2
Effect of selected samples on starch digestibility and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	0.0	$0.21^{c}\pm0.01$	$0.29^{c}\pm0.01$	$0.37^{c}\pm0.01$
A. lebbeck	0.0 (100)	$0.12^{a}\pm0.01$ (42.85)	$0.23^{a}\pm0.01$ (20.68)	$0.33^{a}\pm0.01$ (10.81)
M. pruriens	0.0 (100)	$0.14^{b}\pm0.01$ (33.33)	$0.25^{b}\pm0.01$ (13.79)	$0.35^{b}\pm0.01$ (5.40)

Values in parenthesis indicate GDRI.
Mean values ($n=3$) with different superscript letters in columns differ significantly from each other ($P\leq0.05$).

3.4. Effect of A. lebbeck and M. pruriens extracts on glucose transport across yeast cells

The rate of glucose transport across cell membrane in yeast cells system is presented in Figure 2 and Figure 3. The amount of glucose remaining in the medium after a specific time interval serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into the yeast cells was linear in all the 5 glucose

concentrations. The extract of *M. pruriens* exhibited significantly higher ($P \leq 0.05$) activity than the *A. lebbeck* extract at all concentrations. However, the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and was found to decrease with increase in the molar concentration of the glucose solution.

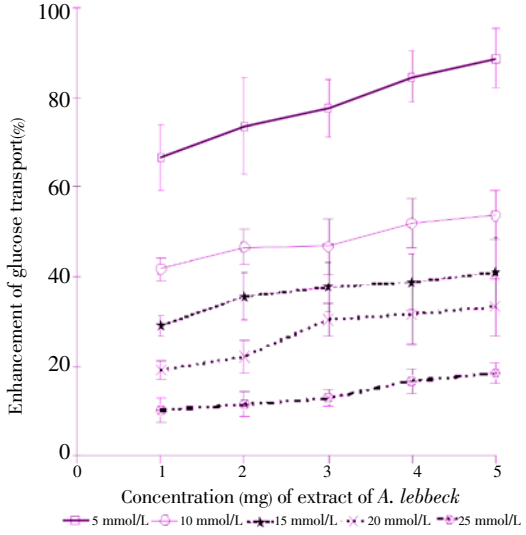

Figure 2. Effect of *A. lebbeck* extract on the uptake of glucose by yeast cells. Values are mean±SD of triplicate determinations.

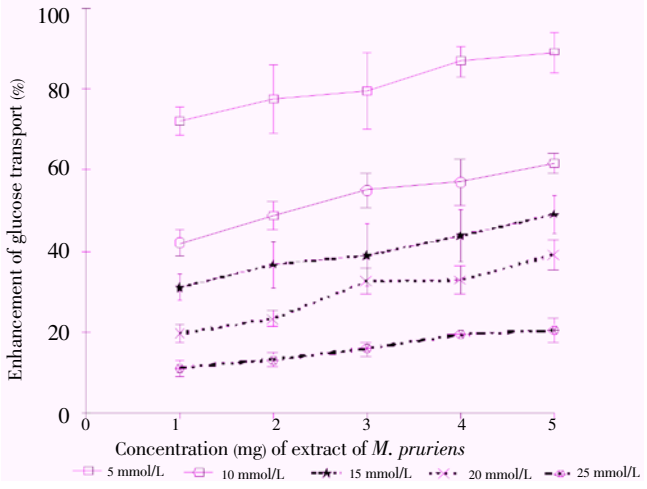

Figure 3. Effect of *M. pruriens* extract on the uptake of glucose by yeast cells. Values are mean±SD of triplicate determinations.

4. Discussion

The higher adsorption capacity of the extracts of *A. lebbeck* and *M. pruriens* may be attributed to their constituents, as both insoluble and soluble constituents and fibers from different sources are reported to adsorb glucose. The results also revealed that the plant extracts under study could bind glucose even at lower concentrations of glucose (5 mmol/L) thereby reducing the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia. Similar observations have been reported by Chau *et al.* for insoluble fiber-rich fractions isolated from *Averrhoa carambola*[11]. GDRI is a useful *in vitro* index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract[12]. A higher GDRI indicates a higher retardation index of glucose by the sample. The GDRI was found to be 42.85% and 33.33% in *A. lebbeck* and *M. pruriens* respectively at 60 min. The retardation of glucose diffusion may also be due to the inhibition of α-amylase by the plant extracts 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[8]. The mechanism of glucose transport across the yeast cell membrane has been receiving attention as a lucrative method for *in vitro* screening of hypoglycemic effect of various compounds/ medicinal plants. Both the extracts promoted glucose transport across the yeast cells. The rate of uptake of glucose into the yeast cells was linear in all the 5 glucose concentrations used in the study. The studies on the transport of non metabolizable sugars, metabolizable glycosides have suggested that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers and takes place by facilitated diffusion process[13,14].

In conclusion, the results of this study verify the antidiabetic properties as implied by the various *in vitro* methods. The hypoglycemic effect exhibited by the extracts of *A. lebbeck* and *M. pruriens* is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells. However, these effects need to be confirmed by employing different in vivo models and clinical trials for their effective utilization as therapeutic agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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support and other necessary facilities for successful completion of work.

Comments

Background

Diabetes has become a major health problem worldwide. None of the current antidiabetic therapies is considered to be ideal, due to their side effects and sometimes diminution in response after prolonged use. Thus, there is an urgent need of new natural compounds with antidiabetic properties to overcome any resistance developed by patients to the currently used drugs.

Research frontiers

The present research work depicts the hypoglycemic effects of aqueous extracts of stem bark of *A. lebbeck* and seeds of *M. pruriens* using various in vitro techniques.

Related reports

Glucose diffusion rate, glucose dialysis retardation index, glucose uptake by the yeast cells, glucose adsorption capacity has been reported as some of the *in vitro* methods to assess hypoglycemic activity.

Innovations and breakthroughs

The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control. In this context the present investigation is an attempt to explore the possible mechanism of action of *A. lebbeck* and *M. pruriens* which are traditionally known for their antidiabetic potential.

Applications

The present investigation verified the antidiabetic properties of *A. lebbeck* and *M. pruriens* as implied by the various *in vitro* methods. These effects need to be confirmed by employing different *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

Peer review

This is a valuable research work in which authors have demonstrated the hypoglycemic activity of *A. lebbeck* and *M. pruriens* using various *in vitro* methods. The activity was assessed by studying the effects of plant extracts on glucose adsorption, diffusion amylolysis kinetics and glucose transport across yeast cells. It was found that the hypoglycemic effect exhibited by the plant extracts is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells.

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