

Antihyperglycemic Potential of Saponin-enriched Fraction from *Pithecellobium dulce* Benth. Seed Extract

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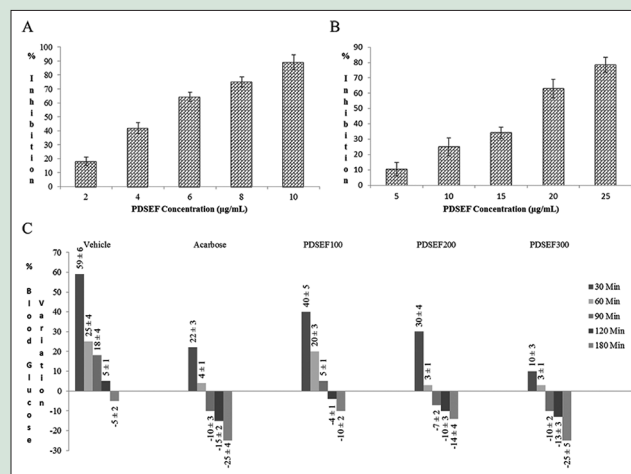
ABSTRACT

Background: Indian traditional system of medicine uses *Pithecellobium dulce* for the treatment of diabetes mellitus. **Objectives:** This study aims to develop an extract rich in saponins derived from seeds of the plant and to evaluate its antihyperglycemic potential *in vitro* and *in vivo*. **Materials and Methods:** Defatted seeds were extracted with methanol and processed to afford saponin-enriched fraction (*Pithecellobium dulce* saponin-enriched fraction [PDSEF]). This fraction was evaluated for its potential to inhibit enzymes such as α -glucosidase and α -amylase, *in vitro*. The fraction was subjected to oral toxicity study followed by *in vivo* sucrose tolerance test. An analytical high-performance liquid chromatography method was developed for fingerprinting of the fraction. **Results:** The method adopted for enrichment of saponins was robust enough to enrich saponin content to $96.37\% \pm 1.21\%$ w/w. PDSEF displayed superior inhibition of enzymes (α -glucosidase and α -amylase with IC_{50} of 5.12 ± 0.15 μ g/ml and 17.28 ± 0.23 μ g/ml, respectively) compared to acarbose. It was found to be safe in mice up to 2000 mg/kg and significantly prevented blood glucose level in sucrose tolerance test by inhibiting enzymes responsible for hydrolysis of sucrose. **Conclusion:** PDSEF displayed excellent antihyperglycemic activity *in vitro* and *in vivo* and should be evaluated further to develop it as a promising drug for the management of diabetes mellitus.

Key words: Antihyperglycemic, *Pithecellobium dulce*, saponins, α -amylase, α -glucosidase

SUMMARY

- Saponin enriched fraction from *P. dulce* seeds showed significant inhibition of key enzymes responsible for digestion of polysaccharides. The saponin enriched fraction was found to be safe in mice and prevented blood glucose level in mice in sucrose tolerance test.



Abbreviations Used: PDSEF: Pithecellobium dulce saponin-enriched fraction, IC_{50} : Inhibitory concentration 50, HPLC: High performance liquid chromatography

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INTRODUCTION

Pithecellobium dulce Benth. (family: Fabaceae), commonly known as "Jungle Jalebi," is widely distributed throughout India and also found in South Africa, Australia, and other parts of Asia. Fruits of *P. dulce* have been consumed as a dietary supplement for its high nutritive and medicinal value. In traditional system of medicine, it is used for the treatment of diabetes, toothache, earache, leprosy, peptic ulcer, and used as an astringent, emollient, and abortifacient.^[1,2] Seed extract displayed potent antidiabetic and antihyperlipidemic activity in streptozotocin-induced diabetic rats.^[1] Bark of the plant is used as an astringent that cures dysentery, febrifuge, and eye inflammation.^[3] It is also reported to possess free radical scavenging and H^+ , K^+ -ATPase inhibitory activity;^[4] the fruit extract possesses antiulcer activity^[5] and it is also reported to prevent CCl_4 -induced renal oxidative impairments and necrotic cell death.^[6] Saponin fraction of *P. dulce* fruits was reported to possess anti-inflammatory activity.^[7] Several phytochemical studies on seed have resulted in isolation and characterization of oleanane-type triterpene glycosides, pithedulosides A-K, bisdesmodic triterpenoid saponin, dulcin, and other saponins.^[8-11] The present study aims to investigate the antihyperglycemic activity of saponin-enriched fraction from the *P. dulce* seed extract.

MATERIALS AND METHODS

Chemicals and reagents

α -Glucosidase (EC 3.2.1.20, Maltase, ex microorganism), α -amylase (ex-porcine pancreas, EC 3.2.1.1), *p*-nitrophenyl α -D-glucopyranoside, 3,5-dinitrosalicylic acid, and oleanolic acid were purchased from SRL India. Acarbose was purchased from Sigma India. Starch, sucrose, NaOH pellets, $KNaC_4H_4O_6 \cdot 4H_2O$, Na_2HPO_4 , NaH_2PO_4 , NaCl, dimethyl sulfoxide (DMSO), Na_2CO_3 , and other chemicals and solvents were of highest purity grade and purchased from Merck, India. Milli-Q water was used for all the enzymatic assays and high-performance liquid chromatography (HPLC).

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Plant material

The ripen fruits of *P. dulce* were purchased from the local market of Jaipur, India, and a voucher specimen was submitted at institute's herbarium (ACP/2014/M-01).

Extraction and preparation of saponin-enriched fraction

The seeds were separated from the fruits and powdered using a mechanical grinder and sieved to mesh size 40. The powdered seeds (2.5 kg) were defatted using petroleum ether (7L × 3) for 48 h and each time. The defatted seeds were air-dried for 72 h and then extracted with methanol (7L × 4) at room temperature for 48 h and each time. The methanolic extract was concentrated under reduced pressure and lyophilized to afford the crude methanolic extract (198 g; yield, 9.9% w/w). A part (150 g) of it was then suspended in milli-Q water (300 ml) and partitioned successively with petroleum ether (650 ml × 3), EtOAc (650 ml × 3), and n-butanol (650 ml × 3). The n-butanol fraction was dried under vacuum to constant weight (70 g). Then, the dried n-butanol extract was dissolved in methanol (50 ml) and slowly added to acetone (200 ml) with constant stirring for 30 min so as to precipitate the saponins. The precipitate was filtered under vacuum, dried (44 g; *Pithecellobium dulce* saponin-enriched fraction [PDSEF]), and stored at 4°C till further use.

Determination of total saponins

The content of total saponins in PDSEF was determined approximately using the method described earlier by Wang *et al.* using oleanolic acid as standard to compare absorbance at 554 nm.^[12,13]

Preparation of enzyme, samples, and standard solution

Stock solutions of α -glucosidase with 1 mg/ml (1 mg = 60 U) and α -amylase with 0.5 mg/ml (1 mg = 15 U) concentrations were prepared at the time of assay.^[14-17] The PDSEF was dissolved in DMSO and diluted to desired concentrations with respective buffers. The final DMSO concentration was maintained below 1% (v/v) for all enzymatic assays. For HPLC analysis, the PDSEF was accurately weighed and dissolved in methanol to get a concentration of 5 mg/mL. PDSEF was dissolved in saline solution for oral administration. Acarbose was employed as standard antihyperglycemic drug at the dose of 5 mg/kg. Sucrose (3 g/kg) was used as carbohydrates to carry out the sucrose tolerance test.

High-performance liquid chromatography profiling of the saponin-enriched fraction

HPLC profiling was done using a Shimadzu system equipped with SCL-10A VP Shimadzu system controller, SPD-M10A VP Shimadzu diode array detector, LC-10AT VP Shimadzu liquid chromatography pump, Class VP software, and a Rheodyne injector with 20 μ l loop. The analysis was carried out at 30°C \pm 1°C using sample solutions filtered through 0.45 μ m membranes. Separation was achieved using Discovery RP amide C16, 5 μ m, 25 cm \times 4.6 mm; gradient elution (0–5 min solvent B 100%; 5–15 min solvent B 100%–70%; 15–25 min solvent B 70%–45%; 25–35 min solvent B 45%–10%; 35–45 min solvent B 10%–0%; and 45–50 min solvent B 0%–100%) was carried out using the mobile phase of acetonitrile (solvent A) and 1% acetic acid in water (solvent B) at a flow rate of 1 ml/min. The eluate was monitored at 210 nm.

α -glucosidase inhibition assay

The α -glucosidase inhibitory assay was performed in a 96-well plate as described earlier.^[14-17] The uninhibited enzyme was taken as control; an appropriate DMSO control was used, wherever applicable. Appropriate

blank was used for all the samples. The assay was performed in triplicate. Acarbose was used as standard inhibitor of the enzyme.

α -amylase inhibition assay

The assay was performed as described earlier.^[14] The uninhibited enzyme was taken as control. Appropriate blank was used for all the samples. Acarbose was used as standard inhibitor of the enzyme. The assay was performed in triplicate.

Experimental animal

Swiss albino mice of either sex, approximately of the same age weighing between 25 and 30 g, procured from the Animal Research Division of the Institute, were used in this study. They were acclimated for 1 week in a temperature- and humidity-controlled facility (temperature: 22°C \pm 1°C) housed under standard animal house conditions (12 h light-dark cycle) and maintained on standard pellet diet and water *ad libitum*. After the experiments, all animals were sacrificed in a CO₂ chamber. The animal protocols for this study were approved by the Institutional Animal Ethics Committee (ACP/MK/001).

Acute toxicity study

Acute oral toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) guideline^[18] for testing of chemicals by Acute Oral Toxicity–Acute Toxic Class method (OECD, 2001). Twenty-four male mice starved overnight were divided into four groups (six in each group) and orally fed with PDSEF in increasing dose levels of 250, 500, 1000, and 2000 mg/kg. The mice were continuously observed for the first 30 min and periodically during the first 24 h. Thereafter, they were observed for a total of 14 days for any physical signs of toxicity. After 14 days, the lethality or death was calculated.

Oral sucrose tolerance test

The oral sucrose tolerance test (OSTT) was performed on overnight-fasted mice. Different doses of PDSEF and acarbose were administered 60 min before sucrose administration (3 g/kg). The blood samples were collected from the orbital venous plexus just before sucrose load (0 min) and at 30, 60, 90, 120, and 180 min after glucose administration. Serum glucose concentrations were determined using Accu-Chek Active Glucometer.^[19]

Statistical analysis

The data obtained were analyzed and expressed as mean \pm standard error of mean of six animals in each group. Statistically significant differences between groups were determined by an ANOVA (one or two ways) followed by Dunnett's or Bonferroni *post hoc* tests; $P < 0.05$ was considered statistically significant. GraphPad Software, Inc. USA was used for all the statistical calculations.

RESULTS AND DISCUSSION

Total saponin content

The experiment was performed in triplicate in three independent experiments, and the total saponin content in PDSEF was found to be 96.37% \pm 1.21% w/w. This suggests that the method adopted for the preparation of saponin-enriched fraction is up to snuff. Furthermore, HPLC method developed for profiling of the PDSEF is efficient enough to distinctly separate all the compounds [Figure 1].

α -Glucosidase and α -amylase inhibitory activities of *Pithecellobium dulce* saponin-enriched fraction

α -Glucosidase and α -amylase are the key enzymes involved in hydrolysis/digestion of polysaccharides. α -Amylase hydrolyzes the

complex polysaccharides into oligosaccharides and α -glucosidase releases monosaccharides from oligosaccharides, which are absorbable forms of carbohydrates from intestine.^[14-17] Inhibition of α -glucosidase and α -amylase suppresses the meal-induced increase of plasma glucose level and inhibition of these enzymes exerts antihyperglycemic effect.^[14-17] In the present investigation, the PDSEF was evaluated for its potential to inhibit these enzymes; it has demonstrated potent inhibition of both enzymes, α -glucosidase and α -amylase, in concentration-dependent manner [Figure 2] with IC_{50} of $5.12 \pm 0.15 \mu\text{g/ml}$ and $17.28 \pm 0.23 \mu\text{g/ml}$,

respectively, for α -glucosidase and α -amylase. The PDSEF was found to exert inhibition of these enzymes greater than that of standard inhibitor acarbose (α -glucosidase IC_{50} : $8.23 \pm 0.09 \mu\text{g/ml}$ and α -amylase IC_{50} : $38.42 \pm 2.53 \mu\text{g/ml}$). Further to establish antihyperglycemic activity *in vivo*, the PDSEF was evaluated in mice loaded with sucrose in OSTT.

Acute toxicity study

Oral administration of PDSEF at four different doses did not provoke any physical signs of toxicity in mice, and moreover, no death was

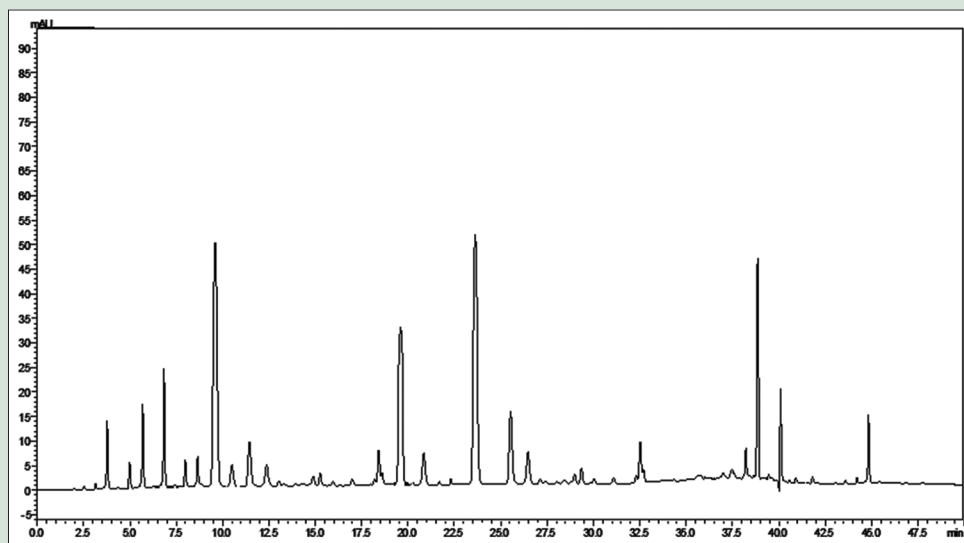


Figure 1: High-performance liquid chromatography profiling of *Pithecellobium dulce* saponin-enriched fraction at 210 nm

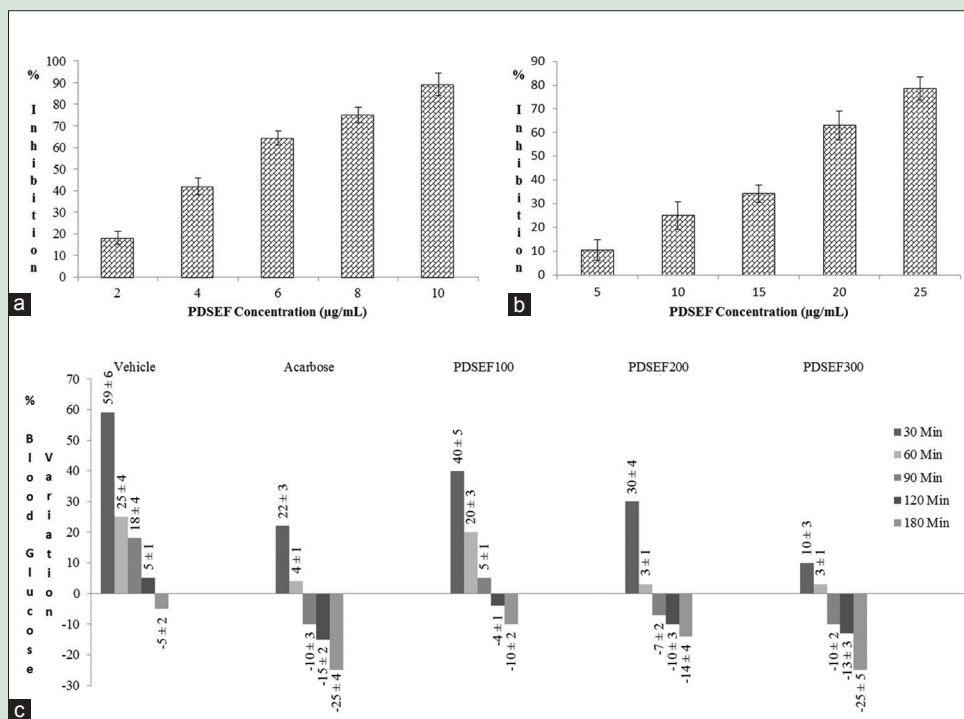


Figure 2: Inhibitory effect of *Pithecellobium dulce* saponin-enriched fraction on (a) enzyme α -glucosidase 0.5 U/mL using the substrate p-nitrophenyl α -D-glucopyranoside 0.5 mM (b) enzyme α -amylase (0.5 mg/mL) using substrate starch 0.5% w/v (c) effect of *Pithecellobium dulce* saponin-enriched fraction at three different concentrations on mice loaded with sucrose at 30, 60, 90, 120, and 180 min. Acarbose was used as a standard reference. All the data showed significant difference as compared to control at $P < 0.05$

reported up to 14 days, indicating PDSEF to be nontoxic and safe in mice up to an oral dose of 2000 mg/kg. Therefore, investigation of antihyperglycemic activity of PDSEF at 100, 200, and 300 mg/kg dose levels was considered safe.

Oral sucrose tolerance in mice

PDSEF was evaluated at three different concentrations under specified conditions in mice loaded with sucrose and it was able to prevent rise in blood glucose level. The observations led to conclude that the PDSEF inhibited the enzymes responsible for hydrolysis of sucrose into absorbable monomers.

CONCLUSION

This study indicates that PDSEF is effective in combating meal-induced blood glucose rise in experiment conditions (*in vivo* and *in vitro*). The studied experimental conditions and data generated suggest that this effect is due to inhibition of key enzymes (α -glucosidase and α -amylase) involved in the digestion of polysaccharides. Altogether, these results strongly recommend that *P. dulce*, which is a commonly available fruit, is of great importance and could be used alone or in combination with other herbs for the management of sudden rise in blood glucose level in diabetic patients. This study also provides further scope for a detailed investigation of saponin/s in different diabetic models.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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