In vitro (anti-alpha-glucosidase) activity and in vivo anti-diabetic activity of Androsace foliosa (common rock jasmine) in alloxan-induced diabetic BALB/c mice

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Abstract

Androsace foliosa syn. Androsace sarmentosa (botanical name of common rock jasmine) (Primulaceae) is used in the treatment various disorders. The aim of this study is to evaluate in vitro anti-diabetic activity of crude methanolic extract of leaves and roots of A. foliosa by anti-alpha-glucosidase (α-Glc) and in vivo anti-diabetic activity of n-hexane fraction on alloxan-induced diabetic mice. Results of in vitro anti-diabetic (α-Glc) activity showed that n-hexane leaves fraction was most potent among all the fractions and showed IC50 (half maximal inhibitory concentration) value of 64.91 ± 0.16 µg and % inhibition of 89.35 ± 0.45, comparable to that of standard acarbose. In vivo n-hexane leaves fraction decreases blood glucose level and reduces body weight similar to that of standard drug glibenclamide. Based on the conclusion of both in vitro and in vivo activities, it can be accomplished that the plant A. foliosa acquires noteworthy anti-diabetic action and can be used to treat diabetes mellitus type II and to reduce body weight.

Keywords

Androsace foliosa, anti-alpha-glucosidase, diabetes

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Introduction

Diabetes mellitus is a persistent metabolic disease resulting from insulin insufficiency. Worldwide occurrence of diabetes in adults in 1995 was estimated to be 4% and might elevate to 5.4% by year 2025.1,2 Diabetes is highly prevalent in developed countries than in developing countries. The percentage rise in type I diabetes will be 42 in developed countries and 170 in developing countries. The prevalence of diabetes is highest in countries such as India, China, and United States.3 Postprandial hyperglycemia (PPHG) is a condition in which the level of blood glucose remains high after the ingestion of meal and it is an essential component to be considered in the management of diabetes and diabetes-related complications such as diabetic neuropathy, diabetic retinopathy, and cardiovascular diseases.4 α-Glc enzyme present in epithelial mucosa of small intestine is responsible...
for conversion of complex carbohydrates to simple sugar by breaking glycosidic bonds. A beneficial effect on body weight is observed by α-Glc inhibitors due to their lowering effect on postprandial elevations of insulin level. Inhibition of the enzyme α-Glc delays or inhibits the absorption of glucose, and this strategy is the striking therapeutic approach for the management of type 2 diabetes. Currently, acarbose, voglibose, and miglitol are the enzyme inhibitors that are used to treat PPHG effectively, but due to their gastrointestinal side effects, these inhibitors are not desirable for long term.5

Identifying potential inhibitors of α-Glc and controlling the glucose level in blood are important therapeutic approaches for reducing diabetic complications. Plants are good and alternative source of drugs, have an imperative function in the treatment of various diseases, and can be used as alternative approaches to treat diabetes.6 Androsace foliosa syn. Androsace sarmentosa (botanical name of common rock jasmine) (Primulaceae), grows in India and Pakistan. A. foliosa has a bitter taste and possess a cooling and coarsening strength. It is used in the treatment of disorders from tumors, fluid inflammation, and other serous fluid syndromes. It is also used to cure amenorrhea, skin allergies, and leucorrhoea and acts as an abortifacient.7 In addition, it is used in treating diabetes mellitus, but the scientific investigation on its effects on diabetes mellitus was not performed. Thus, the present study was carried out to evaluate in vitro anti-diabetic activity of crude methanolic extract of leaves and roots of A. foliosa by anti-alpha-glucosidase (α-Glc) and in vivo anti-diabetic activity of n-hexane fraction on alloxan-induced diabetic mice.

Materials and methods
This study was undertaken at Department of pharmacy, COMSATS Abbottabad in April 2015.

Materials
The plant A. foliosa was collected from Donga Gali, Ayubia National Park, Pakistan, in July 2014. The plant was identified by Dr. Qazi Najam-us-Saqib as A. foliosa. Voucher specimen has been deposited in herbarium of Botany Department (G.P.G.C #1). Glibenclamide (10 mg/kg) was purchased from local market. Other chemicals and drugs Alloxan (ALX 90.5% w/v in Tween 80 solution), Acarbose, sodium azide, NaCl, bovine serum albumin, and substrate (para-nitrophynyl-α-D-glucopyranoside) were purchased from Merck Germany.

Plant extraction
After washing carefully with tap water, plant was shade dried and chopped into a moderately coarse powder. The powder was retained in air tight container. Methanolic extract was prepared by maceration of powder (1000 g) in 2000 mL methanol for 7 days. The process was repeated 3 times for a total of 21 days. Extract was allowed to dry. The semi-solid crude extract was weighed and stored at room temperature in well-closed inert container for further examination. The methanolic extract was further fractionated with diverse organic solvents such as n-hexane, chloroform, and acetone.

5In vitro anti-diabetic activity (anti-α-glucosidase assay)
α-Glc acted on crude methanolic extract of leaves and roots of A. foliosa and subsequent organic fractions, for example, n-hexane leaves, n-hexane roots, and acetonic leaves. A complete 85-µL test solution was blended together with 10 µL of phosphate buffer of pH 7.0. Furthermore, in all well –1 in the 96-well plate followed by totaling of 10 µL of taster adding together and sodium azide 10 µL of enzyme elucidation (Sodium azide 0.0135 units). Ingredients were pre-incubated at 37°C for 5 min. Then, 20 µL of bovine serum albumin stock solution was added per well. In addition, incubation was performed for 10 min at 37°C. Subsequent to incubation, 50 µL of substrate (para-nitrophenyl-α-D-glucopyranoside) was added and additionally incubated for 5 min at 25°C. Incubation was continued further for 10 min at 37°C.

Absorbance was calculated at 625 nm by means of the 96-well plate reader (Synergy HT BioTek, USA). All the interpretations were performed with their controls in triplicates. The concentration of acarbose that was used as a positive control was 0.5 mM.8 The outcomes were determined as per the following formula.

\[
\text{Inhibition} \% = \frac{\text{Control} - \text{Test}}{\text{Control}}
\]
EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) was used to calculate $IC_{50}$ (half maximal inhibitory concentration) value. Furthermore, $IC_{50}$ value was determined by successive strength of the compounds from 0.5 mM to 0.25, 0.125, 0.0625, 0.03125, 0.015625 mM. The concentration at which the enzyme inhibition was 50% is the $IC_{50}$. Values are mean of three autonomous experiments, and this value is determined with the help of graph.

**Phytochemical analysis**

*A. foliosa* n-hexane leaves fraction was screened for various phytochemical constituents using standardized protocol.9

**In vivo anti-diabetic activity in mice**

Male Balb$^6$ Albino mice weighing 30 to 40 g were used in the existing revision. During the reading, animals were fed unrestricted standard diet and water ad libitum. Animals were retained under standard animal housing circumstances, that is, with 12 h dark/light cycle. The study was undertaken with due approval from Institution Animal Ethics Committee.

**Induction of diabetes**

Diabetes was induced in 12-h fasted mice by administration of alloxan monohydrate dissolved in 0.9% NaCl solution, at a dose of 150 mg/kg (S/C). After 3 days of alloxan monohydrate introduction, glucose concentration (GC) was determined in samples of blood. Blood samples were obtained by tail bleed, and Swiss albino mice possessing blood glucose level (BGL) of $\geq$200 mg/dL after 3 days were considered diabetic and chosen for the current research.

**Experimental design**

Animals were classified into six groups possessing six mice in each group.

*Group I.* Normal healthy mice given simply vehicle standard diet (normal control).

*Group II.* Diabetic control mice given alloxan only.

*Group III.* Diabetic mice of this category were given a solitary dose of glibenclamide (10 mg/kg body weight (BW)) 1 mL with vehicle by oral dose every day for 15 days.

*Group IV.* Diabetic mice of this category were given solitary dose of *A. foliosa* n-hexane leaves fraction (100 mg/kg BW) 1 mL with vehicle by oral dose every day for 15 days.

*Group V.* Diabetic mice of this category were administered with single dose of *A. foliosa* n-hexane leaves fraction (250 mg/kg BW) 1 mL with vehicle by oral dose every day for 15 days.

*Group IV.* Diabetic mice of this category were given single dose of *A. foliosa* n-hexane leaves fraction (500 mg/kg BW) 1 mL with vehicle by oral dose every day for 15 days.

**Acute toxicity study**

Acute toxicity study was done as per guideline 423 of Organization for Economic Cooperation and Development for fixed-dose procedure.10 After administration of single oral dose of 100, 250, and 500 mg/kg extract, rats were observed for any adverse signs and symptoms at hourly intervals for the next 24 h and thereafter for 2 days.

**Evaluation of anti-diabetic activity**

After treating the diabetic mice groups with diverse doses of *A. foliosa* n-hexane leaves fraction (100, 250 and 500 mg/kg BW) and standard drug, for example, glibenclamide for 15 days, glucose level was determined with the help of NIPRO blood glucose monitoring system. BGL was determined for all six groups and for every mice individually and recorded. Body weight and BGL of experimental mice were recorded before initiating the dose. Body weight and glucose level were also recorded at 3rd, 6th, 9th, 12th, and 15th day of the research.

**Statistical analysis**

Mean ± standard deviation (SD) and analysis of variance (ANOVA) test were applied for each value of body weight and fasting blood sugar concentration. Differences between groups were measured considering $p < 0.05$ levels as significant.
Diabetic control group was compared with the diabetic *A. foliosa* n-hexane leaves fraction–treated group and glibenclamide-treated group. Diabetic control group was also compared with normal control group, and data were analyzed.

## Results

### Phytochemical analysis

Phytochemical screening of the *A. foliosa* n-hexane leaves fraction has revealed the presence of saponins, flavonoids, and terpenes.

### Acute toxicity study

Acute toxicity study revealed that the administration of methanolic and other organic fractions (n-hexane leaves, n-hexane roots, and acetonic leaves) did not produce significant changes in the behavior of animals. All the animals were physically active and no deaths were recorded up to the dose of 500 mg/kg, which may be considered as a therapeutic advantage.

### In vitro anti-diabetic activity

Anti-α-Glc activity of *A. foliosa*'s crude methanolic extract of leaves and roots and different organic fractions (n-hexane leaves, n-hexane roots, and acetone leaves) was measured at % inhibition level of 0.5 mg/mL and their IC$_{50}$ (µg/mL) was also calculated. The n-hexane leaves fraction demonstrated IC$_{50}$ of 64.91 ± 0.16 µg/mL and n-hexane roots fraction showed IC$_{50}$ of 156 ± 0.12 µg/mL, while these fractions exhibited % inhibition of 89.35 ± 0.45 µg/mL and 87.47 ± 0.26 µg/mL at 0.5 mg/mL respectively. Results indicate that n-hexane leaves fraction was relatively more potent than all other fractions and showed highest IC$_{50}$ of 64.91 ± 0.16 µg/mL. Acarbose was used as standard that showed IC$_{50}$ of 38.25 ± 0.12 µg/mL and % inhibition of 92.23 ± 0.14 µg/mL at 0.5 mg/mL. Results are given in Table 1.

### In vivo anti-diabetic activity

As n-hexane leaves fraction was most potent among all the fractions, this fraction was chosen for in vivo anti-diabetic action. In vivo anti-diabetic action was conducted for 15 days on diabetic mice. Outcome elaborates that n-hexane leaves fraction lessen the blood GC in a dose-dependent mode. Results of findings indicate that n-hexane leaves fraction (500 mg) decreased BGL from 373 ± 16.4 to 235 ± 16.4 (mg/dL). Glibenclamide (10 mg/kg) was used as standard and reduced BGL from 333 ± 16.4 to 186 ± 16.4 (mg/dL). No alteration was observed in BGL of normal and diabetic control groups. Outcome of the existing revision indicates that n-hexane leaves fraction at the dose of 500 mg/kg indicates analogous findings with the standard (glibenclamide) in decreasing blood GC in alloxan-induced diabetic mice. Results are tabulated in Figure 1. Consequences of revision display that *A. foliosa* n-hexane leaves fraction reduces body weight of diabetic mice in a linear and dose-dependent behavior. Results show that body weight was extensively reduced from 36 ± 1.30 to 29 ± 2.40 g by the induction of *A. foliosa* n-hexane leaves fraction at the dose of 500 mg/kg, while glibenclamide 10 mg/kg was used as standard and reduces body weight from 36 ± 0.80 to 29 ± 1.4 g. Results of body weight reduced by *A. foliosa* n-hexane leaves fraction (500 mg/kg) were equivalent to that of glibenclamide. Findings are tabulated in Table 2.

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**Table 1.** Anti-α-Glc activity of *Androsace foliosa*’s crude methanolic extract of leaves and roots and different organic fractions.

| Sr no | Sample code                        | α-Glc activity |
|-------|------------------------------------|----------------|
| 1     | *Androsace foliosa* n-hexane leaves fraction | 64.91 ± 0.16 | 89.35 ± 0.45 |
| 2     | *Androsace foliosa* n-hexane roots fraction | 156.27 ± 0.12 | 87.47 ± 0.26 |
| 3     | *Androsace foliosa* methanolic leaves (crude) | 48.12 ± 0.16 | − |
| 4     | *Androsace foliosa* acetone leaves fraction | 75.12 ± 0.16 | − |
| Standard | Acarbose                              | 38.25 ± 0.12 | 92.23 ± 0.14 |

IC$_{50}$, half maximal inhibitory concentration. All extracts were dissolved/suspended in methanol and tests carried out in triplicates. Data are signified as mean ± SEM, and IC$_{50}$ values of active extracts/fractions were calculated after appropriate dilutions of the active extracts/fractions only.
Discussion

High cost and undesirable side effects of currently available synthetic α-Glc inhibitors drive the need to investigate the natural sources of new efficient inhibitors. This study demonstrated that both n-hexane leaves fraction and n-hexane roots fraction showed α-Glc inhibitory activity (Table 1). In addition, n-hexane leaves fraction concentration of 0.5 mg/mL had the highest inhibitory action (89.35%), among all fractions and crude extracts. Meanwhile, n-hexane roots fraction indicates inhibitory activity of 87%. α-Glc blocking activity of n-hexane leaves fraction was potent than methanolic extract and n-hexane roots portion. Results of the finding noticeably indicate that IC50 of n-hexane leaves was equivalent to that of acarbose. As this fraction hinders α-Glc enzyme, it decreases the absorption of carbohydrates in the upper portion of small intestine. As a potent α-Glc inhibitor, A. foliosa blocks the activity of enzyme in small intestine. α-Glc activity is the rate limiting step in the alteration of oligosaccharides into monosaccharides essential for gastrointestinal absorption. A. foliosa also mitigates the PPHG by delaying the liberation of D-glucose of liposaccharides and disaccharides. Furthermore, it delays the glucose absorption. A. foliosa (n-hexane leaves fraction) in alloxan-induced diabetic mice causes drop in glucose level. The anti-hyperglycemic consequence of n-hexane leaves fraction of 500 mg/kg BW dose was comparatively more valuable than 250 mg/kg and 100 mg/kg BW in this regard. The glibenclamide (standard) works oppositely by promoting insulin discharge by shutting of potassium-ATP (adenosine triphosphate) channels. Furthermore, membrane depolarization and stimulation of calcium influx are the fundamental steps in insulin discharge.11 Phytochemical screening of the A. foliosa n-hexane leaves fraction has revealed the presence of sapo- nins, flavonoids, and terpenes. The presence of these phytochemicals in this fraction is possibly responsible for hypoglycemic action. Saponins are supposed to be more responsible for anti-diabetic activity as mentioned in the literature.12 The current result reveals that A. foliosa powerfully inhibits α-Glc enzyme in vitro in a dose-dependent manner. The n-hexane leaves fraction is the most potent and shows IC50 value in comparison to that of standard drug acarbose. So, this fraction is effective in the treatment of PPHG. The in vivo experiments of

Table 2. Anti-diabetic activity of Androsace foliosa n-hexane leaves fraction (100, 250 and 500 mg/kg)–treated and standard drug glibenclamide (10 mg/kg)–treated, normal control and diabetic control groups showing mean, SD, and variance of body weight on alloxan-induced diabetic mice.

| Groups                        | Body weight in grams |
|-------------------------------|----------------------|
|                               | Initial  | 3rd day  | 6th day  | 9th day  | 12th day | 15th day | VR (%) |
| Normal control                | 35 ± 1.8 | 34 ± 1.5 | 33 ± 1.8 | 33 ± 0.8 | 32 ± 1.8 | 31 ± 1.7 | 3.26   |
| Diabetic control              | 37 ± 0.9 | 36 ± 0.9 | 35 ± 1.2 | 34 ± 1.3 | 33 ± 1.3 | 32.1 ± 0.8 | 7.38   |
| DB + Androsace foliosa n-hexane (100 mg/kg) dose | 37 ± 0.9 | 36 ± 1.0 | 35 ± 1.2 | 34 ± 1.2 | 33 ± 1.34 | 32 ± 1.5 | 5.6    |
| DB + Androsace foliosa n-hexane (250 mg/kg) dose | 36 ± 1.2 | 34 ± 1.28 | 33 ± 1.33 | 32 ± 1.40 | 31 ± 1.50 | 30 ± 1.67 | 6.54   |
| DB + Androsace foliosa n-hexane (500 mg/kg) dose | 36 ± 0.8 | 34 ± 0.90 | 33 ± 0.1 | 32 ± 1.21 | 30 ± 1.32 | 29 ± 1.4 | 9.63   |
| Diabetic + GB (10 mg/kg) dose | 36 ± 1.3 | 34 ± 1.5 | 33 ± 1.7 | 32 ± 1.9 | 31 ± 2.21 | 29 ± 2.4 | 7.35   |

Abbreviations: GB: glibenclamide; DB: diabetic; VR: Variance.
n-hexane leaves fraction (500 mg/kg) of A. foliosa show maximum reduction of blood GC in alloxan diabetic mice in analogous to that of the standard drug glibenclamide. Further research is needed to develop anti-diabetic drug from this plant.

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