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Antidiabetic Effect of *Asparagus adscendens* Roxb. in RIN-5F Cells, HepG2 Cells, and Wistar Rats

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Abstract

Background: The antidiabetic effect of *Asparagus adscendens* root ethanolic extract (AAE) was evaluated in this study using both *in vivo* and *in vitro* models. The effect of AAE on carbohydrate metabolizing enzymes (α-amylase and α-glucosidase) was determined. The safety of AAE was tested on Wistar rats and two different cell lines. Some mechanisms of action were also investigated with AAE's dose-response.

Methods: Glucose-loaded (10 g/kg) and streptozotocin-induced (60 mg/kg) diabetic Wistar rats were used in the *in vivo* model, whereas RIN-5F pancreatic cells and HepG2 liver cells were used in the *in vitro* model. Nontoxic mass value of AAE was used in the *in vitro* (from 0.625 to 2.5 μg/100 μl) and *in vivo* (up to 400 mg/kg) studies. The inhibitory activity of AAE on α-amylase and α-glucosidase was examined by spectrophotometric and microplate reader techniques.

Results: The AAE inhibited α-amylase and α-glucosidase, two key enzymes of the carbohydrate metabolism, and stimulated the release of insulin in RIN-5F cells line and glucose uptake in HepG2 cells in a concomitant way. Lower mass values of the extract caused no significant change in the viability of the cells, whereas 5 μg caused a significant reduction in the viability of RIN-5F (59.78%) and HepG2 (56.87%) when compared to the control. The 2.5 μg extract stimulated 91% insulin release in RIN-5F cells when compared with the control. Also, 2.5 μg extract induced 86% and 83% glucose uptake in HepG2 cells in the presence and absence of insulin, respectively, when compared with the control. The median lethal dose of AAE was ≥5000 mg/kg in Wistar rats. AAE caused a decrease in fasting blood glucose level from 30 min in glucose-loaded Wistar rats and from the 4th day in streptozotocin-induced diabetic rats when compared with the control. There was also an increase in serum insulin and serum α-amylase level in streptozotocin-induced diabetic rats, compared to the control, at the end of the study.

Conclusion: *A. adscendens* root exerts its antidiabetic effect by inhibiting α-amylase and α-glucosidase enzymes, inducing insulin secretion in RIN-5F pancreatic cells, and enhancing glucose uptake in HepG2 liver cells.

Keywords: *Asparagus adscendens,* diabetes, α-amylase, α-glucosidase, RIN-5F cells, HepG2 cells, Wistar rats

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by increased fasting blood glucose level [1]. The number of adults (above 18 years) with diabetes has increased substantially between 1980 and 2014, rising from 108 million to 422 million [2]. The drugs involved in the treatment of type 2 diabetes include insulin secretagogues (sulfonylureas and meglitinides), α-glucosidase inhibitors (acarbose), insulin sensitizers (thiazolidinediones), inhibitors of hepatic glucose production (biguanides), incretin mimetics (glucagon-like-peptide-1 [GLP-1] agonist), enhancers of incretins (dipeptidyl-peptidase-4 [DPP-4] inhibitors), and enhancers of glucose elimination in urine (sodium-glucose cotransporter 2 [SGLT2] inhibitors) [3–7]. However, none of these pharmacological drugs have total control on blood glucose regulation, and these drugs exert some undesirable side effects, including hypoglycemia, weight loss, heart failure, and gastrointestinal side effects [6–8]. In India, *Asparagus adscendens* Roxb. (Asparagaceae) is commonly known as Safed musli, shatavari, jhirni, shatamuri, sahasrapal, and sainsabuti. *A. adscendens* is a flowering perennial shrub with spines and woody stem that

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is found in India and Himalayan Mountain ranges [9–11]. *A. adscendens* root is traditionally orally used by human for the treatment of sexual debility, urinogenital disorders, diarrhea, dysentery, and general debility [10,11]. Negi et al. [12] reported that *A. adscendens* have immunostimulant, anti-inflammatory, anti-hepatotoxic, anti-cytotoxic, and anti-bacterial effect. The use of *A. adscendens* root in ayurvedic medicine in India has given rise to various commercial formulations, but its mechanism of antidiabetic effect is yet to be evaluated. However, the antidiabetic activity of the aqueous extract of *A. adscendens* rhizome on starch digestion, BRIN-BD11 clonal pancreatic cells, and 3T3-L1 adipocytes cells have been documented [13]. The *in vivo* antidiabetic activity of *A. adscendens* root ethanolic extract (AAE) and its mechanisms mediating the antidiabetic effect on inhibition of α-amylase and α-glucosidase enzymes, glucose uptake in hepatic cells (HepG2 liver cells), sensitivity of HepG2 liver cells to insulin, stimulation of insulin secretion in RIN-5F pancreatic cells, and the safety of AAE in Wistar rats, HepG2 cells, and RIN-5F cells is yet to be evaluated.

In this study, the antidiabetic mechanisms of AAE were investigated on the α-amylase enzyme, α-glucosidase enzyme, RIN-5F pancreatic cells, HepG2 hepatic cells, and Wistar rats.

Materials and Methods

Plant collection and authentication
A. adscendens pulverized root was a kind gift from AMSAR Private Limited, Indore, India. The photograph of the plant root available on the website of the company was also confirmed by Dr. Navin K. Jain at the Department of Botany, Holkar Science College (HSC), Indore, India. A herbarium sheet of *A. adscendens* (N.K. Jain 02-*Asparagus*, HSC) was preserved at the Department of Botany, HSC. The dried pulverized root of *A. adscendens* was macerated in 70% ethanol for 72 h, and the suspension was then filtered using Whatman No.1 filter paper. The filtrate was concentrated to a solid form at 45ºC and stored in a refrigerator at 4–8ºC before use.

Cell lines, media, assay kits, and reagents
RIN-5F pancreatic cells and HepG2 liver cells were purchased from National Center for Cell Science, Pune, India. The reagents 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), insulin testing kit, α-amylase enzyme, α-glucosidase enzyme, and p-nitrophenyl glucopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), and glucose testing kit were procured from Hi-Media (Mumbai, India).

Carbohydrate metabolizing enzymes inhibition assay

α-amylase inhibition assay
The α-amylase inhibition assay was performed, as described by Oboh et al. [14], with slight modifications. The AAE,

enzyme, and the substrate (starch) were separately dissolved in 20 mM NaH_2PO_4-Na_2HPO_4 buffer, pH 6.9 having 6 mM NaCl. The 1 ml α-amylase solution (1 Unit α-amylase/ml) was added to 1 ml of different mass values of AAE (62.5, 125, 250, 500, and 1000 μg/ml) in different test tubes. The mixed contents were incubated at 25°C for 5 min, and thereafter, 1 ml of 1% (w/v) soluble starch was mixed and then further incubated at 25°C for 10 min. The reaction was stopped by using 1 ml of dinitrosalicylic acid (DNS) reagent. After that, the test tubes were incubated at 90°C for 5 min in a water bath and subsequently were brought to room temperature by gradual cooling in air. Thereafter, 5 ml of water was added to dilute the contents, and color intensity was determined by measuring optical density (OD) at 540 nm in a spectrophotometer (UV-1700 PharmaSpec., Shimadzu, Kyoto, Japan). A parallel test under identical conditions was also performed where acarbose (100 μg/ml) was used in place of AAE. Another set was also performed simultaneously under identical conditions where neither AAE nor drug (acarbose) was added to consider it as 100% enzyme activity. The percentage of enzyme inhibition was calculated using the following equation:

% Enzyme inhibition =
Absorbance at 100% enzyme activity – Absorbance of test samples / Absorbance at 100% enzyme activity x 100

α-glucosidase inhibition assay
The α- glucosidase enzyme inhibition assay was performed, as described by Kim et al. [15], with slight modifications. A synthetic substrate, p-nitrophenyl glucoside (pNPG), was used, and its 10 mM solution was prepared by dissolving in 20 mM NaH_2PO_4-Na_2HPO_4 buffer, pH 6.9. A 20 μl of α-glucosidase solution (0.2 Units α-glucosidase/ml buffer) was pre-incubated at 37ºC for 3 min with 20 μl of different mass values of AAE (3.125, 6.25, 12.5, 25, and 50 μg/20 μl). Thereafter, the reaction was started by adding 20 μl of 10 mM pNPG. The enzyme reaction was allowed for 15 min at 37°C, followed by the addition of 80 μl of 0.2 M Na_2CO_3 to inactivate the enzyme. The color intensity was determined by measuring OD at 405 nm using a microplate reader. A parallel test (positive control) under identical conditions was also run simultaneously by using 20 μl acarbose (250 μg/ml) in place of AAE. A set was also run under identical conditions without AAE or drug (acarbose) to consider it as 100% enzyme activity. The percentage of α-glucosidase enzyme inhibition was calculated using the same equation as mentioned above for the α-amylase inhibition assay.

Maintenance of cell culture
RIN-5F pancreatic cells and HepG2 liver cells (National Center for Cell Science, Pune, India) were routinely cultured in EMEM (Cat No. AL047S-100ML, Hi-Media, Mumbai, India) supplemented with 10% FBS and 1% streptomycin and were incubated at 37ºC in a CO_2 incubator.

Cell viability assay

The cell viability assay in the presence of AAE was performed, as described by Mosmann [16], with slight modifications. Both the cell lines (RIN-5F pancreatic cells and HepG2 liver cells) were placed separately into two different 96-well plates having 6×10^3 cells per well in a total 200 μl volume. Two cell-free rows were also included in the 96 well plates to serve as blanks. The cells were incubated for 48 h for adherence and then treated with 100 μl of AAE at various mass values (0.625, 1.25, 2.5, 5, and 10 μg/100 μl) to a specific well for 24 h at 37°C. Thereafter, 50 μl MTT (5 mg/ml) was added in each well (wells treated with AAE and wells without treatment), and the contents were incubated in the dark at 37ºC for another 3 h, as described by Hassan et al. [17]. After that, the spent medium was drawn from each well. The formazan crystals formed in each well were dissolved in 200 μl of 50% dimethylsulfoxide (DMSO), and OD was measured at 540 nm using a microplate reader (ECIL Micro Scan MS5608A). DMSO (200 μl) was also added in the cell-free (blank) wells. The percentage of cell viability was calculated using the following equation [18]:

$$\% \text{ Cell viability} = \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

where At is OD of treated cells, Ab is OD of Blank (DMSO only in cell-free wells), and Ac is OD of control (untreated cells).

RIN-5F cells insulin secretion assay

RIN-5F pancreatic cells were placed, on an average 6×10^3 cells per well, in a 96 well plate and incubated for 72 h at 37ºC in a CO_2 incubator. Thereafter, the spent medium was drawn by aspiration, and cells settled in the wells were washed thrice with 200 μl of wash buffer (Kreb's Ringer bicarbonate (KRB) buffer, pH 7.4, which was consisted of 115 mM sodium chloride, 4.7 mM potassium chloride, 1.28 mM calcium chloride, 1.2 mM potassium dihydrogen phosphate, 1.2 mM magnesium sulfate, 24 mM sodium bicarbonate, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) along with 1% BSA and 1.1 mM glucose). Thereafter, these cells were pre-incubated at 37ºC for 40 min with the wash buffer. After that, these cells were incubated at 37ºC for 20 min with 100 μl KRB buffer having 1.1 mM glucose and 100 μl of different mass values of AAE (0.625, 1.25, 2.5, 5, and 10 μg/100 μl), 100 μl glibenclamide (1 μg/100 μl) [positive control], and 100 μl phosphate-buffered saline in place of AAE (negative control) all in different test wells [17]. Thereafter, a portion of the contents was drawn from each well and stored at –20ºC before doing insulin assay. The insulin assay was done by using an insulin ELISA kit (Cat no. KAP1251, Sigma-Aldrich, St. Louis, MO, USA).

HepG2 cells glucose utilization assay

The assay for glucose utilization in HepG2 cells was carried out using the method of Zheng et al. [19], with slight modifications. It was performed in the absence and presence of insulin (10 μM/100 μl) in the wells treated with the root extracts and standard (test wells). The assay, carried out with insulin, was to determine the effect of the root extract on the increase in the sensitivity of HepG2 liver cells to insulin. Insulin was also added to two wells without test samples. In summary, HepG2 liver cells were seeded at a density of 6×10^3 cells per well in a 96 well plate. After 48 h, the spent medium was aspirated, and the 150 μl EMEM containing 0.2% bovine serum albumin (BSA) and 8 mm glucose was added. After replacing the medium, a volume of 100 μl of different mass values of AAE (0.625, 1.25, 2.5, 5, and 10 μg/100 μl), 100 μl positive control (1 μg/100 μl metformin without insulin and 1 μg/100 μl pioglitazone with insulin), and 100 μl phosphate-buffered saline in lieu of root extracts (negative control) was added to different wells. After 24 h, 10 μl of the contents was taken from each well and transferred into another 96 well plate. Thereafter, 200 μl of glucose oxidase reagent was added. The plate was further incubated for 15 min at 37°C, and the OD was determined at 492 nm using Micro Scan MS5608A. Glucose test kit (Cat. No. GLUL 05 100, Robonik India Pvt. Ltd., Thane, India) was used for determining the amount of glucose in the contents. The percentage of glucose utilization was calculated in relation to the untreated wells (negative control) using the following equation [18]:

% Glucose utilization = Concentration of glucose in treated wells / Concentration of glucose in untreated wells x 100

The wells treated with AAE, metformin, pioglitazone, and insulin were considered as treated wells, while the wells treated with only phosphate-buffered saline were considered as untreated wells.

Assay for *in vivo* antidiabetic studies

The experiments using Wistar rats were carried out after approval (approval no. 779/CPCSEA/IAEC/2018/010) from the Institutional Animal Ethics Committee, Devi Ahilya University, Indore, India. The approved guidelines were strictly followed.

Animals

Wistar rats of both sexes (200–220 g) were procured from the Institutional Animal house of the University. These rats were kept in well-ventilated polypropylene rat cages, fed with broilers mash, and were given water *ad libitum.* The rats were allowed to acclimatize with the environment under natural daylight and night conditions for two weeks before starting the experiments.

Acute toxicity studies

Three Wistar rats per group (6 groups) were orally (p.o.) acutely administered 10, 100, 1000, 1600, 2900, and 5000 mg extract/kg body weight (group 1 to group 6, respectively) and were monitored for signs of toxicity/mortality every hour for the first 4 h and daily for 3 days after acute administration of the ethanolic extract [20].

Oral glucose loading

A mass of 10 g glucose/kg body weight (b.wt) was administered (p.o.) to Wistar rats that were fasted overnight (12 h). After 30 min of administration, blood was drawn from the vein of the tail, and the level of blood glucose was checked using glucometer and glucose strips (Accu-Check Active Glucometer, model: GC0088, Mannheim, Germany) [21]. Wistar rats with fasting blood glucose level (FBGL) above 7 mmol/l were randomly divided into 6 groups of five rats per group; the extracts and glibenclamide were orally administered once. Group 1; normoglycemic Wistar rats (5 ml distilled water only), group 2–4 (100, 200, and 400 mg ethanolic extract/kg b.wt, respectively), group 5 (5 mg glibenclamide/kg b.wt), and group 6; control (10 ml distilled water/kg b.wt). The FBGL was measured at 0, 30, 60, 120, and 240 min.

Assay for the antidiabetic effect of AAE in streptozotocin-induced diabetic rats

Wistar rats were fasted overnight (for 12 h), and diabetes was induced by a single intraperitoneal injection of 60 mg streptozotocin/kg b.wt. After seventy-two hours, blood was drawn from the vein of the tail, and the FBGL was measured. Animals with FBGL above 11.1 mmol/l were selected for the experiment. The streptozotocin-induced diabetic rats were randomly divided into groups as in glucose-loaded rats above. The extracts and glibenclamide were administered (p.o.) daily for 21 days, and the FBGL was measured on day 0, 4, 7, 10, 14, 18, and 21 [22]. On the 21st day, animals were sacrificed, and blood samples were collected by cardiac puncture into plain bottles. The serum was collected for insulin [23] and α-amylase assay [24].

Analysis of data

Data for each group were collected and summarized in tabular and graph forms for each treatment group. The data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett post hoc multiple comparisons tests at 95% ($p<0.05$) level of significance using Primer (version 3.01). The data have been shown as the mean ± standard error of the mean (SEM). The student's t-test was also used to determine the significant difference between the two treatment groups.

Results

Effect of AAE on α-amylase enzyme

AAE significantly ($p<0.05$) inhibited the α-amylase enzyme in a concentration-dependent manner (Table 1). The lowest concentration (62.5 μg) of AAE inhibited 27% of the α-amylase enzyme, whereas the highest concentration (1000 μg) caused 57.43% inhibition. The positive control (acarbose) also significantly ($p<0.05$) inhibited an α-amylase enzyme (Table 1).

Effect of AAE on α-glucosidase enzyme

Increasing mass values of AAE significantly ($p<0.05$) increased the inhibition of α-glucosidase (Table 2). AAE at a concentration of 3.125 μg (lowest concentration) exerted 11.79% inhibition of α-glucosidase, whereas 50 μg (the highest concentration) caused 50.06% inhibition. The positive control (acarbose) also significantly ($p<0.05$) inhibited an α-glucosidase enzyme (Table 2).

Effect of AAE on the viability of RIN-5F pancreatic cells and HepG2 liver cells

The AAE exerted a significant ($p<0.05$) concentration-dependent decrease in the viability of RIN-5F and HepG2 cells (Table 3). AAE at concentrations of 0.625, 1.25, and 2.5 μg caused no significant effect on the viability of RIN-5F and HepG2 cells compared to untreated cells (control; Table 3). While at higher mass values (5 and 10 μg), there was a significant ($p<0.05$) decrease in the viability compared to untreated cells (Table 3). The IC$_{50}$ of AAE for RIN-5F cells and HepG2 cells was 7.49 μg/20 μl and 7.29 μg/20 μl, respectively.

Table 1: Effect of *Asparagus adscendens* root ethanolic extract (AAE) on the α-amylase enzyme.

Sample concentration	Percentage inhibition of the α-amylase enzyme	IC$_{50}$ of AAE
AAE 62.5 μg	27.00 ± 0.56	
AAE 125 μg	30.14 ± 0.21	
AAE 250 μg	38.45 ± 0.51	731.29 μg
AAE 500 μg	44.23 ± 0.62	
AAE 1000 μg	57.43 ± 0.81	
Positive control (100 μg acarbose)	60.69 ± 0.31	

The data are represented as the mean ± SEM, n = 3. IC$_{50}$: half maximal inhibitory concentration.

Table 2: Effect of AAE on the α-glucosidase enzyme.

Sample concentration	Percentage inhibition of the α-glucosidase enzyme	IC$_{50}$ of AAE
AAE 3.125 μg	11.79 ± 0.41	
AAE 6.25 μg	26.31 ± 0.33	
AAE 12.5 μg	34.04 ± 0.09	45.02 μg
AAE 25 μg	40.52 ± 0.31	
AAE 50 μg	50.06 ± 0.01	
Positive control (100 μg acarbose)	56.56 ± 0.01	

The data are represented as the mean ± SEM, n = 3.

Table 3: Effect of AAE on the viability (%) of RIN-5F pancreatic cells and HepG2 liver cells.

Concentration (μg)	RIN-5F % cell viability	HepG2 % cell viability
AAE 0.625	99.11 ± 4.11	99.30 ± 3.42
AAE 1.25	97.22 ± 3.22	98.45 ± 4.29
AAE 2.5	96.78 ± 5.11	97.02 ± 5.34
AAE 5	59.78 ± 3.11*	56.87 ± 4.49*
AAE 10	33.44 ± 4.11*	31.99 ± 4.67*
Control	100 ± 5.21	100 ± 5.11

The data are represented as the mean ± SEM, n = 3. *$p < 0.05$ compared to the untreated cells (control).

Effect of AAE on insulin secretion in RIN-5F pancreatic cells

The AAE at concentrations of 0.625 μg (0.59 μIU/ml), 1.25 μg (0.62 μIU/ml), and 2.5 μg (0.65 μIU/ml) exerted a significant ($p < 0.05$) increase in insulin secretion in RIN-5F pancreatic cells compared to the untreated cells; control (0.54 μIU/ml). Glibenclamide at 1 μg (0.72 μIU/ml) concentration also caused a significant ($p < 0.05$) increase in insulin levels when compared to the control (Figure 1). The AAE at a concentration of 2.5 μg exerted a higher significant ($p < 0.05$) percentage increase in insulin secretion in RIN-5F pancreatic cells when compared with other mass values of the extract (Figure 1).

Effect of AAE on glucose utilization in HepG2 liver cells

The AAE at concentrations 0.625 μg (11.94 mg/dl), 1.25 μg (9.75 mg/dl), and 2.5 μg (6.46 mg/dl) exhibited a significant percentage increase in glucose utilization ($p < 0.05$) compared to the untreated cells; control (144.8 mg/dl) (Figure 2). Metformin at 1 μg (2.08 mg/dl) concentration also caused a

significant ($p < 0.05$) percentage increase in glucose utilization when compared to the control (Figure 2). Also, 2.5 μg AAE exerted a higher significant ($p < 0.05$) percentage increase in glucose utilization in HepG2 cells when compared with other mass values of the extract (Figure 2).

Effect of AAE on the sensitivity of HepG2 liver cells to insulin

The AAE at concentrations 0.625 μg (13.03 mg/dl), 1.25 μg (10.84 mg/dl), and 2.5 μg (7.56 mg/dl) exerted a significant ($p < 0.05$) percentage increase in glucose uptake in HepG2 liver cells due to the sensitivity of the cells to insulin when compared with the control (44.78 mg/dl) (Figure 3). Ten microgram insulin (6.46 mg/dl) and 1 μg pioglitazone (0.99 mg/dl) also caused a significant ($p < 0.05$) percentage increase in glucose uptake when compared to the control (Figure 3). The AAE at a concentration of 2.5 μg exerted a higher significant ($p < 0.05$) percentage increase in glucose uptake in HepG2 liver cells due to the sensitivity of the cells to insulin

when compared with other mass values of the extract (Figure 3). However, when the effect of AAE at different concentrations on glucose uptake in HepG2 liver cells was tested in the presence and absence of insulin, no significant increase in uptake of glucose was observed in the presence of insulin compared to its absence (Table 4).

The median lethal dose (LD50) of AAE

The ethanolic extract caused no mortality in Wistar rats after acute administration of the extract orally, and the LD_{50} of the extract was ≥5000 mg/kg b.wt (p.o.).

Effect of AAE on FBGL of Wistar rats

The extracts and glibenclamide caused a significant reduction in the FBGL from 30 min when compared with the control (untreated Wistar rats) and 240 min in glucose-loaded rats (Figure 4). In streptozotocin-induced diabetic rats, AAE caused a significant reduction in FBGL when compared with the control and on the 21st day when compared with day 1, 4, 7, 10, 14, and 18 (Figure 5).

Effect of AAE on serum insulin and serum α-amylase levels

The AAE and glibenclamide caused a significant increase in serum insulin and serum α-amylase level in Wistar rats when compared with the untreated diabetic rats (control; Table 5).

Discussion

The results of this *in vitro* study showed that AAE inhibited α-amylase and α-glucosidase enzymes, induced the secretion of insulin in RIN-5F pancreatic cells, and enhanced the glucose intake in HepG2 liver cells in a concomitant way. In the *in vivo* study, the AAE caused a reduction in FBGL (in both glucose-loaded and streptozotocin-induced diabetic rats) and increased serum insulin and serum α-amylase levels in streptozotocin-induced diabetic rats.
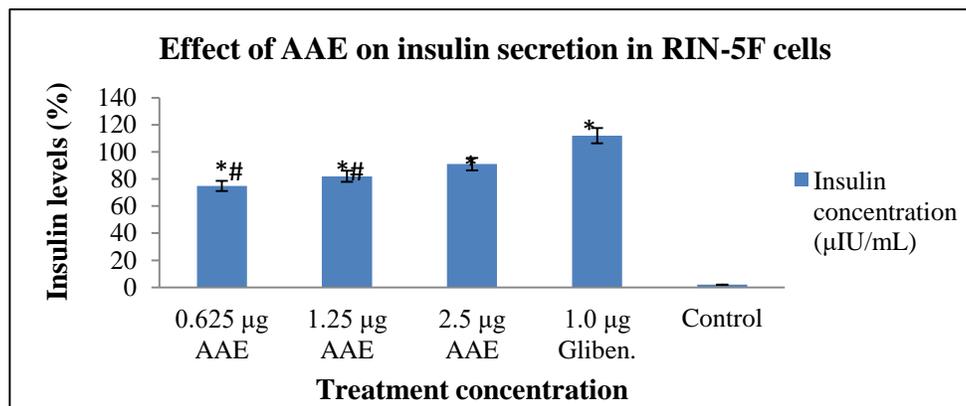

Figure 1. Effect of *Asparagus adscendens* root ethanolic extract (AAE) on insulin secretion in RIN-5F pancreatic cells. The data are represented as the mean ± SEM, n = 3. *$p<0.05$ compared to the control (untreated cells); #$p<0.05$ compared to 2.5 μg AAE.
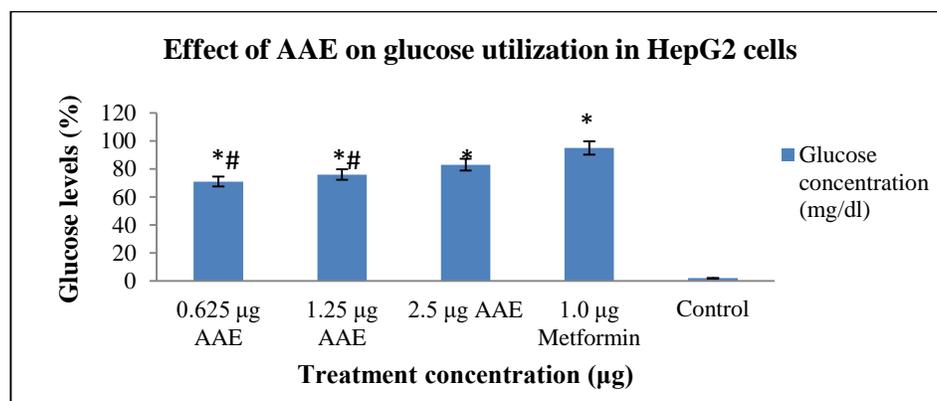

Figure 2. Effect of AAE on glucose utilization in HepG2 liver cells. The results are presented as the mean ± SEM; n = 3. *$p<0.05$ compared to the control; #$p<0.05$ compared to 2.5 μg AAE.
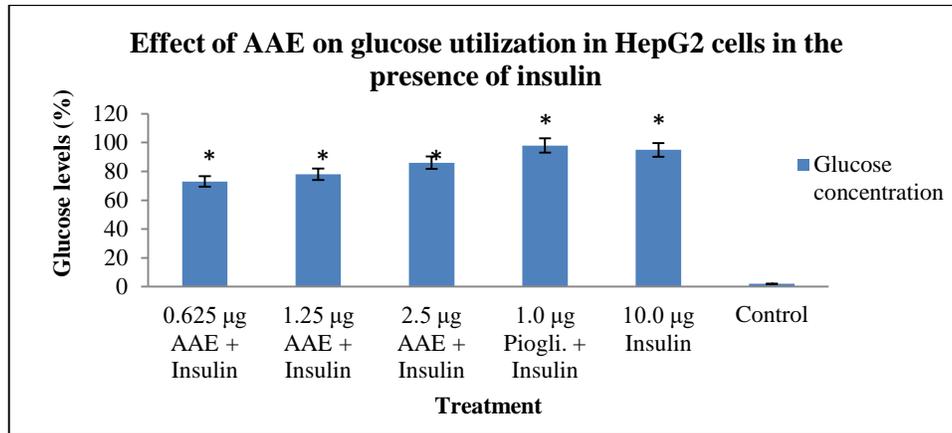
Figure 3. Effect of AAE on glucose utilization in HepG2 liver cells in the presence of insulin. The results are presented as the mean ± SEM; n = 3. Piogli.: Pioglitazone. *$p < 0.05$ compared to the control (untreated cells); #$p < 0.05$ compared to 2.5 μg AAE.

Table 4: Effect of AAE on the sensitivity of HepG2 liver cells to insulin.

Concentration (μg)	Glucose utilization in HepG2 liver cells in the absence of insulin (%)	Glucose utilization in HepG2 liver cells in the presence of insulin (%)
0.625 μg	71 ± 0.04	73 ± 0.05
1.25 μg	76 ± 0.04	78 ± 0.01
2.5 μg	83 ± 0.03	86 ± 0.04
Positive control	95 ± 0.01	98 ± 0.02

The results are presented as the mean ± SEM; n = 3. Positive control: metformin and pioglitazone in the absence and presence of insulin, respectively.
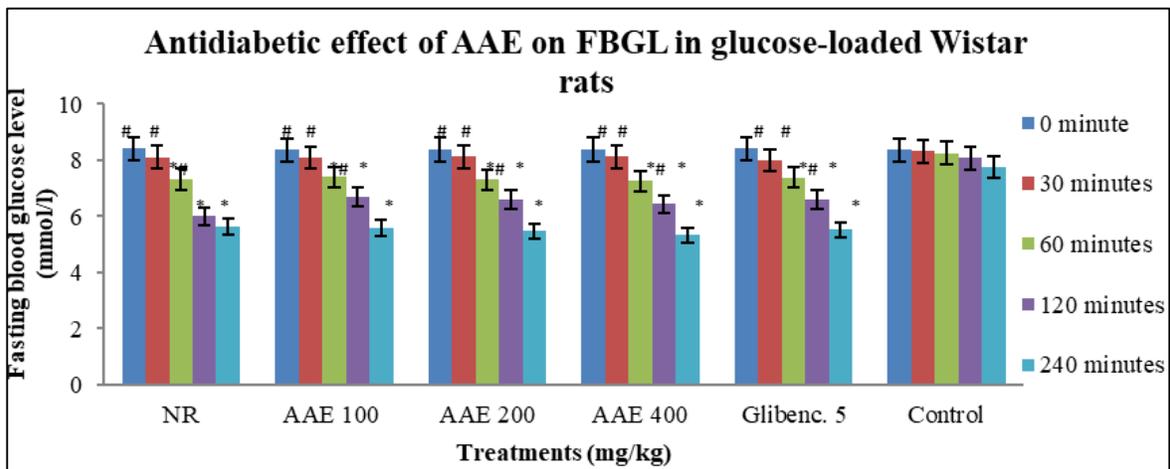
Figure 4. Effect of AAE on fasting blood glucose level (FBGL) in glucose-loaded Wistar rats. Values are given as mean ± SEM; n = 5. NR: Normoglycemic rats. Glibenc.: Glibenclamide. *$p < 0.05$ compared to the untreated diabetic rats (control); #$p < 0.05$ compared to FBGL at 240 min.
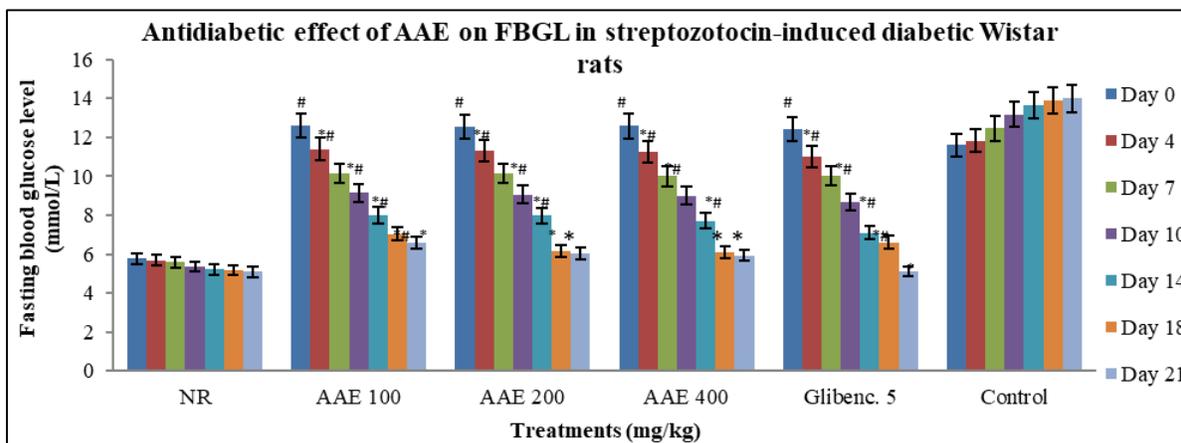
Figure 5. Effect of AAE on FBGL (mmol/l) in streptozotocin-induced diabetic Wistar rats. Values are given as mean ± SEM; n = 5. NR: Normoglycemic rats; Glibenc.: Glibenclamide. *$p<0.05$ compared to the control; #$p<0.05$ compared to FBGL on day 21.

Table 5: Effect of AAE on serum insulin and serum α-amylase levels.

Groups	Serum insulin (μIU/ml)	Serum α-amylase (U/l)
1 (NR)	2.31 ± 0.10*	52.09 ± 0.12*
2 (100 mg AAE)	1.30 ± 0.14*	18.21 ± 0.09*
3 (200 mg AAE)	1.56 ± 0.12*	23.48 ± 0.10*
4 (400 mg AAE)	1.62 ± 0.09*	29.91 ± 0.11*
5 (5 mg/kg glibenclamide)	1.91 ± 0.11*	38.99 ± 0.09*
6 (untreated rats; control)	0.69 ± 0.29	10.93 ± 0.16

Values are given as mean ± SEM; n = 5. NR: Normoglycemic rats. *$p<0.05$ compared to the control.

α-amylase and α-glucosidase are the digestive enzymes that function in the digestion of carbohydrate. These enzymes hydrolyze α-1,4-glycosidic linked polysaccharides and disaccharides to glucose [25–27]. The inhibition of α-amylase and α-glucosidase enzyme activities by the ethanolic extract of *A. adsecendens* root is in line with earlier reports on *Asparagus adscendens* rhizome aqueous extract in the literature. The study showed that *A. adscendens* rhizome aqueous extract causes a reduction in starch digestion by inhibiting α-amylase and α-glucosidase enzymes [13]. Inhibition of the activities of these two enzymes slows down the breakdown of carbohydrate and hence reduces the levels of glucose in diabetic condition [6]. The earlier reports indicated that inhibitors of α-amylase and α-glucosidase bind to the alpha bonds on polysaccharides, preventing the enzymes from hydrolyzing the bond [27,28]. Studies have reported that plant extracts possess α-glucosidase inhibitory activity due to the presence of alkaloids, terpenoids, flavonoids, and phenolic compounds [29]. Also, studies carried out on bioactive compounds from plants reported that plants with mostly phenolics and flavonoids have a correlation with a therapeutic agent for the treatment of diabetes [30,31].

RIN-5F and HepG2 cells were 33% and 32% viable, respectively, at the highest concentration of AAE (10 μg), whereas at lower mass values (below 2.5 μg), there was no significant change in the percentage of viable cells (Table 3). Also, the 50% inhibition concentration (IC$_{50}$) of AAE for the viability of RIN-5F and HepG2 cells was 7.49 μg/20 μl and 7.29 μg/20 μl, respectively. The AAE caused no mortality at up to 5000 mg/kg body weight (p.o.) in Wistar rats.

The pancreas is a vital organ that secretes the hormone insulin, which helps in the uptake of glucose by body cells, including

the liver, muscle, and fat cells [32]. In type 2 diabetic condition, the pancreas is either unable to produce insulin or the cells in the body are resistant to the insulin that is produced by the pancreas [33]. In this study, AAE stimulated RIN-5F pancreatic cells to secrete insulin and uptake of glucose in HepG2 liver cells, and this activity of AAE is similar to the report of previous studies reporting that *A. adscendens* rhizome aqueous extract stimulated BRIN-BD11 clonal pancreatic cells and glucose uptake in 3T3-L1 adipocytes cells [13]. In this study, the AAE also increased serum insulin levels in streptozotocin-induced diabetic rats. The AAE also reduced the FBGL from 30 min in glucose-loaded rats and from the 7th day onwards in streptozotocin-induced diabetic Wistar rats.

The pancreas also secretes the enzyme α-amylase, which catalyzes the digestion of starchy carbohydrate to glucose [25]. In the diabetic condition, the islets of the pancreas are damaged, and this affects the synthesis and release of α-amylase from the pancreas [34]. Recent clinical studies also reported that low serum α-amylase is observed when there is a high blood glucose level in type 2 diabetes, and this signifies a defect in the cells of the pancreas [34,35]. In this study, there was an increase in serum α-amylase levels in diabetic treated animals, and this further reveals the healing effect of the AAE on the pancreatic cells in diabetic condition.

A number of studies have indicated that the antidiabetic effect of natural plant extracts might be through different modes, including inhibition of α-amylase and α-glucosidase enzyme activities, stimulation of the pancreatic cells to produce more insulin, decrease in the liver glucose production, and increase in the sensitivity of the body cells to insulin [3,6,7]. In this study, various biochemical and cell-based assays were employed to identify the probable mechanism(s) of *A. adscendens* root antidiabetic effect. The results of this study suggest that the possible antidiabetic mechanism of action of AAE might be by inhibition α-amylase and α-glucosidase enzymes, stimulation of pancreatic cells to produce more insulin, and increasing the uptake of glucose by liver cells.

Conclusion

The present study indicated that AAE exhibited inhibition of α-amylase and α-glucosidase enzyme activities, induced insulin secretion in RIN-5F pancreatic cells, and enhanced glucose uptake in HepG2 liver cells. The AAE possessed a high lethal dose in Wistar rats (p.o.). The AAE also decreased FBGL and increased serum insulin and α-amylase levels in treated streptozotocin-induced diabetic Wistar rats. Future research on the isolation of the bioactive components might provide a therapeutic agent with antidiabetic activity.

Acknowledgments

Conflicts of interest

The authors declare that they have no conflict of interest.

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