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Research Article
In vitro and *in vivo* Modulation of Postprandial Hyperglycemia by *Solanum incanum* L. (Bitter Apple)

Kumarappan Chidambaram

Department of Pharmacology and Toxicology, College of Pharmacy, King Khalid University, Abha 61421, Saudi Arabia

Abstract

Background and Objective: *Solanum incanum* (Solanaceae) is widely distributed in the Asir Mountains of Abha, Saudi Arabia and for treating liver pain, diabetes and other ailments. The purpose of this study was to assess the hypoglycemic, α-amylase, DPP-IV and free radical scavenging effects of a hydroalcoholic extract of *S. incanum* (HAE-SI) using various *in-vitro* and *in-vivo* models. **Materials and Methods:** The HAE-SI was evaluated for total flavonoid and phenolic content as well as *in-vitro* free radical- scavenging (DPPH, ABTS) and antidiabetic (α-amylase and DPPIV) properties, using established protocols. The *in-vivo* hypoglycemic effect of HAE-SI on normoglycemic, glucose loaded (OGTT) and high fructose-diet (HFD) fed rats was also investigated. **Results:** The HAE-SI dosing at 300 mg kg^{-1} for 4 weeks effectively reduced fasting blood glucose ($p<0.001$) and body weights ($p<0.01$) in HFD-fed. This research suggests that HAE-SI has a hypoglycemic effect on rats fed a high fructose diet. According to the results from *in-vitro* assays, HAE-SI inhibits α-amylase and DPPIV with IC_{50} values of 309.78±3.87 and 327.09±5.32 μg mL^{-1}, respectively. The HAE-SI contains higher levels of total phenolics and flavonoids and it neutralised DPPH (IC_{50} 588.29±4.26 μg mL^{-1}) and ABTS (IC_{50} 963.76±4.63 μg mL^{-1}) radicals in a dose-dependent manner. **Conclusion:** The HAE-SI leaves from this study *in vivo* exhibited a dose-dependent hypoglycemic effect in OGTT and HFD-fed rats, *in vitro* α-amylase, DPP-IV inhibitory and antioxidant properties. Although the traditional claim of *S. incanum* is validated using *in-vitro* assays, extensive *in-vivo* research is needed to clarify its mechanism of action and isolate active constituents.

Key words: *Solanum incanum*, HAE-SI, free radicals, HFD, postprandial hyperglycemia, hypoglycemic, α-amylase, DPPI-IV

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Corresponding Author: Kumarappan Chidambaram, Department of Pharmacology and Toxicology, College of Pharmacy, King Khalid University, Abha 61421, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a chronic metabolic condition characterised by persistent hyperglycemia and it is the major cause of morbidity and mortality in the global[1]. It has complex pathogenesis with various vascular complications and pathogenic pathways in diabetic patients[2]. According to the World Health Organization, China and India are the major epicentres of global diabetes mellitus, whereas, Saudi Arabia is ranked seventh[3]. More than 400 genetic associations have recently been revealed because of a multifactorial disease, accounting for 18% of the risk[4,5]. Advances in the molecular understanding of diabetes mellitus pathogenesis enable a precision medicine approach to diabetes management. Hyperglycemia and insulin resistance have long been recognised as indicators for dysregulated carbohydrate metabolism and inefficient utilisation. Identifying novel regulators of beta-cell function and cellular glucose uptake could be a powerful therapeutic approach for diabetes. The focus should be on decreasing and targeting postprandial hyperglycemic (PPHG) therapy to improve diabete's overall clinical outcomes[6].

Glucose Transporter type 4 (GLUT4) is primarily responsible for glucose uptake in skeletal muscle via insulin-mediated AMPK phosphorylation and GLUT4 translocation[7]. The DPP-IV is a major proteolytic enzyme involved in the inactivation of regulating in cretin peptides (GLP-1) which are essential for insulin-mediated glucose utilisation and signalling pathways in peripheral tissues. The DPP-IV inhibitors have recently become a prominent focus in diabetes research to decrease PPHG by elevating incretin hormones and enhancing islet function. Several studies are presently undertaken to maintain incretin levels by inhibiting DPP-IV, which effectively treats type 2 diabetes in clinical trials. Furthermore, reduced carbohydrate absorption rate reduces postprandial hyperglycemia in diabetes and cardiovascular disease prevention and treatment[8].

Recently, selective amylase enzyme inhibitors have been found to control postprandial glucose levels. However, current clinical research on both enzymatic treatments found that DPP-IV and glycosidase inhibition is associated with gastrointestinal toxicity, cancer and other undesirable effects[9-11]. By altering carbohydrate metabolic pathways, plant-derived enzyme inhibitors (alpha-amylase and DPP-IV) could be an acceptable source for reducing postprandial hyperglycemia and increasing incretin levels[12,13]. As a result, a systematic discovery of enzyme inhibitors from plants is beneficial for developing novel antidiabetic therapies.

Solanum incanum L. (Solanaceae) has been commonly known as yellow-fruit nightshade and used as a traditional medicine for treating hepatitis, mycotic infection, diabetes mellitus, Snake envenomation and other ailments[14,15]. Several phytocompounds have been isolated and shown to have anticancer, hypoglycemic, antimicrobial and anti-ulcer activities[16-19]. The therapeutic activities of *S. incanum* have been attributed to their content of and related glycoalkaloids (solanine) and polyphenolic compounds[20]. It has been used as an alternative medicine for treating diabetic patients in Kenya, Ethiopia and other several countries[21,22]. However, the scientific rationale and evidence for this diabetic indication are limited. Hence, the current work used *in-vitro* and *in-vivo* combined methods to assess the antidiabetic efficacy of the aqueous extract from *S. incanum* leaves (HAE-SI).

MATERIALS AND METHODS

Study area: The study was performed at the Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia from January-June, 2020.

Plant collection: The fresh leaves of *Solanum incanum* were collected from the nearby area of King Khalid University, Grieger. Later the collected plant material was submitted for identification and authentication by a taxonomist/ pharmacognosist. The collected fresh leaves were further cleaned and washed with tap water and dried under shade for seven days. Further, the leaves were ground to coarse powder for hydroalcoholic extraction.

Preparation of hydroalcoholic extract of *S. incanum* (HAE-SI): The powdered plant materials were soaked in 70% ethanol and 30% distilled water for 3-4 days. The extract was filtered through muslin cloth and then passed through Whatman's filter paper (Merck, Darmstadt, Germany) to get clear filtrate. The excess solvent was removed with a rotary vacuum evaporator (BUCHI Rotavapor, Flawil, Switzerland) at 40°C. The resulting crude extract was stored in the refrigerator at -4°C and frozen until used for the experimental study.

Estimation of total flavonoid content: The content of flavonoids in the examined plant extracts was determined spectrophotometrically based on the earlier procedure with modifications[23]. About 1 mL of HAE-SI (1 mg mL^{-1} in methanol) was mixed with 1 mL of 2% $AlCl_3$ (Sigma-Aldrich, St. Louis, USA) solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using a spectrophotometer at $\lambda_{max} = 415$ nm.

The HAE-SI samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin (Sigma-Aldrich, St. Louis, USA) and the calibration line was construed. Rutin was used for constructing the standard curve (10-100 µL) ($Y = 0.0102X+0.0222$, $R^2 = 0.9979$) and the total flavonoid concentration in each extract was expressed as milligrams of rutin equivalent per 100 mg of dried extract (mg RE/100 mg).

Determination of total phenolic content: The total phenolic content of HAE-SI was determined using Folin-Ciocalteu's (Sigma-Aldrich, St. Louis, USA) method as stated earlier[24]. The gallic acid was used as the standard by which the stock gallic acid was made by mixing 10 mg of gallic acid in 10 mL of methanol (1000 µg mL^{-1}). The standard solution (250, 125, 62.5, 31.25, 15.62 and 7.81 µg mL^{-1}) was then prepared through serial dilution from the stock solution. The final volume of the standards was made into 2 mL with 1 mL of diluted solution and 1 mL of methanol. Then 0.5 mL from each gallic acid standard, plant extract (1 mg mL^{-1}) and methanol used to prepare the blank were pipetted into different test tubes. Then, 2.5 mL of 10% of Folin-Ciocalteu's reagent was diluted in water and 2.5 mL of 7.5% of $NaHCO_3$ (Sigma-Aldrich, St. Louis, USA) was added to the respective test tubes to make up the final volume to 5.5 mL. The sample and blank standards were then incubated for 15 min at 45°C using the water bath. This was followed by measuring the absorbance value at 765 nm using the UV-Vis spectrophotometer (Shimadzu UV1800, Kyoto, Japan). The sample was prepared in triplicate and the average value was then calculated. The total phenolic content present in the HAE-SI was expressed as milligram gallic acid equivalents (mg GAE/g of extract).

In-vivo-studies

Animals: Male Wistar albino rats (n = 120) weighing about 200-250 g per body weight were used in the present study (Procured from Central Animal House Facility, King Khalid University). Animals were collected from the breeding colony and acclimatised to the laboratory condition for 2 weeks. They were housed in macrolon cages under standard laboratory conditions (12 hrs light and 12 hrs dark cycle, 21±2°C and relative humidity 55-70%). During the experiments, the animals were fed with commercial diet from local suppliers and free access to water (*ad libitum*). Experiments were performed complied with the rulings of the OECD and the study was permitted by the institutional ethical committee (IEC) of the King Khalid University, Saudi Arabia.

Acute toxicity study: Under OECD Test Guidelines 425 (Up and Down Procedure), nulliparous and non-pregnant female Wistar rats weighing 150-200±4 g having ages 8 to 10 weeks were randomly selected[25]. Animals were kept under standard conditions for 5 days. The limit test was performed at 2000 mg kg^{-1} p.o., as a single dose. The rats were kept without food for 3-4 hrs before dosing but had access to water *ad libitum*. The dose was administered to a single rat according to body weight. The animals were closely observed for the first 30 min, then 4 hrs. Food was provided after 1-2 hrs of dosing. After the survival of the treated rat, five additional rats were administered with the same dose under the same conditions. The same procedure was followed for a vehicle-treated control group of 6 rats to whom distilled water (DW, 2 mL kg^{-1}) was administered in the same volume as the treated group. Both the groups were observed closely for any toxic effect within the first 6 hrs and then at regular intervals for 14 days. Surviving rats were observed to determine the onset of the toxic reaction.

OGTT and hypoglycemic activity assay in normal healthy rats: After the acclimatisation period, healthy Wistar albino male rats will be tested for OGTT and hypoglycemic effect[26]. Experimental rats were divided into 5 and 4 groups (n = 6), respectively, for OGTT and hypoglycemic studies (Table 1). Overnight fasted normal rats will be used in the experiment for OGTT and hypoglycemic studies. The animals were then given 2 g kg^{-1} of glucose orally for the OGTT assay. The oral glucose tolerance test was studied after oral glucose load for FBG levels at 0, 30, 60, 120 and 180 min after the administration of the HAE-SI and metformin using an Accu-Check OneTouch glucometer (Roche, Basel, Switzerland). For the hypoglycemic assay, FBG levels of all the groups were checked at 0, 30, 60, 120 and 180 after HAE-SI/metformin treatment.

Antihyperglycemic activity of HAE-SI in HFD treated rats: After the acclimatisation period, Wistar albino male rats will be made insulin resistant (pre-diabetic) by feeding 66% (w/w) fructose and 1.1% (v/w) coconut oil mixed with a normal rat pellet diet (NRPD) for 4 weeks. The high fructose diets (HFD) will be prepared fresh daily and fed to rats ad libitum (Fig. 6). The experimental rats will be divided into five groups of 6 each and treated as follows:

- **Group 1:** NRPD-fed rats received water (2 mL/kg/b.wt./day, p.o., for 4 weeks)
- **Group 2:** HFD-fed rats received (2 mL/kg/day, p.o., for 4 weeks)

Table 1: Experimental design of oral glucose tolerance test (OGTT) and hypoglycemic assay

Treatment	Effect on OGTT	Hypoglycemic assay
Group 1	NRPD-fed (Water 2 mL kg^{-1}, p.o.)	NRPD-fed (water 2 mL kg^{-1}, p.o.)
Group 2	Glucose-fed (3 g kg^{-1}, p.o.)	HAE-SI (150 mg kg^{-1}, p.o.)
Group 3	Glucose-fed+HAE-SI (150 mg kg^{-1}, p.o.)	HAE-SI (300 mg kg^{-1}, p.o.)
Group 4	Glucose-fed+HAE-SI (300 mg kg^{-1}, p.o.)	Metformin (100 mg kg^{-1}, p.o.)
Group 5	Glucose-fed+Metformin (100 mg kg^{-1}, p.o.)	-

- **Group 3:** HFD-fed rats treated with HAE-SI (150 mg/kg/b.wt./day, p.o., for 4 weeks)
- **Group 4:** HFD-fed rats treated with HAE-SI (300 mg/kg/b.wt./day, p.o., for 4 weeks)
- **Group 5:** HFD-fed rats treated with pioglitazone (Sigma-Aldrich, St. Louis, USA) (2 mg/kg/b.wt./day, p.o., for 4 weeks)

All the animals will have fasted for half an hour before drug administration. During the experimentation, blood glucose levels will be measured on the 1st, 7th, 14th, 21st and 28th days after fasted using an Accu-Chek OneTouch glucometer (Roche, Basel, Switzerland). Blood will be collected by tail vein puncture from anesthetized rats in fasting conditions[27]. The body weight will be measured at weekly intervals for 4 weeks and the feed and water will be given *ad libitum* during the experiment.

DPPIV inhibitory assay: The DPPIV inhibitory activity of Sitagliptin and the extract of HAE-SI leaves were tested in a DPPIV inhibitor screening assay kit *in-vitro* as stated in protocol[28]. In brief, the assay was performed in half volume 96-well white plate. The 100% initial activity wells, background wells and inhibitor wells were set up by adding the different volumes of reagents into the wells correspondingly. Triplicates were done for each sample. The mixture reaction was initiated by adding 50 µL of diluted substrate solution to all the wells being used. The plate was incubated for 30 min at 37°C. Finally, the fluorescence was measured using an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.

α-amylase inhibition assay: The α-amylase inhibitory activity was measured according to the previously described methods with some modifications[29]. A 200 µL of sample solution (0.2 mg of sample or acarbose in 20 mM sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride) was premixed with 200 µL of α-amylase (Sigma-Aldrich, St. Louis, USA) solution (0.5 mg mL^{-1} in the pH 6.9 buffer) and incubated at 25°C for 10 min. After pre-incubation, 400 µL of a 0.25% starch solution in the pH 6.9 buffer was added to each tube to start the reaction. The reaction was carried out at 37 for 5 min and terminated by adding 1.0 mL of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then incubated in a water bath at 60°C for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm using a UV-Vis spectrophotometer. The control had 200 µL of buffer solution in place of the α-amylase solution. The % inhibition was calculated by:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Protein denaturation assay: Protein denaturation assay was done according to the previously described method with some modifications as described by Mirshafie *et al.*[29]. The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine serum albumin (Sigma-Aldrich, St. Louis, USA) and 4.78 mL of phosphate-buffered saline (PBS, pH 6.4) and 0.02 mL of PE-EVOO/indomethacin and the mixture was mixed. It was incubated in a water bath (37) for 15 min and then the reaction mixture was heated at 70 for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Biorad). The phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula[30]:

$$\text{Inhibition of denaturation (\%)} = \frac{1 - A_2}{A_1} \times 100$$

Where:

A_1 = Absorption of the control sample

A_2 = Absorption of the test sample

DPPH scavenging activity: The HAE-SI's DPPH free radical scavenging activity measured hydrogen donating or radical scavenging ability using the stable radical DPPH with slight modifications[31]. About 0.2 mM solution of DPPH in methanol was prepared and 2.0 mL of the solution was added to 3.0 mL of *Coptidis rhizoma* extract solution in water at different concentrations ranging from 7.81-1000 µg mL^{-1}. After 30 min,

the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu). Methanol is used as blank. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of DPPH radical scavenging activity of the positive control and test sample was calculated.

$$\text{Radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100$$

Where:

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of positive control or test sample

ABTS radical decolourisation assay: The ABTS radical scavenging activity was determined using the ABTS radical decolourisation assay with slight modification[32]. The ABTS·+ stock solution was prepared by dissolving 0.0768 g of ABTS with 0.0132 g of $K_2S_2O_8$ in 20 mL of ultrapure water. The stock solution was then kept in the dark area at room temperature for 16 hrs. Before usage, the ABTS+solution was dissolved in PBS (1:100 v/v) (pH 7.4) to obtain the absorbance of 0.700±0.004 at 734 nm. Then 1 mL of gallic acid which is used as positive control (0.02, 0.03, 0.06, 0.12, 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63 and 31.25 μg mL^{-1}) and plant extract (7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 μg mL^{-1}) was mixed with 2 mL of diluted ABTS·+. Immediately after that, the absorbance was measured at 734 nm. The control contains all the reagents except for gallic acid and plant extract. The ABTS radical scavenging activity was determined using the same formula as the DPPH scavenging activity.

Statistical analysis: All statistical analysis will be made using the software GraphPad Prism (Version 7.0) for windows. All results will be expressed as mean±SEM. The statistical difference between means will be analyzed using a One-way Analysis of Variance (ANOVA) followed by a post hoc Dunnett's test to determine statistical significance. The values will be considered statistically significant when $p<0.05$. The results were expressed as Mean±SED obtained from three independent analysis. Statistical significance of the differences was evaluated using Student's t-test for *in vitro* antioxidant assay.

RESULTS

A linear calibration curve for gallic acid was constructed in 0-250 g mL^{-1} with an R^2 value of 0.9985. Extrapolating from the graph, HAE-SI contains a considerable amount of phenolic metabolites in 15±0.0996 mg GAEs/g dry weight of leaves. The total flavonoid content of the HAE-SI, calculated from the calibration curve (R^2 = 0.9935), was 769±0.032 mg REs/g dry weight of leaves. The results from a 14-day acute toxicity study on HAE-SI suggested that a single 2000 mg kg^{-1} (limit dose) of the extract is devoid of any adverse effects in rats (classified as Category 5 as per OECD, LD_{50} is >2000 mg kg^{-1}). The current study also suggests that HAE-SI leaf extract may be a safe potential candidate for further antidiabetic investigations (Table 2). However, a further sub-acute/chronic study using various dosages and repeated dosing must be conducted to ascertain the pathological, biochemical and haematological parameters that HAE-SI affects.

Table 2: Effect of HAE-SI on body weight (g) general characteristics and behavioural observations at 14 days using OECD-425 methods in Wister albino rats

	Body weight (g)	
Days	Control (DW 2 mL kg^{-1})	HAE-SI (2000 mg kg^{-1})
0 day (initial)	272.00±4.68	276.33±4.27^{NS}
7th day (week 1)	271.12±7.82 (1.3%)	279.33±8.6^{NS} (0.33%)
14th day (week 2)	273.28±6.25 (1.12%)	279.45±5.94^{NS} (0.44%)
	General characteristics and Behavioral observations on 14 days	
Observations		
Body weight	No Significant change	No Significant change
Food intake	Normal	Normal
Water intake	Normal	Normal
Changes in skin/fur	Normal	Normal
Urination/colour	Normal	Normal
Faecal consistency/diarrhoea	Normal	Normal
Drowsiness/sedation	Not observed	Not observed
General physiques	Normal	Normal
Epilepsy/convulsions	Not observed	Not observed
Coma	Not observed	Not observed
Mortality	Not observed	Not observed

DW: Distilled water, NS: Not significant vs. Control, values are expressed as Mean±SEM (n = 6) and student's t-test (unpaired test)

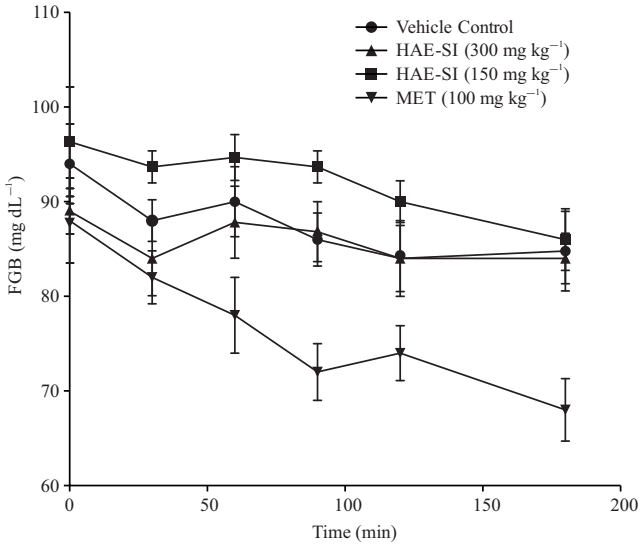

Fig. 1: Effect of HAE-SI on Fasting blood sugar of normoglycemic rats

Data were analysed by One-Way Analysis of Variance (ANOVA), and Dunnett's t-test was used as the significance test, $p<0.05$* (300 mg kg^{-1}) and $p<0.001$** (MET 100 mg kg^{-1}) were considered significance compared to the vehicle-treated group. Data are the Mean±SEM of 6 rats per group. One way ANOVA followed by Dunnett's multiple comparison test was applied

In Fig. 1 shows the hypoglycemic action of HAE-SI on FBG levels in normoglycemic rats. The HAE-SI did not have a significant hypoglycemic action at 100 mg kg^{-1}. At 90 min of oral administration, a dose of 300 mg kg^{-1} of HAE-SI resulted in a modest reduction in FBG ($p<0.05$). Nevertheless, after 3 hrs of HAE-SI treatment, a rise in glucose level was detected. However, metformin's standard treatment significantly lowered FBG in most periods ($p<0.001$). It is evident from the findings that HAE-SI (150 and 300 mg kg^{-1}) had a moderate hypoglycemic effect on FBC in normoglycemic rats when compared with metformin. In Fig. 1 demonstrates the findings of OGTT studies on healthy rats. After 1 hr, oral loading of 2 g kg^{-1} glucose resulted in hyperglycemia and increased FBG. At 90 min, Group 2) in OGTT-grouped rats, the hypoglycemic effects of HAE-SI (150 and 300 mg kg^{-1}) and metformin (100 mg kg^{-1}) were investigated. The FBS of the vehicle-treated group was statistically ($p<0.01$**) greater than other treatment groups after 90 min of glucose feeding. At 60 and 90 min, pretreatment with HAE-SI at a 300 mg kg^{-1} dose effectively reduced glucose-load induced hyperglycemia (Fig. 2). However, compared to other groups of rats, the FBS of the 300 mg kg^{-1} HAE-SI treated group was not significant. After 180 min, the mean FBG level in most of the groups declined significantly to near normal. Furthermore, metformin greatly reduced FBG at all time points from the peak hyperglycemic state compared with other treatments ($p<0.01$**).

The effect of HAE-SI on fasting blood glucose levels and body weight in normal and experimental rats is shown in Fig. 3 and 4, respectively. After 4 weeks of the HFD diet, the fasting blood glucose levels were elevated to 26.11%, compared to 5.87% in the NRPD group. Oral administration of HAE-SI at 150 and 300 mg kg^{-1} for 4 weeks resulted in decreased fasting plasma glucose rises of 18.78 and 14.84%, respectively, compared to HFD-treated rats. Compared to the NRPD-fed rats (2.58%), the HFD-fed rats had a considerable increase in body weight (35.56 %) after 4 weeks of treatment. The increase in body weight caused by HFD feeding was reduced to 20.26 and 7.28% after 4 weeks of HAE-SI treatment at 150 and 300 mg kg^{-1}, respectively. Nevertheless, the overall percentage reduction in the rise of FBG levels and body weight of MET (100 mg kg^{-1}) treatment with HFD fed rats was significant compared with all other treatment groups.

The α-amylase inhibitory activity of HAE-SI was analysed in different concentrations (7.5-960 μg mL^{-1}), where acarbose was used as a positive control. The results of the α-amylase inhibitory effect have been illustrated in Fig. 5. The IC_{50} for the HAE-SI was estimated at 309.78±3.87 μg mL^{-1}, compared to acarbose (262.47±2.89 μg mL^{-1}) as the positive standard. Moreover, the HAE-SI was found to be a moderate inhibitor of DPPIV with an IC_{50} value of 327.09±5.32 μg mL^{-1}, while Sitagliptin showed the strongest inhibitory activity at141.15±3.95 μg mL^{-1}. The DPPIV inhibitory activity of HAE-SI and Sitagliptin is shown in Fig. 6.

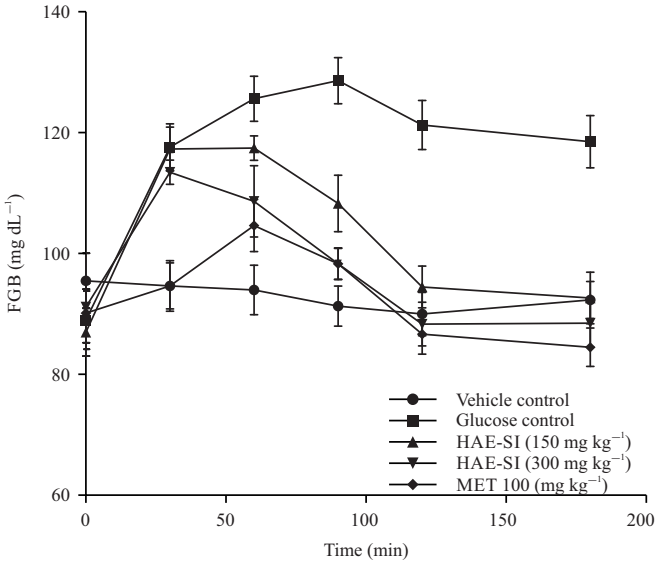

Fig. 2: Effect of HAE-SI treatment on the oral glucose tolerance curve during acute treatment

Glucose solution (2 g kg^{-1} b.wt.) was administered orally, and fasting blood glucose concentrations were measured at different time intervals (0-180 min). Data are the Mean±SEM of six rats per group, *p<0.05 (300 mg kg^{-1}) and **p<0.01 (MET 100 mg kg^{-1} were considered significant compared to the glucose loaded group. One way ANOVA followed by Dunnett's multiple comparison test was applied

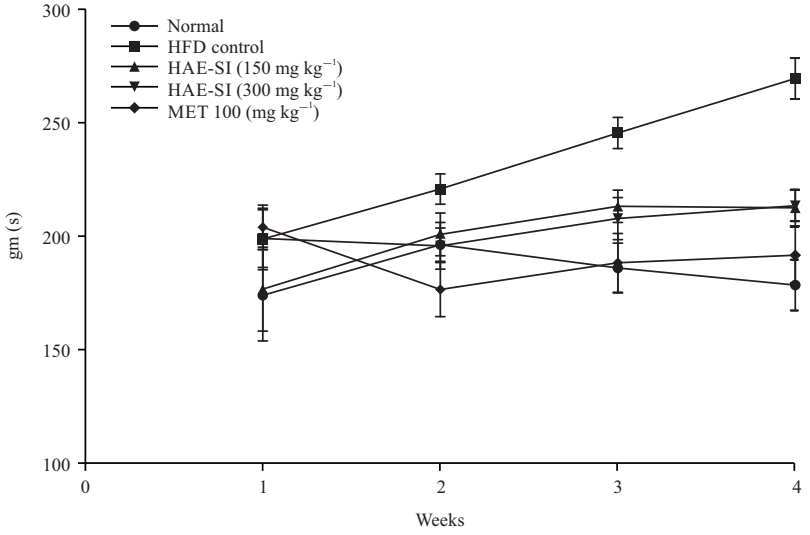

Fig. 3: Effect of HAE-SI treatment on bodyweight indices of rats fed with HFD for 4 weeks of treatment

Data are the Mean±SEM of six rats per group, *p<0.05 (300 mg kg^{-1}) and **p<0.01 (MET 100 mg kg^{-1} were considered significant compared to the HFD treated group and One way ANOVA followed by Dunnett's multiple comparison test was applied

The HAE possessed a weak anti-inflammatory effect on heat-induced protein denaturation compared to the standard anti-inflammatory drug diclofenac sodium. The DPPH scavenging potential of the GA and HAE-SI at varying concentrations, ranging from 7.81-1000 µg mL^{-1}, was measured and the results are depicted in Fig. 7. Sigmoidal calibration curves of GA and HAE-SI were constructed and the IC_{50} values of scavenging DPPH radicals for GA and HAE-SI were 210.60±9.24 and 588.29±14.62 µg mL^{-1}, respectively. The ABTS radical-scavenging abilities of HAE-SI and gallic acid were tested at the concentration range of 7.81-1000 µg mL^{-1} using the ABTS cation assay. In Fig. 8 depicts the ABTS radical scavenging effect of HAE-SI, which shows the half-maximal inhibitory concentration of ABTS-scavenging capacity is 963.76±4.63 µg mL^{-1}. However, the IC_{50} values of ABTS radical-scavenging activity of GA were found to be

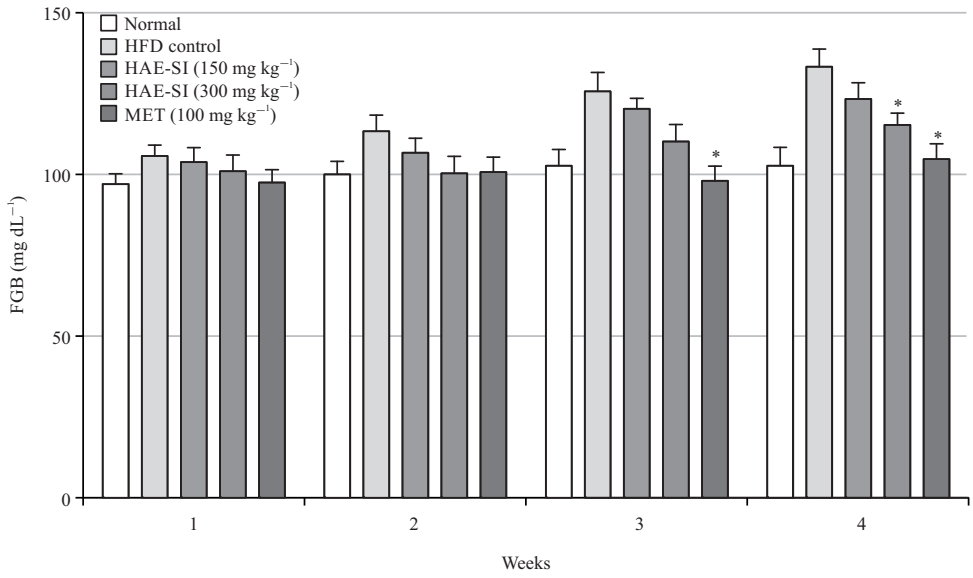

Fig. 4: Effect of HAE-SI treatment on fasting blood glucose of rats fed with HFD for 4 weeks of treatment

Data are the Mean±SEM of six rats per group, *p<0.05 (300 mg kg^{-1}), *p<0.01 (MET 100 mg kg^{-1} were considered significant compared to the HFD treated group at weeks 3 and 4 and One way ANOVA followed by Dunnett's multiple comparison test was applied

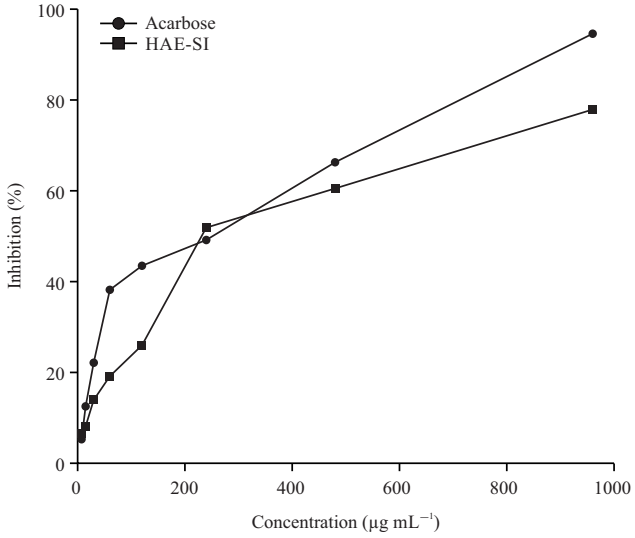

Fig. 5: *In vitro* alpha-amylase inhibitory effect of HAE-SI and acarbose at various concentrations

Values represent the means (SEM) of 3 independent experiments performed in triplicates

137.41±3.87 µg mL^{-1}. The half-maximal inhibitory concentration of HAE-SI against various *in-vitro* assays is shown in Table 3.

DISCUSSION

The T2DM (Type 2 Diabetes Mellitus) is a global health concern with complex pathogenesis that results in cardiometabolic syndrome illnesses and is a major therapeutic challenge[33]. Long-term complications of diabetes mellitus are mainly driven by persistent hyperglycemia and impaired glucose tolerance. Traditional antidiabetic medicines are linked to potential drug interactions and cardiovascular side effects, prompting researchers to investigate for natural compounds to treat diabetes mellitus.

The *S. incanum* is traditionally used with multiple therapeutic applications for various ailments in several African countries[34]. The current experiment was performed based on

Table 3: Summary of half-maximal inhibitory concentration (IC_{50}±SEM) of HAE-SI and reference standards against various *in-vitro* assays

In vitro assay	IC_{50} for HAE-SI (µg mL^{-1})	IC_{50} for HAE-SI (µg mL^{-1})
DPPH scavenging assay	588.29±4.26	210.60±2.94
ABTS discolouration assay	963.76±4.63	137.41±3.87
α-amylase inhibitory assay	309.78±3.87	262.47±2.89
DPP-IV inhibitory assay	327.09±5.32	141.15±3.95

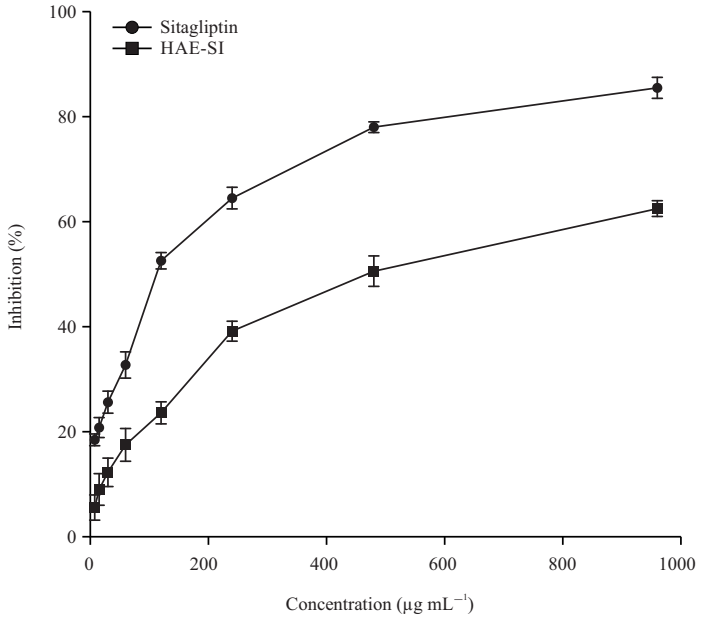

Fig. 6: *In vitro* DPP-IV inhibitory effect of HAE-SI and Sitagliptin at various concentrations
Values represent the means (SEM) of 3 independent experiments performed in triplicates

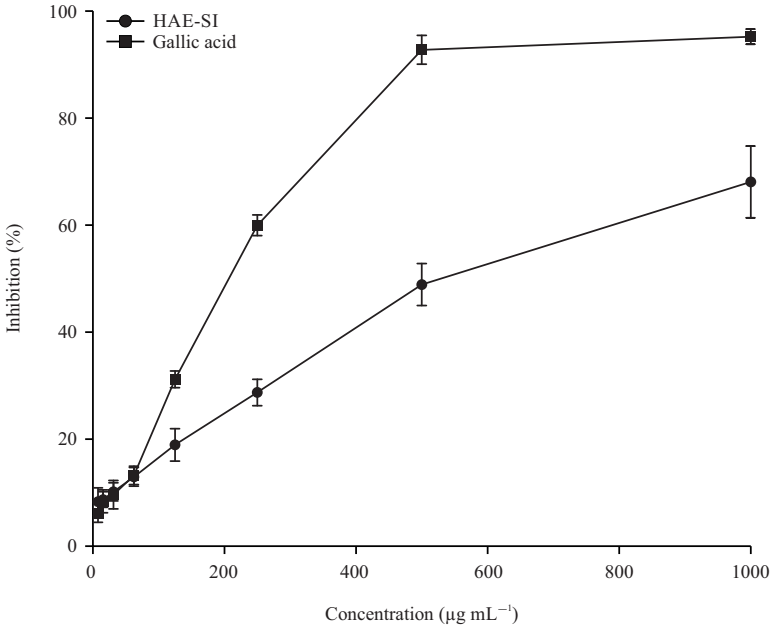

Fig. 7: Percentage of DPPH radical scavenging effect of HAE-SI and gallic acid at various concentrations (7.81-1000 µg mL^{-1})
Gallic acid in 50% methanol served as a positive control and values are means of 3 replicates±SEM

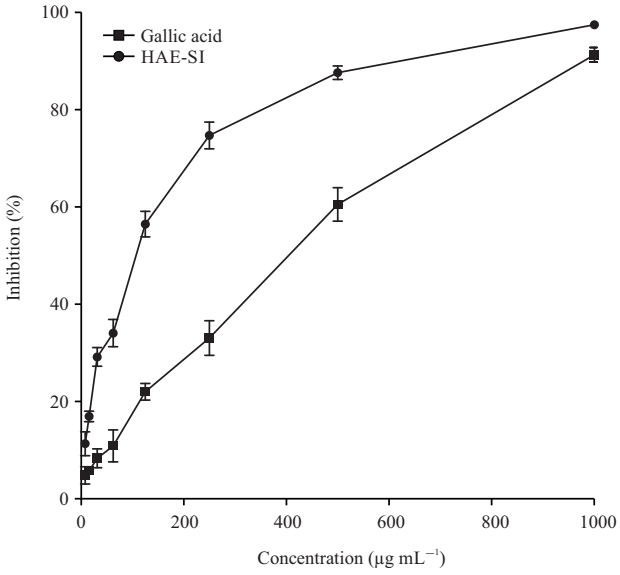

Fig. 8: Percentage of ABTS+radical scavenging effect of HAE-SI and gallic acid at various concentrations (7.81-1000 µg mL^{-1})
Gallic acid in 50% methanol served as a positive control and Values are means of 3 replicates±SEM

ethnopharmacological claims of *S. incanum* to ascertain the hypoglycemic effects and thus validate its traditional use against diabetes mellitus. In diabetes mellitus, the OGTT is an important diagnostic test for determining insulin release and function[35]. The oral treatment with HAE-SI improved hyperglycemia state and glucose tolerance in both normoglycemic and glucose-loaded rats. However, the hypoglycemic effect was not as significant as in the metformin-treated rats.

Increased consumption of high-fructose diets has been strongly associated with insulin resistance, abnormalities in glucose metabolism and metabolic syndrome development. Furthermore, an excessive fructose diet may impair the liver by causing oxidative stress, insulin resistance, inflammatory and metabolic abnormalities[36,37]. According to our findings, a high-fructose diet resulted in a significant increase in fasting blood glucose levels and body weight. The HAE-SI treatment for 4 weeks reduced blood glucose levels which a high-fructose diet had increased. Thus, treatment with HAE-SI increases glucose tolerance and prevents hyperglycemia in high-fructose diabetic rats.

Another popular pharmaceutical method for controlling diabetes and its complications is to inhibit alpha-amylase and DPPIV enzyme activity from reducing postprandial hyperglycemia. Numerous plant-based enzyme inhibitors have been shown to decrease postprandial hyperglycemia and could be used to treat diabetes[13,38]. Targeting alpha-amylase may reduce chronic postprandial hyperglycemia and slow the progression of diabetes mellitus by delaying glucose absorption at the intestinal brush barrier. Similarly, inhibiting DPP-IV could increase insulin secretion by prolonging the half-life of GLP-1, providing it with a promising target for diabetes treatment. The HAE-SI effectively inhibits alpha-amylase and DPP-IV might decrease postprandial blood glucose spike in a dose-dependent mechanism. In alloxan-treated diabetic rats, the aqueous extract of *S. incanum* elicited a significant reduction in postprandial blood glucose at various time intervals, which supports our findings and its traditional use[21,39].

Excessive oxidative stress caused by reactive oxygen species and other free radicals can result in various inflammatory disorders and diseases[40]. These reactive oxygen species (ROS) are major contributing important factors to the development of diabetes and play a significant role in diabetic complications[41]. Natural antioxidants derived from plants are currently getting prominence as a strategy to overcome excessive ROS and its toxic effects on cells. Several species of the *Solanum* genus have been found to have antioxidant properties, many have been shown to have antidiabetic properties in animal studies[42]. Flavonoids and polyphenolic compounds, which are key sources of natural antioxidants, were quantified in abundance in this investigation on HAE-SI. The presence of polyphenolic chemicals has a strong antioxidant effect against various ROS due to their phenolic contributing nature. Our results show that HAE-SI has a dose-dependent free radical scavenging effect on $DPPH^{-}$ and $ABTS^{+}$

radicals due to its ability to donate electrons via phenolic groups. However, a further detailed study on HAE-SI is warranted to evaluate its antioxidant and hypoglycemic effect using an animal model of diabetes mellitus.

These findings imply that HAE-SI is a more effective hypoglycemic drug, nevertheless, more research is needed to determine the efficacy of long-term treatment in animal models of insulin resistance and the safe dose in chronic toxicity models. The *S. incanum* bioactive compounds quantification and isolation using a variety of separation approaches would have provided a possible mode of action for HAE-SI.

CONCLUSION

In conclusion, the HAE-SI has a hypoglycemic effect by lowering postprandial glucose levels via increasing glucose uptake and inhibiting alpha-amylase and DPPI-IV enzymes. Furthermore, due to phenolic compounds in the leaves, HAE-SI has a significant free radical scavenging activity. As a result, the findings of this study confirm its traditional uses and suggest that it could be a promising therapeutic agent in the treatment of diabetes mellitus. Further mechanistic investigation on animal models of diabetes mellitus and the isolation of bioactive compounds from HAE-SI are enormously essential to prove its pharmacological potential.

SIGNIFICANCE STATEMENT

Inhibition of key glucose metabolism-regulating enzymes and suppression of HFD-mediated postprandial hyperglycemia may contribute to the anti-diabetic benefits of *S. incanum*. However, additional long-term research is needed on whether *S. incanum* can be used effectively to manage diabetes. The antioxidant and free radical scavenging effects of HAE-SI leaves extract *in vitro* demonstrated that it has potential as a natural antioxidant source. The *S. incanum*'s high content of polyphenols or flavonoids as well as their numerous phenolic hydroxyl groups, scavenge free radicals and may be effective to prevent oxidative stress-mediated disorders of vital organs. These findings would support researchers to further investigate the possible use of the S. incanum leaves for the treatment of diabetes.

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