Possible role of a compound identified from *Pericampylus glaucus* plant in controlling blood glucose in experimental animals

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Objective
The aim of the present research work was to determine the possible mechanism of an active fraction identified from *Pericampylus glaucus* plant in controlling blood glucose in experimental animals.
Materials and methods
The effect of an active fraction from *P. glaucus* on intestinal absorption of glucose at a dose of 50 mg/kg (body weight) was investigated using an in-vivo method. The identification of the compound was carried out with gas chromatography mass spectrometry followed by ^{1}H nuclear magnetic resonance. The active fraction and standard drug were given daily for 7 days. The animal groups were anesthetized through intraperitoneally injection with sodium pental at a dose of 40 mg/kg (body weight) after they were made to fast overnight to evaluate the effect of the active fraction on the absorption of glucose in comparison with the diabetic group.
Results and discussions
A single dose of 50 mg/kg (body weight) of the active fraction and standard drug acarbose produced significant ($P<0.001$) attenuations in the intestinal absorption of glucose as compared with the diabetic group. The amount of glucose absorbed was 94.05±0.9 mg/g tissue (weight) in the diabetic group, whereas that absorbed in the active fraction-treated group was 55.80±1.2 mg/g tissue (weight). However, the animals treated with standard drug acarbose showed a significant reduction in glucose absorption rate at 38.21±2.5 mg/g tissue (weight). The inhibition of glucose absorption by standard acarbose and active fraction was 59.37 and 40.66%, respectively, as compared with the diabetic control group. The gas chromatography mass spectrometry analysis indicated a single compound propanoic acid in plant sample. The molecular weight was 234, ^{1}H nuclear magnetic resonance was 452 MHz, %area was 3.57, retention time was 2.990, and total area was 1 015 462. The molecular formula was $C_9H_{22}O_3Si$ at *m/z* 73 (high resolution). The presence of this compound in *P. glaucus* might be responsible for the inhibition of glucose absorption by blocking Na^+, K^+ ATPases activity.
Conclusion
The present experiment confirmed reduced glucose absorption rate and the antidiabetic activity is due to this possible mechanism.

Keywords:
^{1}H nuclear magnetic resonance, acarbose, glibenclamide, glucose absorption, *Pericampylus glaucus*

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Introduction

Diabetes mellitus is a chronic metabolic hereditary disorder, which is characterized by variations in the metabolism of carbohydrates, protein, and fats that result in rise in blood sugar level [1]. All over the world, 382 million of population are suffering from diabetes and it will affect almost 592 million in 2035 [2]. Malaysia is also being counted among those countries having a large number of people suffering from diabetes. The Malaysian diabetic populations have become almost double in a duration of two spans from 1985 to 2006 [3]. To manage diabetes mellitus without any adverse effect still remains a challenge to all medical healthcare specialists. In recent times, treating diabetes mellitus with drugs available in the market has shown an extensive progress, but still remains problematic to manage the postprandial hyperglycemia as compared with fasting hyperglycemia. It has been confirmed by many researchers that various diabetic complications along with coronary heart diseases are connected with postprandial hyperglycemia [4]. Until the present time, the search for novel drugs for the treatment of diabetes mellitus still continues; because of the presence of several

limitations of allopathic drugs [5], there are decreases in the response of individuals to drugs over a long period of use [6]. The plant-based medicines may give a valuable source of novel drug for the ailment of hyperglycemia and provide a new way for the advancement of pharmaceutical entities to the present remedies. The ethnobotanical reports indicate that about 800 of natural products on this planet may have antihyperglycemic prospects [7], but with inadequately understandable mechanisms. Recently, many scientists in the field of diabetic research are investigating various plants to derive novel drugs from traditionally used plants and may provide a new possibility in the modern healthcare system.

The plant *Pericampylus glaucus* belongs to the family of Menispermaceae and is commonly distributed throughout Malaysia [1]. The plants' leaf extract was scientifically investigated for antidiabetic activity in streptozotocin (STZ)-induced high-fat diet diabetic rats [1]. Previously, the plant was investigated against excessive free radicals, cancer, AIDS, hepatitis B virus, and hepatitis C virus [8,9]. Therefore, the present study is an attempt to determine the possible mechanism of the ethanolic extract of *P. glaucus* on reducing blood glucose level using the in-vivo method and identification of the active compound.

Materials and methods

Drugs, chemicals, and apparatus

Acarbose, glibenclamide, sodium pental, and silica gel mesh size (230–400) were purchased from Santa Cruz (Santa Cruz Biotechnology, Inc., 10410 Finnell Street Dallas, Texas 75220 USA) and Sigma-Aldrich (3050 Spruce St. Louis, MO 63103, Missouri, United States) Bio Syn Tech Malaysia Group. The chemicals used were from Astral Laboratory Chemicals (Taren Point, New South Wales, Australia). The column used for column chromatography was supplied from local supplier of Malaysia.

Instruments

The instrument used for the present study was gas chromatography mass spectrometry (GCMS) (GC model 2014 and GCMS-TQ8050, S/N E11484913004, P/N 221-70020-34, GC-2014 AFC 230V; Shimadzu), UV (model 1800, S/N A11454908357, P/N 206-25400-38; Shimadzu; Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, Maryland, USA), and ^{1}H nuclear magnetic resonance (NMR) (JNM-ECZS series, FT-NMR; JEO USA Inc., Peabody, Massachusetts, USA).

Test animals

Adult Sprague–Dawley rats with an average weight of 90–110 g were used. The experimental protocol used was approved by the Lincoln University College, Malaysia, Animal Ethics Committee with reference LUC-AEC number PHARM/2013/MSM/02-November/11/November 2014–October 2016. All animals were made to fast overnight before experiment and were given free excess to water.

Collection of the plant material

The plant *P. glaucus* was collected from village Kampung Jeram Kedah, Negeri Sembilan state of Malaysia in 2014.

Identification of the plant

The plant was authenticated from Forest Research Institute Malaysia by Ms Tan Ai Lee, with specimen herbarium number FRIM/394/490/5/18 (118), deposited to the faculty of pharmacy as a source of reference. The leaves were dried in shade for a period of 20 days and converted into coarse powder with the help of mechanical grinder.

Extraction of plant material

The coarse powder of *P. glaucus* was extracted using the continuous hot extraction method with the soxhlet apparatus at a temperature of 78°C using ethanol solvent for a period of 48 h. The extract was then concentrated under reduced pressure through Rotary evaporator (n-10000 Eyela, Tokyo Rikakikai Co., LTD., Japan) and was preserved in a desiccator for further isolation of active fraction.

Phytochemical screening

The phytochemical constituents in *P. glaucus* ethanolic extract were previously screened using the chemical identification test [9] before identification of compound and determination of possible mechanism.

Identification of active fraction

The antidiabetic activity of ethanolic plant extract was previously investigated scientifically in STZ-induced high-fat diet diabetic rats [1]. Therefore, ethanolic extract (20 g) was subjected to fractionation to collect the active fraction as was reported by Kifayatullah *et al.* [10] with some changes in procedure. The column chromatography having silica gel (230–400 mesh size) as stationary phase in various solvents was used. The ethanol plant extract was combined with an equivalent amount of 20 g of silica gel and dried completely [11]. Slurry was prepared from silica gel (230–400 mesh size) in *n*-hexane solvent and was loaded into three-fourths of the total column. Once

the slurry became stable (no breakage), the column was effectively eluted from end to end through different solvents system, starting from nonpolar *n*-hexane followed by chloroform, petroleum ether, ethyl acetate, acetone, and ethanol in different concentration ratios with some modifications in procedure [12]. Four main fractions were collected and were nominated as fractions A, B, C, and D.

Acute toxicity study

In a previous research work, plant extract was investigated for acute and subacute toxicity study [13], and was found safe up to a dose of 4000 mg/kg (body weight). Therefore, a single dose of 100 mg/kg (body weight) for the various fractions and 50 mg/kg (body weight) for the active compound were chosen for the present research work.

Effect of various fractions on blood glucose level in streptozotocin-induced diabetic rats

The effect of various fractions was investigated for antidiabetic activity according to the method used by Islam *et al.* [14] with some changes in procedure. The rats were divided into six groups of four animals each. The first group was the untreated diabetic control group. The second group was the standard group and received glibinclamide at a dose of 20 mg/kg (body weight), whereas groups three to six received various fractions (A, B, C, and D) at a dose 100 mg/kg body weight. After the administration through oral route, blood samples were taken from the tail vein at 0, 2, 4, 6, and 24 h and the total amount of glucose in blood was determined with the help of the glucose oxidase–peroxidase method using glucose standard reactive strips and was compared with the untreated diabetic control group [15,16].

Effect of active fraction on intestinal glucose absorption

The effect of active fraction of *P. glaucus* on intestinal glucose absorption was determined in STZ-induced diabetic Sprague–Dawley rats according to the method used by Das and Barman [17] with slight modifications. The STZ-induced diabetic animal group was divided into three groups of five animals each. The first group was the diabetic control group. The second group was the standard group, treated with acarbose at a dose of 2 mg/ml (oral). The third group was the tested group and was given orally active fraction at a dose of 50 mg/kg (body weight). The active fraction, standard and normal saline, was given daily as a single dose for 7 days. On the eighth day of treatment, all treated rats were treated with thiopental sodium [40 mg/kg (body weight) for inducing anesthesia. The abdomen of each animal was opened and, from the pyloric part, an intestinal loop of about 10 cm was made. Care was taken when making the intestinal loop to ensure normal supplies. Thereafter, 1 ml of 0.25 g% d-glucose in normal saline was introduced into the intestinal loop with the help of tuberculin syringe at 37°C. The animals were killed after an absorptive period of 15 min and the loop from each was removed. The excised loop was weighed after removing the fat and mesentery, and cut open to recover the fluid left after absorption. The glucose absorption was calculated as a difference between the total amount of glucose introduced into the lumen and the total amount recovered. The absorption was expressed in mg/g dry weight of the tissue. The dry weight of the tissue segment was measured after dehydrating it with 95% ethyl alcohol for 24 h, followed by drying for 2 h at 120°C in the hot air oven.

The amount of glucose absorbed by the intestine was calculated using the following formula [18]:

$$\text{Amount of glucose absorbed(mg/g tissue weight)} = \frac{G_{\text{before}} - G_{\text{after}}}{W_{\text{intestine}}},$$

where, G_{before} is the glucose concentration before incubation period, G_{after} is the glucose concentration after incubation period (15 min), and $W_{\text{intestine}}$ is the weight of intestinal segment after drying.

Identification of compound

The active fraction was further subjected to the identification, characterization, and structural determination of compound using GC chromatogram, MS spectra, and ^{1}H NMR spectroscopic techniques [19]. The name, molecular weight, and structure of the compound were determined.

Statistical analysis

Statistical analysis was performed as mean of variance±SEM (N=4), followed by the analysis of variance test using Graph Pad Prism (Graph Pad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA), and, for multiple comparison test among the groups, the Bonferroni test was used. A probability level of $P<0.05$ was accepted statistically as significant.

Results and discussion

At present, the investigations on plant-based medicines are developing rapidly, as a portion of drug innovation. The previous research approved the attenuation effect

of ethanolic extract of *P. glaucus* on blood glucose level in STZ-induced high-fat diabetic rats. This research work to determine the effects of various fractions on blood sugar level in single low dose of STZ- induced diabetic rats was not performed previously. In order to confirm the previous research, here, an attempt was made to investigate the effect of various fractions of Pericampylus glaucus on blood glucose levels, effect of active fraction on absorption of glucose in intestine for understanding the possible mechanism of glucose lowering in animal models and the identification of the active compound.

The effects of Malaysian native *P. glaucus* plant on blood glucose levels and on intestinal glucose absorption was investigated *in vivo*.

Effects of various fractions on blood sugar level in streptozotocin-induced diabetic rats

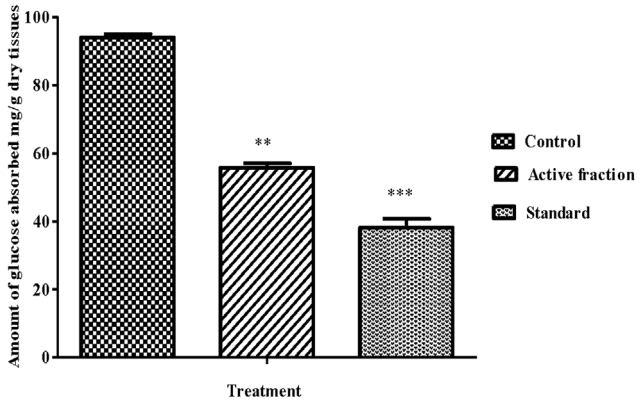
The effects of various fractions on blood glucose level in experimental animals are presented in Table 1. There was no significant ($P>0.05$) difference in blood glucose level in the animal group that received various fractions for the first 2 h of treatment. Fraction B reduced the blood glucose level significantly ($P<0.01$) from 16.20 ±0.30 mmol/l to 14.75±0.64, 13.75±0.55, and 12.85 ±0.15 mmol/l at 4, 6, and 24 h as compared with other fractions and the untreated diabetic group. The fraction D also produced a slight attenuation in blood glucose level significantly ($P<0.01$) at 24 h of treatment as compared with fractions C and A, but was less than that produced by fraction B. There was no significant ($P>0.05$) attenuation in blood glucose level in the animal group that received fractions A and C. The percentage of reduction of blood glucose level was 14.33 and 19.20% at 6 and 24 h after receiving fraction B at a dose of 100 mg/kg (body weight) as compared with the control group. The percentage of reduction of blood glucose level was 12.20% at 24 h after receiving fraction D compared with the untreated diabetic control group. The attenuation in blood glucose level produced by different fractions of *P. glaucus* was found to be less than that produced by standard drug glibenclamide ($P<0.001$) at a dose of 20 mg/kg (body weight, oral).

Data were expressed as mean±SEM; the number of animals in each group was 4. *P*-value of less than 0.01 was considered significant as compared with the normal control group. The statistical test used was the analysis of variance test, followed by the Bonferroni test.

Effects of active fraction on intestinal glucose absorption in streptozotocin-induced diabetic rats

The effects of active fraction and glibenclamide on absorption of glucose in the intestine in experimental animals are summarized in Table 2 and graphically in Fig. 1. The active fraction and standard drug acarbose produced an effective attenuation in glucose absorption compared with diabetic controls. The results showed that the active fraction at a dose of 50 mg/kg (body weight) for 7 days caused significant ($P<0.01$) attenuations in the absorption of glucose in comparison with the diabetic control group. The amount of glucose absorbed in the small intestine was 55.80±1.2 mg/g tissue weight in the animal group receiving the active fraction. The animals that were treated with the standard drug acarbose at a dose of 2 mg/ml caused more significant ($P<0.001$) (38.21±2.5 mg/g tissue) attenuations in glucose absorption compared with the diabetic and active fraction-treated group. There was a

Figure 1



Effect of active fraction and acarbose on inhibition of glucose absorption in small intestine. Data are expressed as mean±SEM, $N=5$. Statistically significant $^{**}P<0.01$, significant as compared with controls.

Table 1 Effect of various fractions of *Pericampylus glaucus* on blood glucose level in streptozotocin-induced diabetic rats

Treatments	Time (h) after administration of various fractions on blood glucose level (mmol/l)				
	0	2	4	6	24
Fraction A (100 mg/kg body weight)	16.45±0.45	15.85±0.15	15.45±0.15	15.05±0.05	14.90±0.01
Fraction B (100 mg/kg body weight)	16.20±0.30	15.55±0.55	14.75±0.64	13.75±0.55 (14.33%)**	12.85±0.15 (19.20%)***
Fraction C (100 mg/kg body weight)	16.80±0.40	15.85±0.15	15.35±0.15	14.95±0.05	14.60±0.15
Fraction D (100 mg/kg body weight)	16.40±0.44	15.75±0.25	15.15±0.04	14.50±0.19	13.90±0.20 (12.30%)**
Glibenclamide	16.05±0.44	15.35±0.65	13.85±0.85*	12.55±0.44 (21.80%)***	11.45±0.15 (29.20%)***
Diabetic control	16.20±0.69	15.65±0.15	15.55±0.15	16.05±0.05	15.85±1.25

*$P<0.05$, **$P<0.01$, ***$P<0.001$.

significant difference in the absorption of glucose ($P<0.05$) between the active fraction (*P. glaucus*) and acarbose-treated groups. The inhibition of glucose absorption produced by the standard drug-treated animal group was 59.37% compared with the diabetic control group. The active fraction-treated animal group caused an inhibition of glucose absorption in small intestine reaching 40.66% as compared with the diabetic control group. The active fraction was effective in reducing intestinal glucose absorption but was less than the effect produced by standard acarbose. The effect of active fraction and standard drug on intestinal glucose absorption was determined on the jejunum part that was about 10 cm in length from the pyloric end of the treated animals and revealed that the duodenum and the upper jejunum have the highest ability to absorb sugars in the intestine [20]. The animal group that was treated with active fraction caused significant ($P<0.01$) diminishes in glucose absorption as compared with the diabetic group. The attenuation in absorption of glucose through the intestine may be caused by inhibiting specific sodium/glucose-linked transporters, an intestinal glucose transporter that is situated in the mucosal of the small intestine [21]. Thus, it may be suggested that the reduction in absorption of glucose by active fraction of *P. glaucus* is due to the inhibition of SGLT1 transporter, and thus delays the process of absorption of glucose in the intestine. The amount of glucose absorbed in the small intestine through the active fraction of *P. glaucus* was less as compared with the diabetic-treated group. It has been confirmed by many researchers that a decrease in absorption of glucose is due to a decrease in sodium gradient leading to a significant inhibition or decrease in $Na^+/K^{+/}$ATPase activity, and thus leads to depolarization of the cell and secretion of insulin [22]. The previous study has reported the presence of various phytochemical constituents in *P. glaucus* extract, and hence it may be suggested that the reduction in absorption of intestinal glucose by *P. glaucus* is due to the inhibition or decrease in $Na^+/K^{+/}$ATPase activity due to the presence of these compounds [23]. The amount of glucose inhibition produced by active fraction indicates that *P. glaucus* contain certain compounds, which are responsible for increasing the uptake of glucose by activating 5′-AMP-activated protein kinase in skeletal muscle and adipose tissues [24]. The present in-vivo test findings also suggest that the antihyperglycemic activity of *P. glaucus* was due to the suppression of postprandial hyperglycemia through the inhibition of the intestinal glucose transporter 2 [25].

Table 2 Effect of active fraction and standard drug on intestinal glucose absorption in streptozotocin-induced diabetic animals

Groups	Treatment	Glucose absorption (mg/g tissue)
Diabetic group	Normal saline	94.05±0.95
Tested	Active fraction (50 mg/kg body weight)	55.80±1.2*
Standard	Acarbose (2 mg/ml (oral)	38.21±2.5***

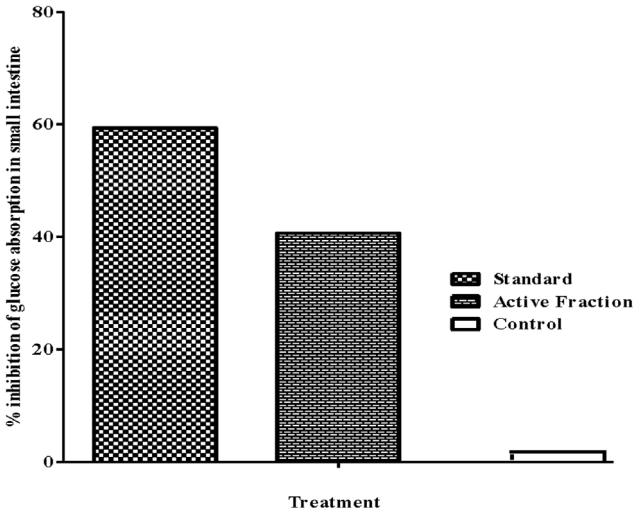
Values are expressed as mean±SEM, $N=5$. *$P<0.05$, significant as compared with control; statistical test used was the one-way analysis of variance test, followed by the Bonferroni test. ***$P<0.001$.

Identification and structure elucidation of compound

The active fraction collected from column chromatography through petroleum ether and ethyl acetate solvent system was subjected to identification, characterization, and structural determination using various spectroscopic techniques. The GCMS and ^{1}H NMR spectral data indicate single compound, which was propanoic acid. The International Union of Pure and Applied Chemistry name was propanoic acid, 2-[(trimethylsilyl)-oxy]-, trimethylsilyl ester. The compound was identified on ^{1}H NMR at 452 MHz; the molecular weight was 234, retention time was 2.990, % area was 3.57, and the total area of the compound was 1 015 462. The molecular formula of the compound was $C_9H_{22}O_3Si$ and was determined using high-resolution E IMS at m/z 73. The MS spectrum, molecular formula, molecular weight, and molecular structure of the compound are shown in Fig. 2.

The GC chromatogram followed by mass spectra and ^{1}H NMR showed the presence of propanoic acid, 2-[(trimethylsilyl)-oxy]-, trimethylsilyl ester in the active fraction of *P. glaucus*. The propanoic acid

Figure 2



Mass spectrums the identified compound.

compound was approved for antioxidant activity and hence the possible action of the compound on inhibition of intestinal glucose absorption and hyperglycemia due to its free radical scavenging activity.

The literature review study indicates that research has approved the role of propanoic acid in scavenging of free radicals in body and the antiproliferative properties that was isolated from plant *Rheedia brasiliensis* [26].

The present research strongly suggests that the presence of propanoic acid in active fraction might be strongly responsible for the inhibition of the membrane Na^+, K^+ ATPases activity that are responsible for providing the driving force for glucose into intestinal epithelium.

Conclusion

It is concluded from the present research work that the active fraction significantly decreases the intestinal absorption of glucose and hence justified the traditional use of plant against high levels of blood glucose due to reduction of intestinal absorption of blood glucose. The GC/MS followed by ^1H NMR indicates the presence of a single compound in active fractions. However, further research is needed to determine other possible mechanisms that are responsible for lowering of blood glucose level.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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