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Original article

The antidiabetic effect of methanolic extract of *Holarrhena pubescens* seeds is mediated through multiple mechanisms of action

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ABSTRACT

Holarrhena pubescens is widely used in Indian and Chinese medicine in the treatment of diabetes. The current work determined the oral hypoglycemic and antidiabetic effects of seed extract in rats. The probable mechanism of action was evaluated *in-vitro* by α - glucosidase inhibition, glucose metabolism in insulinoma (INS-1) cells to reflect secretion of insulin, and protein glycation inhibition. Its potential for herb-drug interaction was evaluated in the cytochrome P450 3A4 (CYP3A4) inhibition assay. The seed extract increased serum insulin levels and reduced serum blood glucose levels in the oral glucose tolerance test. It also reduced the serum glucose levels in streptozocin-induced diabetes. The extract also inhibited α –glucosidase enzyme activity and demonstrated that it can increase the secretion of insulin from INS to 1-rat insulinoma cell line cells *in-vitro* in a concentration-dependent manner. However, it had a very weak inhibitory effect on protein glycation and it did not affect the activity of CYP3A4. The results of the study showed that *H. pubescens* seed extract increases insulin secretion and inhibits glucose absorption both *in-vivo* and *in-vitro* with a weak protein glycation inhibitory effect. The herb is devoid of CYP3A4 inhibitory effect indicating that it may not have pharmacokinetic interaction with the drug metabolized by this enzyme.

1. Introduction

Holarrhena pubescens Wall. ex G. Don, (Family-*Apocynaceae*) commonly known as Tellicherry bark (English) is a medicinally significant plant native to Africa and Asia's tropical and subtropical areas(Zahara et al., 2020). It is also available in the Middle East where it is popularly known as '*the tongue of the bird*'. *H. pubescens* is widely used in traditional medicine in the treatment of gastrointestinal disorders (Jamadagni et al., 2017). The herb has been reported for several pharmacological effects such as antipyretic, antidysentery, antidiarrheal, anthelmintic, antibacterial, and antiamoebic effects (Jamadagni et al., 2017). It is also known to act as a memory enhancer (Ali et al., 2011a).

Plant products are widely used for the management of diabetes either alone or as supplements with modern drugs (Ansari et al., 2022; Bindu and Narendhirakannan, 2019). *H. pubescens* is used traditionally used in the treatment of diabetes (Ocvirk et al., 2013). There are several reports on the antidiabetic effect of this herb on streptozocin-induced diabetes (Ali et al., 2011a; Hegde et al., n.d.; Keshri, 2012; Kumar and Yadav, 2015; Pathak et al., 2015; Sheikh et al., 2013). Another study found that a hydromethanolic seed extract of *H. pubescens* inhibits glucosidase (Ali et al., 2011b).

Several drugs are available in the market for the management of diabetes and each of these drugs acts through different mechanisms ranging from prevention of glucose absorption, increasing insulin secretion, and enhancing glucose transport into the cells (DeFronzo et al., 2019).

Despite the wide use of this plant in the management of diabetes traditionally, the pharmacological studies reported so far have several flaws. All the studies in diabetic rats were conducted after the induction of diabetes by a single dose of streptozocin which results in the loss of all pancreatic cells in about 5 days and is a model for type-1 insulin-dependent diabetes mellitus (Deeds et al., 2011). Though the administration of nicotinamide

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before streptozocin to induce diabetes in adult rats is reported to protect some pancreatic insulin-secreting cells, none of the reported studies on *H. pubescens* administered nicotinamide (Furman, 2021). The herb is used traditionally in the management of only type-2 diabetes. None of the studies so far have attempted to identify the phytoconstituents and finally and no attempt was made to understand the mechanism(s) of antidiabetic action of this widely used herbal medicine.

The present study was carried out to confirm the antidiabetic effect of traditionally used *H. pubescens* seeds for the treatment of type-II diabetes. The study determined the hypoglycemic and insulin-secreting effect of the methanolic extract of this herb on oral glucose tolerance test and type-2 diabetes in rats. The mechanisms by which this herb reduces blood glucose levels were also determined by evaluating its effect on different physiologic mechanisms involved in glucose homeostasis such as secretion of insulin, glucose metabolism, α-glucosidase enzyme inhibition, and protein glycation. Since antidiabetic herbs are used along with oral hypoglycemic agents, the effect of *H. pubescens* seed on CYP3A4 inhibition was also determined. An attempt was also made to identify the chemical constituents present in the *H. pubescens* seeds through LC-MS analysis to identify probable chemical constituents contributing to the hypoglycemic and antidiabetic effects.

2. Methodology

2.1. Materials

H. pubescens seeds were purchased online and it was authenticated by a botanist. A voucher specimen is preserved in the college (CAMS/CLS/002–2022). Chemicals and reagents were purchased from different suppliers and these were of analytical or HPLC grade.

2.2. Animals

Albino in-bred Wistar rats were used. The methods and protocols were reviewed and approved by the Ethical Research Committee of Shaqra University (ERC_SU_20220023).

2.3. Preparation of extract and liquid chromatography-mass spectrometry (LC-MS) analysis

Dried seeds of *H. pubescens* were coarsely powdered and extracted using methanol by Soxhlation. The yield was around 11.54% w/w of the seeds.

An LC-MS analysis was carried out using XEVO-TQD#QCA1232 (Waters) with a C_{18} column (SUNFIRE C18, 250 X 2.1, 2.6 µm). The mobile phase consisted of two solvents; solvent A- acetonitrile and solvent B- ammonium formate and gradient elution was used. The column temperature was set at 30 °C with a pressure of minimum bar 0 and maximum bar 300. The detection was done at 280 nm. The gradient conditions are given in Table 1.. The mass spectra were recorded in negative and positive ionization modes between *m*/*z* 150 and 2000.

Table 1
Gradient conditions for LC-MS analysis.

Time	A%	B%	C%	D%	Flow
0.00	0.0	5.0	0.0	95.0	1.500
1.00	0.0	5.0	0.0	95.0	1.500
6.00	0.0	30.0	0.0	70.0	1.500
12.00	0.0	60.0	0.0	40.0	1.500
16.00	0.0	60.0	0.0	40.0	1.500
20.00	0.0	80.0	0.0	20.0	1.500
26.00	0.0	5.0	0.0	95.0	1.500
30.00	0.0	5.0	0.0	95.0	1.500

Solvent A- acetonitrile and solvent B- ammonium formate.

2.4. Oral glucose tolerance test

Male rats were fasted for 16 h and blood glucose level was determined (time 0). Following this, animals were administered orally with extract at two different doses of 250 and 500 mg/kg. Glibenclamide (10 mg/kg) was used as the standard drug. After 30 min of drug administration, a 20% glucose solution (2 g/kg body weight) was given orally. Blood samples were collected at 5, 10, 15, 30, 60, and 120 min after glucose administration. Serum was separated and used for the estimation of insulin and glucose levels. The glucose levels were estimated calorimetrically using a commercial kit (Invitrogen, Cat. No. EIAGLUC, Thermo Fischer Scientific, India), and the insulin was estimated using a rat insulin ELISA kit (Invitrogen, Cat. No. ERINS, Thermo Fischer Scientific, India).

2.5. Streptozocin-induced diabetes in neonatal rats

Albino wistar rat pups that were 5 days old were injected with streptozocin (90 mg/kg) by intraperitoneal route (Nivitabishekam et al., 2009). They were kept with their mothers and were provided with water and feed *ad libitum*. In the 6th week, male animals were selected and fasted for 12 hand glucose levels in the serum were estimated. Animals with serum glucose levels above 150 mg/dl were selected.

The animals were treated as follows; the first group of animals was aged-matched non-diabetic normal animals. The second group was diabetic control and received 0.1% sodium carboxymethylcellulose (vehicle), the third group received the standard drug; glibenclamide at a dose of 10 mg/kg while the fourth and fifth groups received *H. pubescens* extract at 250 mg/kg, *p.o* and 500 mg/kg *p.o*. The treatment continued for 28 days with the determination of blood glucose levels at 7-day intervals.

2.6. α - glucosidase inhibition

The α-glucosidase inhibitory activity was performed using a set of Eppendorf's tubes with extract concentrations of 31.25 µg/ml, 62.5 µg/ml, and 125 µg/ml. To the tubes, potassium phosphate buffer, *H. pubescens* seed extract, and α-glucosidase (1.2 EU/ml) were added at the volumes of 600 µl, 100 µl, and 25 µl respectively followed by incubation at 37 °C for 15 min. Following this, 4-nitrophenyl-β-D- glucopyranoside (25 µl) was added and incubated for another 15 min at 37 °C. For the termination of the reaction, sodium carbonate (750 µl) was used. A blank was prepared by adding the reagents in reverse order and a control without the sample was also prepared. The absorbance was measured at 405 nm using a microplate reader (#EC800, Biotek). The experiments were conducted in triplicates (Akocak et al., 2021; Lolak et al., 2020; Taslimi et al., 2021). The percent inhibition of enzyme activity was calculated by the following formula:

$$\%\ inhibition = \left\{\frac{Mean\ OD\ of\ untreated\ control - mean\ OD\ of\ test\ samples}{Mean\ OD\ of\ untreated\ control}\right\} \times 100$$

2.7. Glucose metabolism in INS-1-rat insulinoma cells to reflect insulin secretion

A 200 µl of INS-1-rat insulinoma cell line cell (Sigma-Aldrich) was suspended in RPMI medium (#AT150, Himedia) with 5% fetal bovine serum ((#RM10432, Himedia). This was seeded onto a

96-well plate at a cell density of 8,000 cells/well. The cells were allowed to grow overnight followed by washing with Dulbecco's phosphate buffer saline (PBS). Serum-free media was added followed by incubation for 3 h. The extract was added at different concentrations and PBS-treated cells were used as a negative control. The plates were incubated for 48 h at 37 °C in a 5% CO_2 atmosphere, with or without glucose (20 mM). Following this, the spent media was taken out and Eagle's minimal essential medium (100 µl) (#AL007A, Himedia) containing 10% fetal calf serum and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (# 4060, Himedia) at a concentration of 0.5 mg/ml was added. The mixture was again incubated at 37 °C for 30 min without exposure to light. A solubilizing agent (dimethyl sulfoxide-100 µl) was added after removing the MTT reagent. The experiments were conducted in triplicates and optical density was determined at 540 nm.

2.8. Protein glycation inhibition

Gelatin (100 mg/50 µl) was prepared in distilled water (5 ml). This was added to black microplates containing glyceraldehydes solution(10 µl) that was prepared by dissolving glyceraldehyde (222 mg) in distilled water (5 ml). After sealing, the plates were incubated for 24 h at 37 °C. Following this, extract (40 µl) was added at different concentrations of 31.25 µg/ml, 62.5 µg/ml, and 125 µg/ml. Negative and positive controls were prepared using distilled water and aminoguanidine (#396494, Sigma) (100 µM) respectively. A 370 nm for excitation and 440 nm for emission were used to measure fluorescence by a microplate reader (MultiscanGo - Thermo). The inhibition (%) was calculated using relative fluorescence unit (RFU):

$$\%\, inhibition = \left\{ \frac{Mean\ RFU\ of\ untreated\ control - mean\ RFU\ of\ test\ samples}{Mean\ RFU\ of\ untreated\ control} \right\} \times 100$$

2.9. CYP3A4 inhibition activity

Seed extract at concentrations of 31.25 µg/ml, 62.5 µg/ml, and 125 µg/ml was used. Ketoconazole, (230 µM) served as positive control. The CYP3A4 inhibition assay described by Sagbo *et al.*, was used (Sagbo et al., 2018).

Statistical analysis: All values are given as mean ± SEM and the number of animals/replicates is given in the footnote under the figures/tables. The statistically significant difference was assessed by using ANOVA followed by Tukey's test using SPSS (version 22 for windows).

3. Results

3.1. LC-MS analysis of the extract

The mass spectra chromatogram in positive and negative mode is given in Figs. 1 and 2 respectively. The LC-MS analysis revealed the presence of 14 different suspected molecules in the positive mode and 12 chemicals in the negative mode (Table 2 and Table 3).

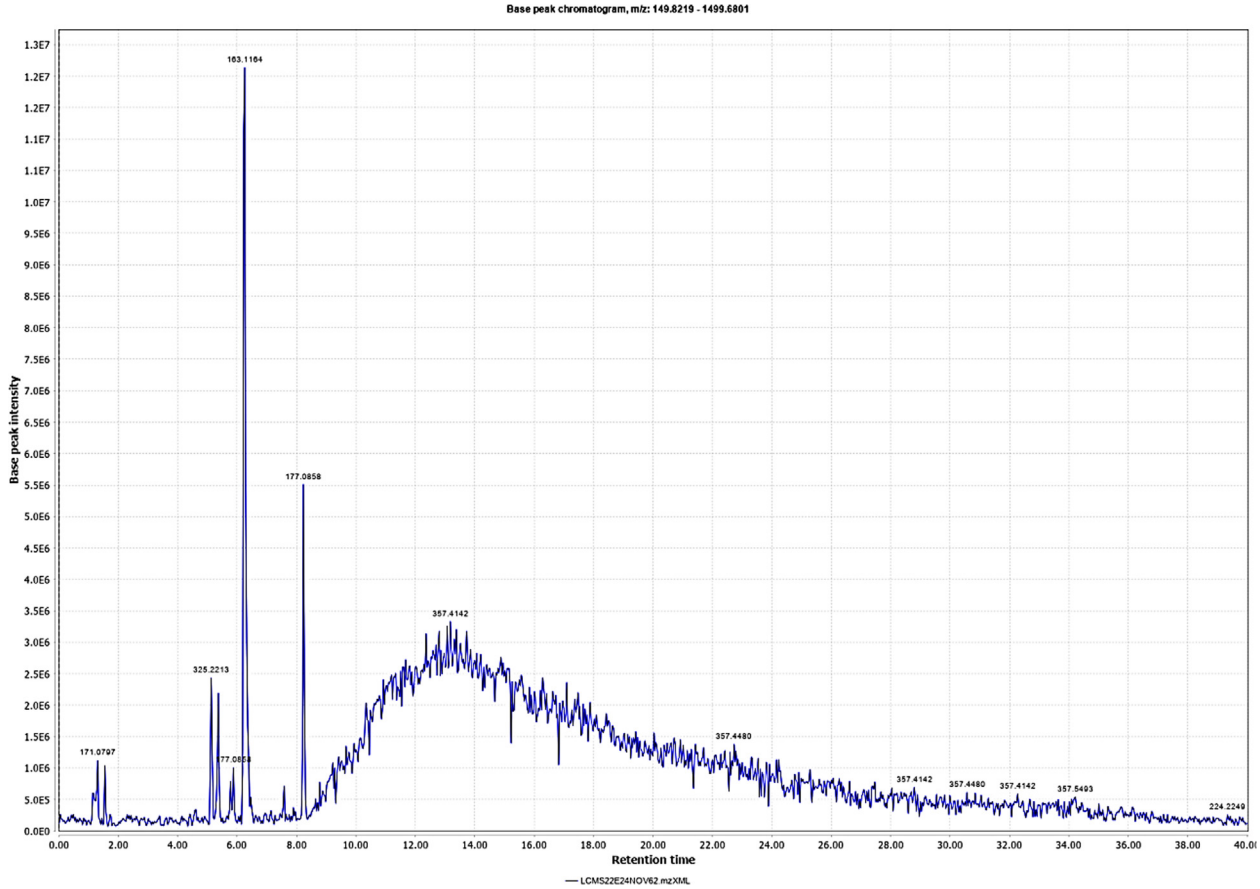

Fig. 1. Chromatogram in positive mode.

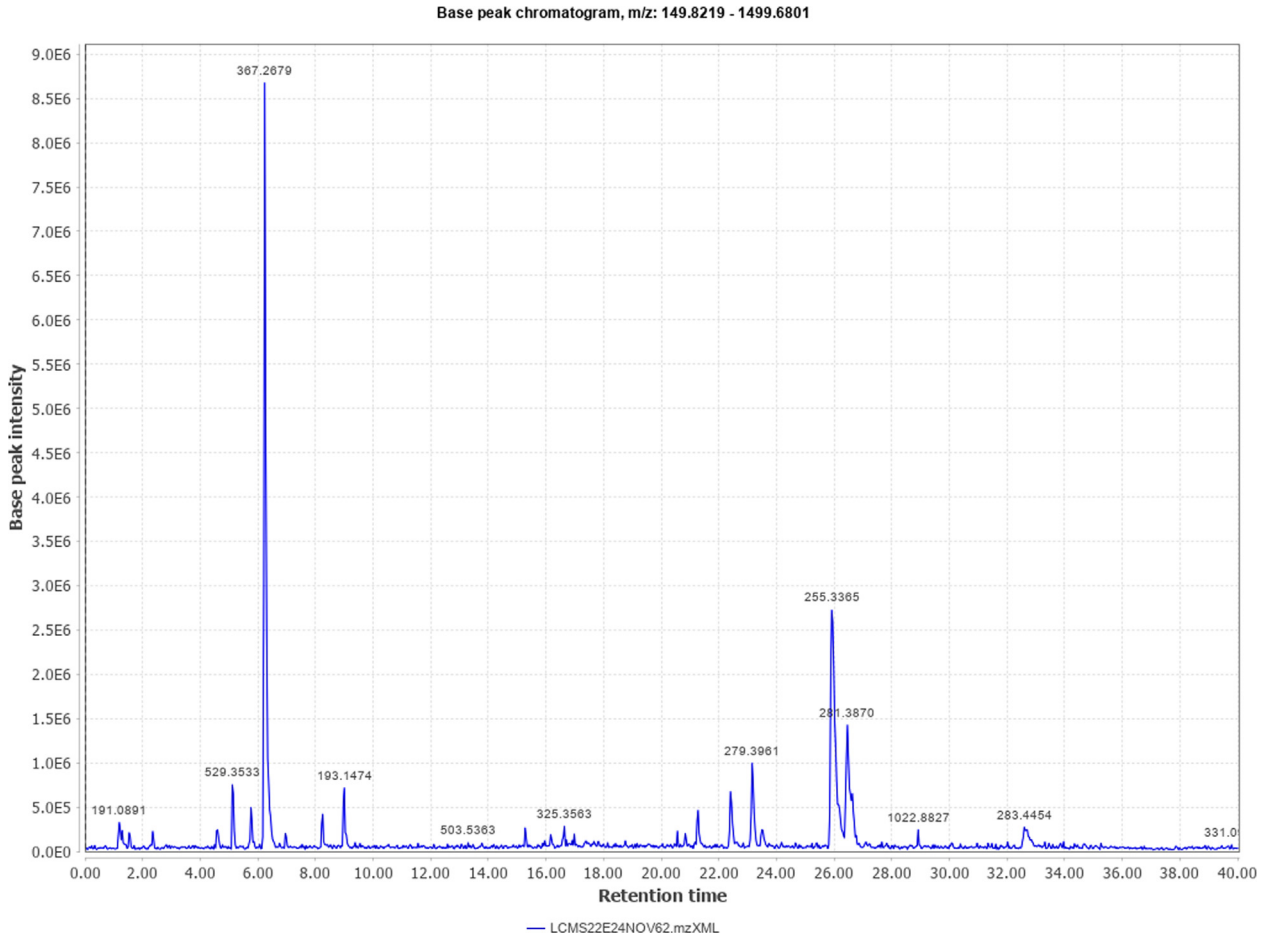

Fig. 2. Chromatogram in negative mode.

Table 2
List of suspected molecules in positive mode.

No.	R.Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Diff
	5.13	0.748	Scoulerin	positive	$C_{19}H_{21}NO_4$	327.147	325.2213	1.93
	5.37	0.816	7-Hydroxy-4-methylcoumarin	positive	$C_{10}H_8O_3$	176.047	175.1288	0.92
	5.88	0.65	Fusaric acid	positive	$C_{10}H_{13}NO_2$	179.094	177.0858	2.01
	15.84	0.827	4-(Methylsulfinyl)butylglucosinolate	positive	$C_{20}H_{41}NO_4$	359.303	357.4480	1.86
	18.43	0.411	Esculin sesquihydrate	positive	$C_{16}H_{18}O_9$	354.095	357.4480	−3.35
	20.72	0.527	Canthaxanthin	positive	$C_{20}H_{41}NO_4$	359.303	357.4142	1.89
	22.42	0.434	Chlorogenic acid Hemihydrate	positive	$C_{16}H_{18}O_9$	354.095	357.4480	−3.35
	26.07	0.811	Sodium Deoxycholate	positive	$C_{24}H_{40}O_4$	392.292	357.4142	34.88
	28.05	0.776	N-Acetyl-Phytosphingosine	positive	$C_{20}H_{41}NO_4$	359.303	357.4818	1.82
	23.55	0.821	Rape seed mixture glucosinolates	positive	$C_{23}H_{24}O_{13}$	347.22	357.5155	−10.3
	34.22	0.766	Cystathionine	[M + H]+	$C_{20}H_{41}NO_4$	359.303	357.5493	1.75
	35.00	0.869	DL-Dihydrozeatin	positive	$C_{13}H_{20}O_3$	224.141	224.1574	−0.02
	35.79	0.896	Farnesol (mixture of isomers)	positive	$C_{13}H_{20}O_3$	224.141	224.1911	−0.05
	36.78	0.891	Methyl Jasmonate	positive	$C_{13}H_{20}O_3$	224.141	224.1236	0.02

3.2. Oral glucose tolerance test

Administration of drugs 30 min before the oral glucose dose did not decrease the serum glucose levels immediately when compared to the control. Glibenclamide (10 mg/kg) showed a highly significant hypoglycemic action starting from 30 min after glucose dosing ($P < 0.001$) while the high dose of the extract (500 mg/kg) showed a significant decrease in serum glucose levels at 30 min when compared to control ($P < 0.05$). At 60 min and 120 min after glucose administration, a highly significant decrease in serum glucose levels was observed in glibenclamide and high dose of the extract (500 mg/kg) administered rats ($P < 0.001$). A significant decrease in serum glucose levels in the low-dose administered group (250 mg/kg) was observed at both 60 min and 120 min (Fig. 3). Unlike serum glucose levels, the effect on serum insulin levels was observed starting at 5 min after oral glucose administration in all the treatment groups with glibenclamide and a high dose of the extract (500 mg/kg) showing a better effect ($P < 0.001$) than a low dose of the extract (250 mg/kg). The decrease in insulin levels was significant in all the treatment groups till 120 min when

Table 3
List of suspected molecules in negative mode.

No	R.Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Diff
	1.19	0.647	D-(-)-Quinic acid	negative	$C_7H_{12}O_6$	192.063	191.0891	0.97
	5.76	0.94	Lignoceric Acid	negative	$C_{24}H_{48}O_2$	368.365	367.2342	1.1
	6.24	0.94	Xanthosine-5′-monophosphate disodium salt	negative	$C_{10}H_{13}N_4O_9P$	364.042	367.2679	−17.23
	8.25	0.892	1-Myristoyl-2-Hydroxy-*sn*-Glycero-3-Phosphate (Sodium Salt) Sodium Salt	negative	$C_{17}H_{35}O_7P$	382.212	381.2724	189.06
	9.00	0.994	D-Glucuronic acid	negative	$C_6H_{10}O_7$	194.042	193.1474	−131.31
	16.64	0.888	Uridine-5′-monophosphate	negative	$C_9H_{13}N_2O_9P$	324.035	325.3563	96.74
	21.28	0.985	2′-Deoxycytidine	negative	$C_9H_{13}N_3O_4$	227.09	227.2955	−26.26
	22.40	0.98	D-Glucosamine-6-phosphate sodium salt	negative	$C_6H_{14}NO_8P$	259.045	253.3457	−20.35
	23.15	0.962	6-Phosphogluconic acid Barium salt hydrate	negative	$C_6H_{13}O_{10}P$	276.024	279.3961	20.69
	25.92	0.978	alpha-D-Galactose-1-phosphate Dipotassium Salt	negative	$C_6H_{13}O_9P$	260.029	255.3365	−21.36
	26.46	0.975	acacetin	negative	$C_{16}H_{12}O_5$	284.068	281.3870	0.62
	32.60	0.885	Xanthosine	negative	$C_{10}H_{12}N_4O_6$	284.075	283.4454	** Expression is faulty **

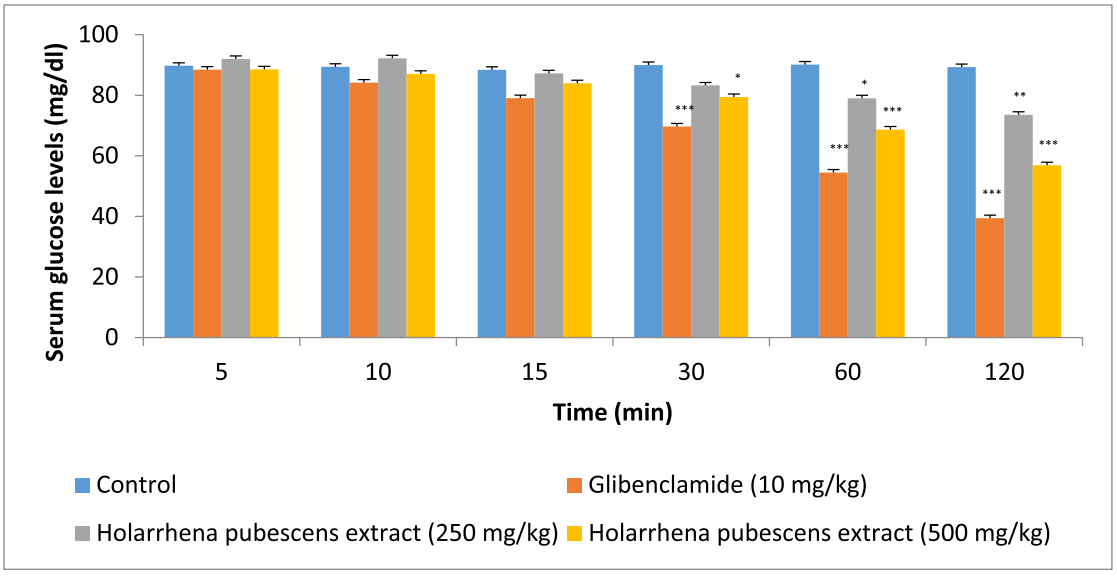

Fig. 3. Serum glucose levels during oral glucose tolerance test. Bars represent mean ± SEM, n = 6, *P < 0.05, **P < 0.01, ***P < 0.001 when compared to vehicle.

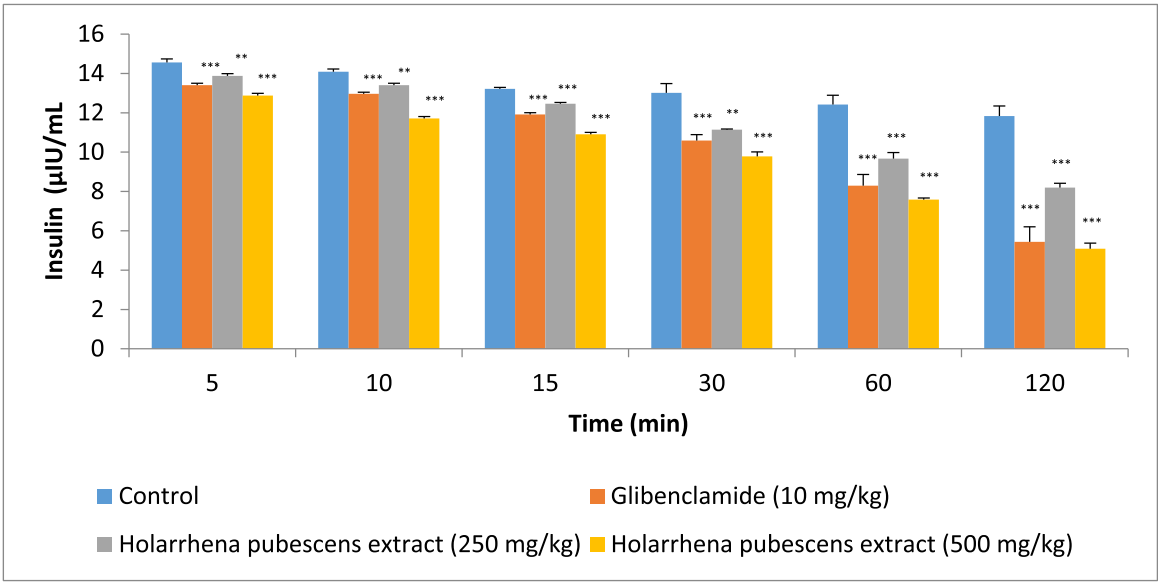

Fig. 4. Serum insulin levels during oral glucose tolerance test. Bars represent mean ± SEM, n = 6, **P < 0.01, ***P < 0.001 compared to vehicle.

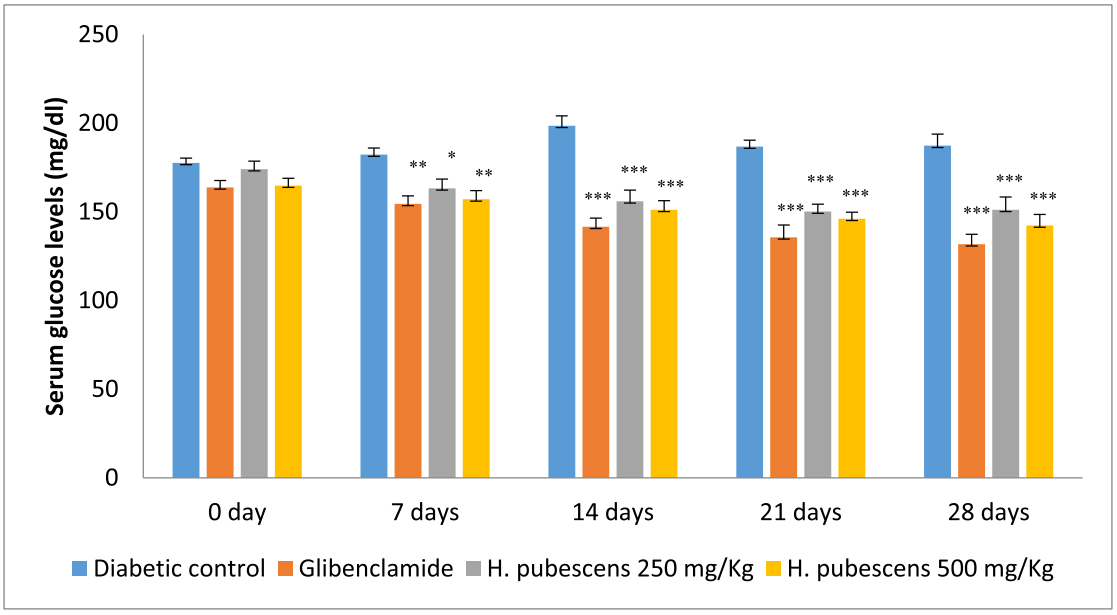

Fig. 5. Serum glucose levels in streptozocin-induced diabetes. Bars represent mean ± SEM, n = 6, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle.

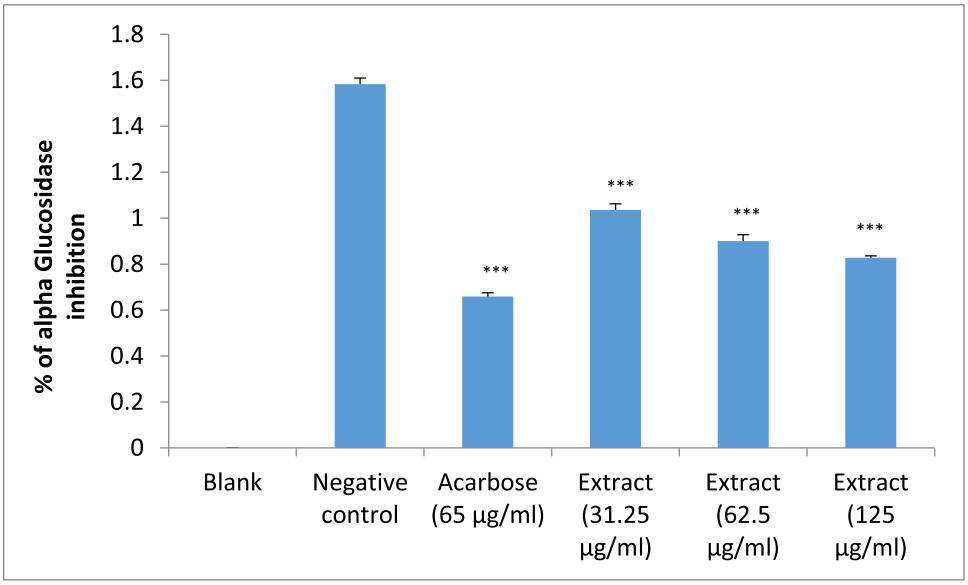

Fig. 6. Alpha-glucosidase inhibition activity Bars represent mean ± SEM, n = 3, $^{***}P < 0.001$ compared to the negative control.

compared to the control. The reduction in the serum insulin levels was maximum in the high dose of extract treatment group (Fig. 4).

3.3. Streptozocin-induced diabetes

The extract at the higher dose of 250 mg/kg and glibenclamide were highly effective in reducing hyperglycemia in diabetic rats starting from day 7. The extract (250 mg/kg) was also effective in controlling blood glucose levels in diabetic rats (Fig. 5).

3.4. α - glucosidase inhibition

H. pubescens extract showed a dose-dependent inhibition of the α–glucosidase inhibition. The concentration of the extract at<31.25 µg/ml did not show a good inhibition while those above 125 µg/ml showed similar inhibition of the enzyme indicating the maximum effective concentration. The effect observed with the highest tested concentration of the extract was similar to that observed with acarbose (Fig. 6.).

3.5. Glucose metabolism in INS-1-rat insulinoma cells to reflect insulin secretion

The extract increased the stimulation of glucose metabolism (with and without glucose) in a dose-dependent manner when compared to PBS-treated control groups (Fig. 7).

3.6. Protein glycation inhibition

The extract showed a very weak protein glycation inhibitory activity. An inhibition of around 36% was observed at 125 µg/ml and concentrations above this such as 250 µg/ml showed a similar

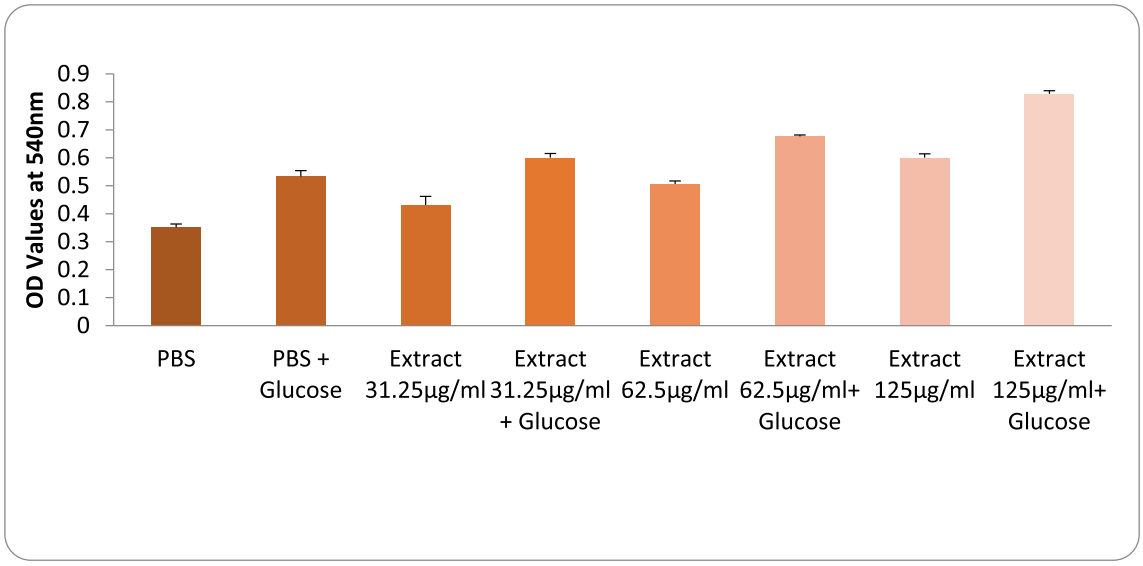

Fig. 7. Effect on MTT reduction in INS-1 cells. Bars represent mean ± SEM, n = 3.

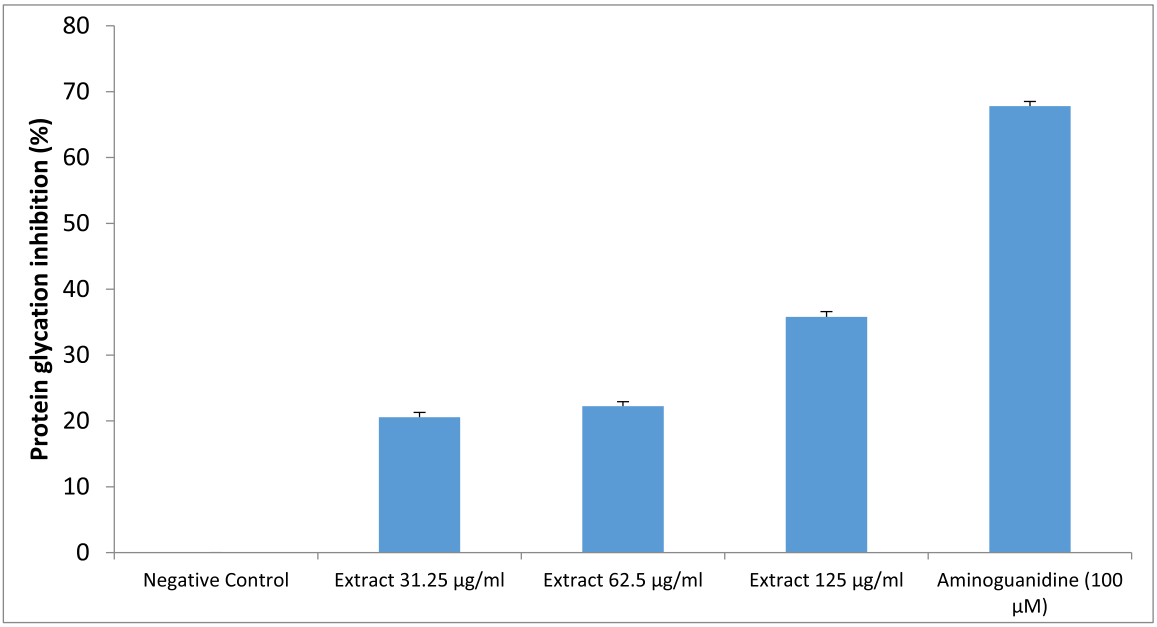

Fig. 8. Protein glycation inhibition, n = 3.

effect indicating the maximum effective concentration of the extract. The activity was much less with lower concentrations. Aminoguanidine showed an excellent inhibitory effect at 100 µM concentration (Fig. 8).

3.7. CYP3A4 inhibition assay

The extract did not show almost negligible inhibition of CYP3A4 activity while ketoconazole, a known CYP3A4 inhibitor showed around 75% inhibition of CYP3A4 activity (Fig. 9).

4. Discussion

The current study is a detailed investigation of *H. pubescens* seeds for the determination of its phytoconstituents and antidiabetic effect. The study was planned based on earlier reports and its traditional use for the treatment of diabetes. Since seeds are the most widely used part of the plant, it was selected for this study. The results revealed that *H. pubescens* seeds contain several phytoconstituents and this explains its varying effect on different parameters related to the antidiabetic effect. Among the suspected constituents identified by LC-MS, some of the constituents are reported to have hypoglycemic effects. Farnesol (Calzada et al., 2019), chlorogenic acid (Yan et al., 2020), D-(-)-Quinic acid (Arya et al., 2014), acacetin (Kwon et al., 2020), and xanthosine (Ahmed et al., 2023) are reported to have antidiabetic action. Furthermore, plants containing butyl glucosinolate (Roughani, 2018), canthaxanthin (Key et al., 2021), fusaric acid (Agrawal and Mourya, 2021), and 4-(Methyl sulfinyl) butylglucosinolate (Akram et al., 2021) are reported for antidiabetic effect though the antidiabetic effect has not been attributed experimentally to

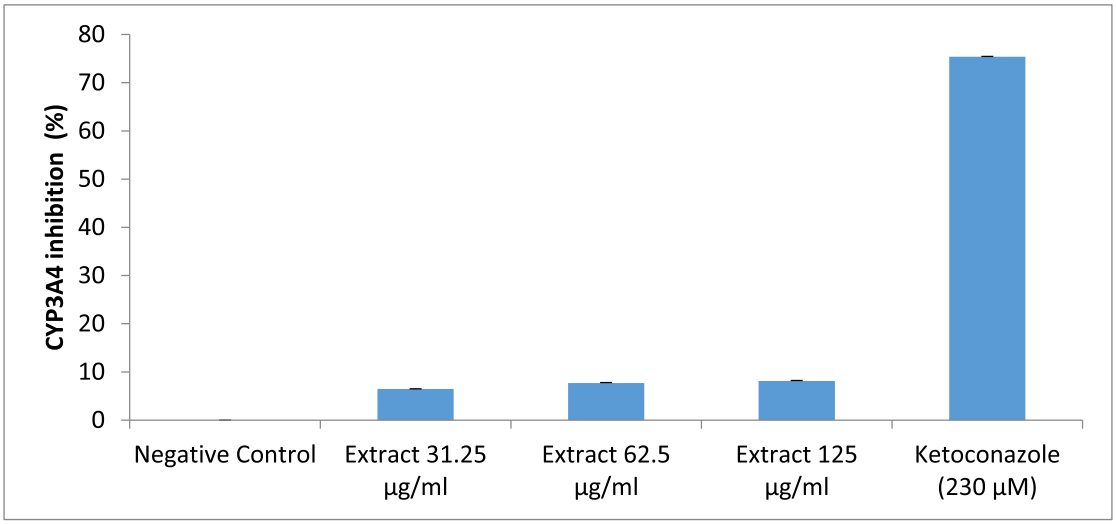

Fig. 9. CYP3A4 inhibitory effect, n = 3.

these constituents. Many of the identified components are reported to possess an antioxidant effect, an action commonly reported for phytoconstituents such as scoulerin (Parvin et al., 2022), 4-Methyl-7-hydroxycoumarin (Šarkanj et al., 2013), Esculin sesquihydrate (Pruccoli et al., 2020), Canthaxanthin (Mayne and Parker, 1989), chlorogenic acid (Bender and Atalay, 2021), cystathionine (Cano-Galiano et al., 2021), farnesol ((Silva et al., 2021), methyl jasmonate (Sá-Nakanishi et al., 2018), D-(-)-quinic acid (Liu et al., 2020) to name a few. A detailed investigation of the potency of these antioxidant effects and their contribution to the cytoprotection of the pancreas may reveal the contribution of these constituents to the observed antidiabetic action.

In the oral glucose tolerance test, the extract reduced the serum glucose levels and increased the serum insulin levels revealing its hypoglycemic potential. The glucose tolerance test determines the speed at which the glucose is removed from the blood. It indicates the level of insulin resistance and beta-cell activity (Eyth et al., 2022). A decrease in serum glucose with a concurrent elevation of serum insulin levels suggests that extract increases glucose uptake into the tissues and accelerates the release of insulin secretion from beta-cells in response to the glucose load.

To evaluate the antidiabetic effect of *H. pubescens* seeds extract, a neonatal-streptozocin rat model was utilized. Injection of streptozocin to neonatal rats is known to produce all the features of type-II diabetes that includes beta-cell dysfunction and insulin resistance along with impaired glucose tolerance (Takada et al., 2007). The neonatal model of type-II diabetes is considered better than other models of type-I and type-II diabetes induced using streptozocin (Takada et al., 2007). The extract at both the tested doses reduced the blood glucose levels significantly confirming its hypoglycemic and antidiabetic effects.

The enzyme α-glucosidase is involved in the digestion of carbohydrates. Agents that inhibit this enzyme reduce glucose absorption consequently producing a decrease in blood glucose levels (Agrawal et al., 2022). In the current study, the *H. pubescens* extract showed moderate inhibition of this enzyme suggesting that this effect may contribute to the overall hypoglycemic effect that was observed in streptozocin-induced diabetes. There is no role of α-glucosidase in oral glucose tolerance test.

Glucose metabolism in the pancreatic cells stimulates insulin release by enhancing ATP production (Röder et al., 2016). This model measures MTT reduction as a measure of insulin release and compounds that stimulate insulin release increase MTT reduction (Janjic and Wollheim, 1992). The *H. pubescens* extract increases the MTT reduction in a dose-dependent manner indicating an increased release of insulin from the INS-1 cells. These results confirm the increased serum insulin levels after the oral administration of the extract in the glucose tolerance test.

Protein glycation leads to several complications in diabetic patients. Glycation is the formation of adducts between reducing carbohydrates and amino acids that are reversible (Sarmah and Roy, 2022). Though the extract increased insulin secretion and inhibited glucose absorption (both *in-vivo* and *in-vitro*), it was not very effective in preventing protein glycation *in-vitro*. The effect on inhibition of protein glycation was observed only at higher concentrations and it was very less when compared to the standard compound; aminoguanidine.

As mentioned earlier, *H. pubescens* has been evaluated for antidiabetic and hypoglycemic effects earlier by several authors. Ali et al., (2011c) reported the hypoglycemic effect of hydromethanolic extract of the seeds on post-prandial glucose levels after starch administration in normoglycemic rats. They also reported α-glucosidase inhibitory effect *in-vitro* and concluded that this plant has α-glucosidase inhibitory *in-vivo* and *in-vitro*. However, the characterization of compounds present in the extract was not done. In the current study, we utilized oral glucose tolerance test to evaluate the overall hypoglycemic effect and demonstrated that *H. pubescens* acts through multiple mechanisms and α-glucosidase inhibition is one of its effects. Furthermore, we determined the different constituents present in the methanolic extract. Keshri (2012) reported the antidiabetic effect of *H. antidysentrica*, a synonym used for *H. pubescens* in rats by studying its effect on oral glucose tolerance and type-I diabetes induced using a single dose of streptozocin. No attempt was made by these authors to determine the mechanism of action or the chemical constituents present in the extract. The current study utilized a type-II diabetic model in rats for which *H. pubescens* is traditionally used and we also determined the chemical constituents present in the methanolic extract by LC-MS and evaluated the mechanism of antidiabetic action of *H. pubescens*. Similarly, Kumar and Yadav (2015) reported the antidiabetic effect of *H. pubescens* on type-I diabetes in rats without evaluation of the mechanism of action or chemical constituents determination. Another similar study on the effect of *H. pubescens* was reported by Pathak et al., (2015) with

the identification of different classes of constituents such as flavonoids, and alkaloids using HPTLC. In our study, we determined individual chemical constituents using LC-MS.

The current study was carried out using methanolic extract. Methanol mainly extracts polar chemical constituents (Abubakar and Haque, 2020). Hence, the observed effect in the present study is due to polar chemical constituents present in the *H. pubescens*. However, the effect of non-polar constituents present in the plant is not known. Further studies carried out using plant extracts prepared with non-polar solvents such as ether, n-hexane, and chloroform may provide insight into the effect of non-polar constituents.

Herb-drug interactions are a big concern in clinical settings, especially in diabetic patients. Both pharmacodynamic and pharmacokinetic interactions between concurrently administered herbs and drugs have been reported (Banerjee et al., 2019). One of the main mechanisms for pharmacokinetic interaction between herbs and drugs is the inhibition of metabolic oxidative enzymes; the cytochrome P_{450} (CYP450) in the liver. The major CYP in the liver that metabolizes the majority of drugs is CYP3A4 (Han et al., 2019). In the current study, the extract did not affect the CYP3A4 activity indicating that it may not show pharmacokinetic interactions with drugs metabolized by this enzyme. However, the activity of the extract on other CYPs may have to be evaluated if the concurrently administered oral hypoglycemic drug is administered by CYP other than CYP3A4. It has to be stressed here that this parameter rules out the pharmacokinetic interaction between *H. pubescens* seed extract and drugs metabolized by CYP3A4. There may be potential pharmacodynamic interaction between *H. pubescens* seed extract and oral hypoglycemic agents as the results of the current study confirmed that *H. pubescens* seed extract increased insulin release and reduced glucose absorption.

5. Conclusion

The results of the study showed that *H. pubescens* seed extract improved oral glucose tolerance in rats mediated through an increase in insulin secretion. An antidiabetic effect was observed in type-II-induced diabetes in rats in a 28-day study with decrease in blood glucose levels from 7th day of treatment onwards. The *in-vitro* study results revealed that the oral hypoglycemic and antidiabetic effects are mediated through a decrease in intestinal absorption of glucose (inhibition of α –glucosidase enzyme activity *in-vitro*) and increase insulin release by stimulating glucose metabolism in pancreatic cells (secretion of insulin from INS to 1-Rat insulinoma cell line cells *in-vitro*). However, the *H. pubescens* extract had a weak protein glycation inhibitory effect *in-vitro*. Determination of its potential for drug interactions showed that extract does not affect the activity of major metabolizing enzyme; the CYP3A4. An investigation of the *H. pubescens* extract by LC-MS for identification of chemical constituents present revealed presence of 14 and 12 different chemicals in negative and positive mode respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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