Alpha glucosidase inhibitory activity of hydro-methanolic (2:3) extract of seed of *Swietenia mahagoni* (L.) Jacq

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ABSTRACT

Objectives: Present study investigated the effect of hydro-methanolic extract of seed of *Swietenia mahagoni* (HMESM) on α-glucosidase inhibition in normal and streptozotocin-induced diabetic rats. **Methods:** Oral carbohydrate tolerance tests were performed in 16h fasted normal and diabetic rats loaded with starch or sucrose or glucose at the dose of 3g/kg, 15min after administration of 250 (S1), 500 (S2), 1000 (S3) mg/kg of HMESM, vehicle (control),or pretreatment at the dose of 10 mg/kg of acarbose (Acar). Blood samples were analyzed for glucose levels at 0, 30th, 60th, and 120th min after respective treatments and the peak blood glucose (PBG) levels and area under the curves (AUC) were determined. **Results:** Results demonstrated that 500mg, 1000mg/kg of HMESM reduced and prolonged the PBG and decreased AUC simultaneously after starch and sucrose loading in normal and diabetic rats. Similarly acarbose also reduce the sucrose and starch induced blood glucose excursion, whereas it had no peak blood glucose suppressive effect after exogenous glucose load in both normal and diabetic rats. On the other hand, phytochemical study of the said extract revealed that it is rich in phenolic compounds (46.25 mg of gallic acid equivalent/g of extract) and flavonoids (231.72mg of quercetin equivalent/g of the extract), which may may responsible for pharmacological activities. **Conclusion:** The HMESM may have PBG suppressive effect in post-carbohydrate challenged state as evidenced by reduced PBG and AUC. This suggest that HMESM may be used effectively as a safer alternative to control postprandial hyperglycemia especially in pre-diabetic and diabetic patients.

Keywords: Streptozotocin, α-glucosidase, Postprandial hyperglycemia, Total flavonoids.

INTRODUCTION

Diabetes is one of the oldest known human diseases whose devastating effects not only increase day by day but its severity is also almost at epidemic level. The number of cases for diabetes that is currently at 171 million is predicted to reach 366 million by the year 2030, and around 3.2 million deaths every year are attributable due to complications of diabetes and it results six deaths every minutes.[1]

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The recent investigation projected that postprandial hyperglycemia (PPH) could induce the nonenzymatic glycation of different proteins, resulting in the development of chronic complications related with cerebrovascular disorder, retinopathy, nephropathy, and neuropathy etc.[2] Hence, the control of PPH is an important strategy for the management of diabetes mellitus, especially type-1 diabetes and to minimize the chronic complications associated with the disease.[3] In this regard, synthetic drugs such as acarbose, miglitol and voglibose are widely used to reduce the PPH by retarding the absorption of glucose through inhibition in the activity of carbohydrate hydrolyzing enzyme i. e. alpha-glucosidase in the digestive tract, but these drugs might induce the onset of symptoms such as abdominal distention, diarrhea, and soft feces etc.[4, 5] Plant extract have long been used for the ethnomedical treatment of diabetes in various system of medicine and are currently accepted as an alternative for diabetic

therapy. However, for many plant extracts, there is no clear understanding of the mechanism of action. Though some preliminary screening studies on α-glucosidase inhibitors from various plant extracts have been reported, the in vitro α-glucosidase inhibitory activity may not always correlate with the in vivo α-glucosidase inhibitory activity.[6] So, it is necessary to investigate the in-vivo action after oral administration on whole live animals, which is an important step in screening plant extracts for physiological and pharmacological effects.

The plant, *Swietenia mahagoni* (L.) Jacq. (Family- Meliaceae) is a beautiful, lofty, evergreen large tree, native to tropical America, Maxico and South America as well as India. *S. mahagoni* is a large medicinally and economically important timber tree native to West Indies. Seed extract of *S. mahagoni* is widely used in Indonesia as folk medicine to cure diabetes.[7] Thus, the current study was designed to determine the possible effect of graded doses of hydro-methanolic (2:3) extract of seeds of *S. mahagoni* after an oral carbohydrate (starch, sucrose, glucose) load on the peak blood glucose (PBG) level and area under the curve (AUC) value in normal and diabetic rats.

MATERIALS AND METHODS

Plant materials

The seeds of *Swietenia mahagoni* (L.) Jacq. (Family- Meliaceae) were collected from Medinipur, District of Paschim Medinipur, West Bengal, India, in the month of December and the materials were identified by Prof. R. K. Bhakat, Department of Botany and Forestry, Vidyasagar University, Medinipur. The voucher specimen was deposited in the Department of Botany, Vidyasagar University (Ref. No. *Swietenia mahagoni* (L.) Jacq./ VU/01/09).

Preparation of hydro-methanolic (2:3) extract from the seeds of Swietenia mahagoni

Fresh seeds were dried in an incubator for 2 days at 40 ^{0}C, crushed separately in an electric grinder and then pulverized. Fifty gm of pulverized powder of the seeds of Swietenia *mahagoni* was dissolved in 250ml of hydro-methanolic (2:3) solution and then kept in incubator at 37^{0}C for 36h. The slurry was stirred intermittently for 2h and left overnight. The mixture was then filtered and filtrate was dried by low pressure of rotary evaporator apparatus (Hahnvapor Scientific Co.). From that amount, 9gm light brown semi-solid residue was collected. The residue was dissolved in water and three separate doses (250mg, 500mg and 1000mg/kg body weight) were used for the treatment.

Chemicals

Streptozotocin (STZ) was purchased from Sigma, USA. Other chemicals were purchased from Sigma –Aldrich Diagnostic Ltd. USA. Blood glucose levels were measured using a one touch electronic glucometer of Ascensia Entrust, Bayer Diagnostics Ltd., Borada, India.

Animals' treatment

Study was conducted on Wistar strain male albino rats, weighing about 190 ± 10g. Animals were acclimatized for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages, at an ambient temperature of 25 ± 2^{0}C with 12h light: 12h dark cycle. Rats have free access to standard food and water *ad libitum*. The principle of laboratory animal care was followed throughout the duration of experiment and instruction given by our Institutional Animal Ethical Committee (Ref. No. VU/IAEC/BMLSM/18/12) was considered regarding injection and relevant treatment of the experimental animals. Diabetes was induced in the animals by a single intramuscular injection of streptozotocin (STZ) at the dose of 4mg/0.1ml of citrate buffer (pH 4.5)/100g body weight/rat. Blood glucose levels were constantly monitored using Ascensia® Entrust® blood glucometer (Bayer Diagnostics India LTD., Baroda, India) and rats showing blood glucose level around 300mg/dl were included in this study as diabetic animal. Acarbose (Bayer Pharmaceuticals, Leverkusen, Germany) was used as a positive control at a dose of 10mg/kg body weight.

The oral carbohydrate tolerance test was carried out in normal and diabetic groups of rats and was equally divided into various treatment groups as mentioned below.

Oral starch tolerance test

Rats were divided into five groups consisting of six rats in each group. The rats were fasted overnight for 16 h but had free access to water. In treatment group I, rats were treated orally with 250mg/kg body weight of HMESM, in treatment group II, rats were treated orally with 500mg/kg body weight of HMESM, and in treatment group III, rats received orally 1000mg kg body weight of HMESM. The groups were designated as S1, S2, S3, respectively. Rats under treatment group IV were treated orally with only distilled water (control) and finally, rat treatment group V, were treated orally with positive control acarbose (Acar) 10mg/kg body weight. After 15 min, all rats were given starch at the dose of 3g/kg (R & M Chemicals, Essex, UK) body weight orally and the tail

was snipped for blood glucose estimation before (0 min i.e. starch loading) and at 30th, 60th, and 120th min post loading state.

Blood glucose levels were recorded and PBG and AUC values were determined. The maximum blood glucose levels found during blood glucose determination was taken as the PBG. The formula for AUC determination is as follows.[8]

$$AUC(mmol/L) = \frac{(BG_0 + BG_{30})}{2} \times 0.5 + \frac{(BG_{30} + BG_{60})}{2} \times 0.5 + \frac{(BG_{60} + BG_{120})}{2} \times 1$$

Oral sucrose tolerance test

The oral sucrose tolerance test was carried out in the same way, but in this test sucrose (R & M Chemicals, Essex, UK) at a dose of 3g/kg body weight was used.[8]

Oral glucose tolerance test

The oral glucose tolerance test was conducted in the same manner where glucose loading (R & M Chemicals, Essex, UK) was performed at a dose of 3g/kg body weight.[8]

Determination of total phenolic compounds (TPC) and total flavonoid compounds (TFC)

The total phenolic content present in the extract was determined using Folin-Ciocalteu reagent.[9] The reaction mixture was prepared by adding 1ml of extract with 0.5ml of Folin-Ciocalteu reagent, 3ml of 20% Na_2CO_3 and 10ml of distilled water. After incubation for 2 h at ambient temperature, the absorbance was measured spectrophotometrically at 765nm. The total phenolic contents were calculated from a gallic acid standard curve and the result was expressed in terms of mg gallic acid equivalents (GAE)/g of extract.

The total flavonoid content was determined by aluminium chloride ($AlCl_3$) using colorimetric method with quercetin as standard.[10] The reaction mixture was prepared using 1ml of extract, 4ml of distilled water, and 0.3ml of $NaNO_2$ and after 5 minutes 3ml of 10% $AlCl_3$ was added to the reaction mixture. After incubation for further 5 minutes the reaction mixture was treated with 2ml of 1 M NaOH and the absorbance was measured at 510nm. The flavonoid content was calculated from a quercetin standard curve and the result was expressed in terms of mg quercetin equivalents (QE)/g of extract.

Statistical analysis

Statistical difference in PBG and AUC values between control and various treatment groups were determined using software in computer (Origin 6.1). One-way analysis of variance (ANOVA) followed by multiple comparison two-tail 't' test was conducted to find out the significant of the result.[11] Values were expressed as mean ± SEM.

RESULTS

Effect of hydro-methonolic extract of seeds of *Swietenia mahagoni* on oral starch tolerance tests in normal and diabetic rats

The results of the oral starch tolerance tests on normal rats demonstrated an inhibition in the increment of blood glucose levels and AUC values at 30th min after HMESM administration followed by oral starch loading in control, S1, and S2 groups. The same nature of curve up to 30th min of HMESM administration was noted in control, S1, and S2 groups in diabetic condition but the blood glucose levels and AUC values were decreased from the starting period in S3 and acarbose treated groups both in normal and diabetic condition(Fig. 1, 2 and Table 1).

In normal condition, blood glucose levels and AUC values were remain stable beyond 30th min up 120th min in the control, S1, and S2 groups but in the S3 and acarbose groups, the levels of blood glucose and AUC values were significantly decreased in that period in respect to control (Fig. 1 and Table 1).

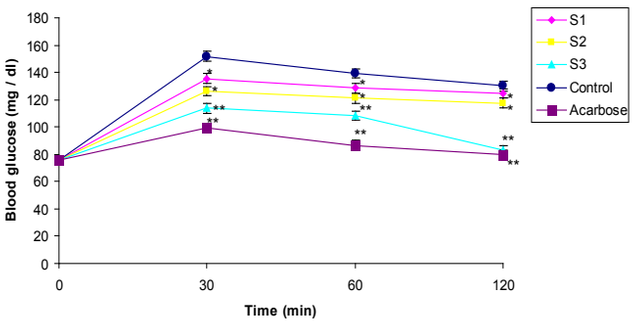

Figure 1. Blood glucose response in oral starch tolerance test after loading with starch at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

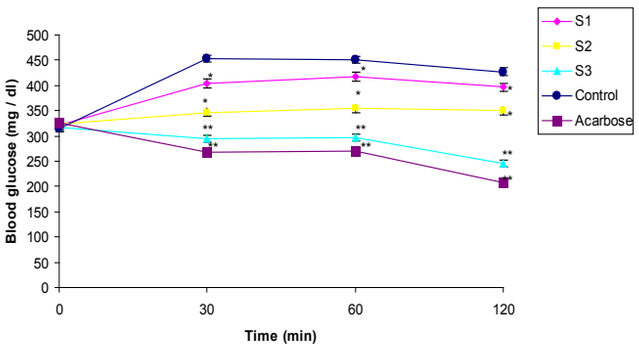

Figure 2. Blood glucose response in oral starch tolerance test after loading with starch at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

Table 1. Effect on PBG and AUC after starch loading at the dose of 3g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg of hydro-methanolic (2:3) extract of seeds of Swietenia mahagoni, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	135.53±3.2*	245.07±3.1*
S2	126.5±2.5*	231.8±2.4*
S3	115.65±2.1**	199.54±2.5**
Control	151.8±3.7	264.26±3.3
Acarbose	99.39±2.3**	173.39±2.2**
Diabetic rats		
S1	404.8±4.1*	792.29±4.3*
S2	346.97±3.7*	692.25±3.8*
S3	294.56±3.1**	573.28±4.1**
Control	453.59±4.1	857.75±5.2
Acarbose	267.45±3.2**	520.41±4.2**

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). *p<0.05 compared with control; **p<0.001 compared with control. ANOVA followed by multiple comparison two-tail 't' test.

In diabetic condition, blood glucose levels and AUC values were remained in flat in S1, and S2 groups in respect to control though the above levels were significantly lower than the control. In contrast, in S3 and acarbose treated groups, the blood glucose levels and AUC values were continuously decreased up to 120th min which were significantly differ in respect to the control (Fig. 2 and Table 1).

Effect of hydro-methonolic extract from seeds of *Swietenia mahagoni* on oral sucrose tolerance tests in normal and diabetic rats

Results of oral sucrose tolerance tests on normal rat's demonstrated an elevation in blood glucose levels and AUC values at 30th min after HMESM administration followed by oral sucrose loading in all the groups as the curve noted in control. The blood glucose levels and AUC values were decreased in normal condition beyond 30th min up to 120th min in S1, and S2 groups. There was no significant difference in the levels of above parameters among S1, S2, and control groups at 120th min of the test. In S3 and acarbose treated groups results were significantly decreased in the levels of said biosensors in that period in respect to control (Fig. 3 and Table 2).

In diabetic condition, blood glucose levels and AUC values were remained in flat in S1, and S2 groups in respect to control though the levels were significantly lower than the control. In contrast, the blood glucose levels and AUC values was continuous decreased up to 120th min in S3 and acarbose treated groups which were significantly less in respect to the control (Fig. 4 and Table 2).

Effect of hydro-methonolic extract of seeds of *Swietenia mahagoni* on oral glucose tolerance tests in normal and diabetic rats

Both in normal and diabetic condition, the blood glucose levels and AUC values attained their peak values at 30th min after the HMESM administration followed by glucose loading in all groups (Fig. 5 and Table 3). After 30th min up to 120th min, the blood glucose levels and AUC values in all the groups remained in flat state which were insignificant differ from the respective control values (Fig. 6 and Table 3).

TPC and TFC compound

The total phenolic compound (TPC) was 46.25mg of gallic acid equivalent and total flavonoid content (TFC) was 231.72mg of quercetin equivalent were present per gram of the hydro-methanolic extract of seeds of *Swietenia mahagoni* (Table 4).

DISCUSSION

The effective management of postprandial hyperglycemia (PPH) in diabetes mellitus is a key problem because high blood glucose levels may cause the stimulation and / or progression of diabetic complications through activation of the polyol pathway, elevation in protein glycation and promotion of hyperinsulinemia.[12–14] A prominent pathway for glucose production from food is the breakdown of carbohydrates by intestinal amylases or glycoside hydrolyses in the intestine. Thus, blood glucose management, prevention in the elevation in postprandial blood

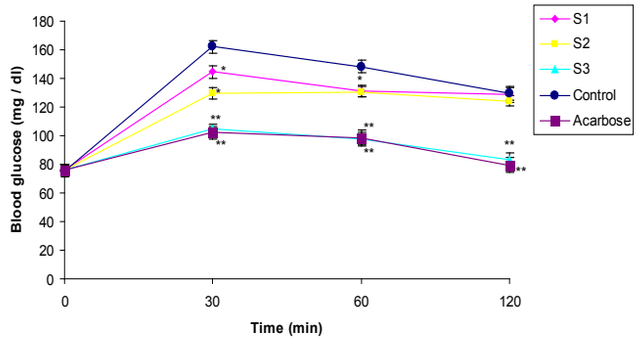

Figure 3. Blood glucose response in oral sucrose tolerance test after loading with sucrose at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10 mg/kg. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

Table 2. Effect on PBG and AUC after sucrose loading at the dose of 3 g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	144.52±3.1*	254.22±3.4*
S2	129.34±3.5*	243.83±2.8*
S3	104.8±2.6**	186.09±2.5**
Control	162.02±3.3	275.88±3.2
Acarbose	102.1±2.7**	183.67±2.6**
Diabetic rats		
S1	421.2±4.2*	790.7±4.8*
S2	364.5±3.7*	691.52±5.1*
S3	297.6±3.8**	527.67±4.6**
Control	448.5±4.4	864.15±5.3
Acarbose	284.3±3.6**	502.37±4.7**

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

glucose level and for maintaining blood glucose level within the normal range by controlling glucose production from food sources using an oral α-glucosidase inhibitor would be an idealistic and effective management for NIDDM patients.

Acarbose-like drugs, those inhibit α-glucosidase activity present in the epithelium of the small intestine, have been demonstrated to decrease postprandial hyperglycemia (PPH) and improve impaired glucose metabolism without promoting insulin secretion in NIDDM patients.[15] Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase

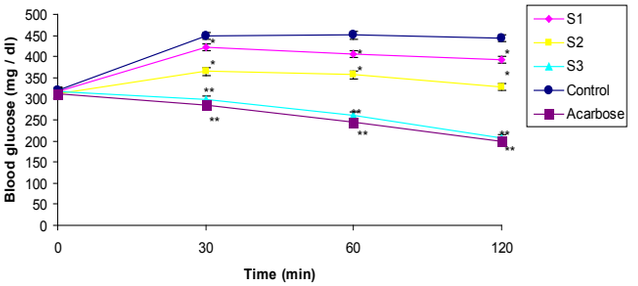

Figure 4. Blood glucose response in oral sucrose tolerance test after loading with sucrose at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). $^{*}p<0.05$ compared with control; $^{**}p<0.001$ compared with control. ANOVA followed by multiple comparison two-tail 't' test.

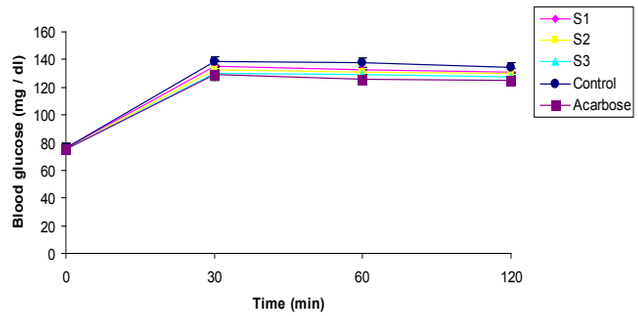

Figure 5. Blood glucose response in oral glucose tolerance test after loading with glucose at the level of 3g/kg in normal rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

Table 3. Effect on PBG and AUC after glucose loading at the dose of 3g/kg in normal and diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg.

Groups	PBG (mg / dl)	AUC (mg / dl)
Normal rats		
S1	133.63±3.3	251.72±4.4
S2	132.81±3.2	249.16±4.2
S3	130.42±2.9	247.62±3.8
Control	136.75±3.6	253.59±4.6
Acarbose	129.31±3.2	245.41±3.7
Diabetic rats		
S1	370.66±4.1	719.11±4.4
S2	369.21±3.8	718.42 ±4.1
S3	367.93±3.6	716.88±3.9
Control	372.47±4.7	721.03±4.8
Acarbose	365.23±3.4	714.12±3.7

PBG: Peak blood glucose; AUC: Area under the curve. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

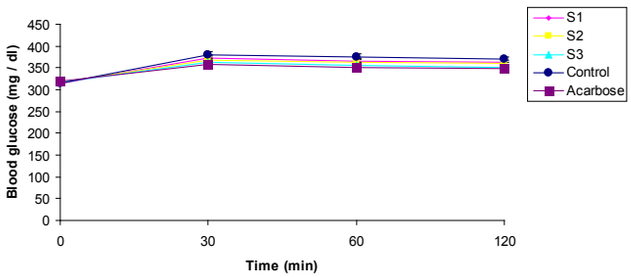

Figure 6. Blood glucose response in oral glucose tolerance test after loading with glucose at the level of 3g/kg in diabetic rats treated with 250 (S1), 500 (S2), 1000 (S3) mg/kg hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni*, (HMESM) or vehicle (water) or acarbose 10mg/kg. Values are the mean ± SEM (n=6). ANOVA followed by multiple comparison two-tail 't' test.

Table 4. Total phenolic and total flavonoid content of hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni* (L.) Jacq.

Samples	Total phenolic content (TPC) (mg GAE / g dried extract)	Total flavonoid content (TFC) (mg QE / g dried extract)
Hydro-methanolic (2:3) extract of seeds of *Swietenia mahagoni* (L.) Jacq.	46.25	231.72

GAE: gallic acid equivalents; QE: quercetin equivalents.

inhibitor offers a prospective therapeutic approach for the management of type -2 diabetes mellitus.[16] We found in the above experiments that S2 and S3 doses of the HMESM reduced the blood glucose excursions and decrease the PBG and AUC after sucrose and starch loading in normal and diabetic rats. The tendency of HMESM to suppress the PBG at 30min in both normal and diabetic rats demonstrates the α-glucosidase inhibitory activity and interfere the PBG. The HMESM seems to delay the quick digestion of starch as well as sucrose and lengthen the duration of carbohydrate absorption and thus reducing the PBG and AUC values. The above results show a striking similarity to the effect of acarbose.

On the other hand phenolic and flavonoids compounds of the extract are responsible for the inhibition in α-glucosidase activity and thereby inhibit glucose absorption in connection with the management of postprandial hyperglycemia may be proposed here as phytochemical analysis of the extract focused the presence of phenolic compounds, i.e., 46.25mg gallic acid equivalents (GAE) and flavonoids 231.72mg quercetin equivalents (QE) per gram of the extract. This proposal is consistent with the findings of others covering other plants.[17] In the field of phytochemistry and herbal medicines, there has been an enormous interest in the development of alternative medicines for type-2 diabetes, especially screening for natural bioactive compounds with the ability to delay or prevent glucose absorption. This is because any control of PPH by these alternative medicines would be much safer and will improve the quality of life of persons with borderline NIDDM. α-glucosidase inhibitors, α-amylase inhibitors, or glucose transport inhibitors have been screened to flatten postprandial blood glucose rise and are in the process of further development.[18–20] At present, only synthetic α-glucosidase inhibitors have been clinically used for management of type 2 diabetes. It is already known that ingestion of α-glucosidase inhibitors like acarbose or voglibiose regularly is more effective in moderating hyperglycemia in borderline NIDDM.[21]

CONCLUSION

The HMESM seems to have α-glucosidase inhibitory activity in diabetic rats because it suppresses the PBG and reduces AUC after simultaneous starch and sucrose loading and significantly affects the absorption of starch and sucrose. It may be used a safer alternative treatment to control PPH particularly in type 2 diabetic patients and also in borderline patients not properly controlled through diet alone. These medications are most useful for people who have just been diagnosed with type-II diabetes. The HMESM are also useful for people taking oral antidiabetic agents who need an additional medication to keep their blood glucose levels within a safe range. However, it is still early to suggest its use in humans, and only a thorough in-depth study can warrant its clinical use.

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