J Acupunct Meridian Stud 2013;6(1):24—30

Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

α-Glucosidase and α-Amylase Inhibitory Activity of *Senna surattensis*

Ellappan Thilagam [1,*], Balasubramaian Parimaladevi [2], Chidambaram Kumarappan [1], Subhash Chandra Mandal [1,†]

[1] *Department of Pharmaceutical Technology, Pharmacognosy and Phytotherapy Research Laboratory, Jadavpur University, Kolkata, India*
[2] *Department of Pharmaceutical Technology, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur, India*

Available online 7 November 2012

Received: May 19, 2011
Revised: Aug 8, 2012
Accepted: Aug 22, 2012

KEYWORDS
α-amylase;
α-glucosidase;
polyphenol;
postprandial hyperglycemia;
rat hemidiaphragm;
Senna surattensis

Abstract
In this study, we investigated the inhibitory effects of ethanolic extract of the leaves of *Senna surattensis* (EESS) on α-glucosidase and α-amylase. We also studied the *in vitro* antidiabetic activity of *S. surattensis* using the glucose uptake by isolated rat hemidiaphragm model. *In vitro* studies using mammalian α-glucosidase extracted from the small intestine homogenate of mouse showed that the extract was found to be more effective in inhibiting the activities of maltase [half maximal inhibitory concentration (IC_{50}): 209.15 μg/mL] and sucrase (IC_{50}: 366.44 μg/mL) when compared with the control group (acarbose). The extract of *S. surattensis* were further quantified with respect to porcine pancreatic α-amylase inhibition using the chromogenic 3,5-dinitrosalicylic acid method. Interestingly, *S. surattensis* was also found to exhibit α-amylase (IC_{50}: 123.95 μg/mL) inhibitory activity. The glucose uptake in the rat hemidiaphragm was significantly ($p < 0.01$) increased by EESS (220.95 ± 5.4 mg/g/30 minute) when compared with the control group. The total polyphenolic content of EESS was found to be 98 μg pyrocatechol/mg of the extract. These results suggest that EESS inhibited carbohydrate digestive enzymes and increased the peripheral uptake of glucose. This study endorses the use of this plant for further studies to determine their potential for managing type II diabetes.

* Corresponding author. Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Faculty of Engineering and Technology, Jadavpur University, Kolkata 700 032, India.
E-mail: thilagampharma@gmail.com (E. Thilagam).

† Authors' contributions: B.P., E.T., and S.C.M. designed the experiments. C.K. and E.T. conducted the experiments. All authors have read, reviewed, and approved the final version of the manuscript. The authors declare that they have no competing interest.

pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2012.10.005

1. Introduction

Noninsulin-dependent diabetes mellitus (NIDDM) is a common disease of the endocrine system caused by the decreased secretion of insulin by the pancreatic Langerhans β cell or by the lowering of insulin resistance due to excessive absorption of glucose [1]. A number of pharmacological approaches are used to control diabetes by different modes of action such as stimulation of insulin release, increase in the number of glucose transporters, inhibition of gluconeogenesis, and reducing absorption of glucose from the intestine [2]. Diabetes is a multifactorial disease leading to several complications and, therefore, it demands multiple therapeutic approaches. In the prediabetic state of insulin resistance, glycemic control can be achieved using oral agents that either interfere with the absorption of glucose (α-glycosidase and/or pancreatic α-amylase inhibitors) or facilitate glucose disposal in peripheral tissues (insulin-sensitizing agents). One of the most beneficial therapies for NIDDM is said to be the control of postprandial hyperglycemia after a meal [3]. In patients with diabetes, postprandial hyperglycemia is most pronounced following a meal due to the absorption of glucose from the gastrointestinal tract. Inhibiting glucose uptake in the intestines and/or promoting glucose disposal in the tissues may be beneficial for these patients to control the blood glucose level in the postprandial state.

Acting as a key enzyme for carbohydrate digestion is intestinal α-glucosidase, a glucosidase secreted in the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in NIDDM [4]. The major source of blood glucose is dietary carbohydrates such as starch, which are hydrolyzed by α-glucosidases and pancreatic α-amylase, so as to be absorbed by the small intestine. Therefore, an effective treatment option for NIDDM is to inhibit the activity of α-glucosidases and pancreatic α-amylase [5]. In this regard, inhibitors can retard the uptake of dietary carbohydrates, suppress postprandial hyperglycemia, and could be useful for treating patients with diabetes and/or obesity [6]. α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the activity of carbohydrate-digesting enzymes and delaying glucose absorption. In addition, numerous α-glucosidase inhibitors have been extracted from plants, which are of clinical importance [7,8].

Diabetes mellitus is also characterized by a diminished reaction of insulin-sensitive peripheral tissues and a marked decrease in glucose uptake and metabolism in response to insulin. The defective glucose transport system may play an important role in the pathogenesis of peripheral insulin resistance, and glucose uptake in target tissues is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load [9]. To enhance the glucose uptake by peripheral cells, biguanides such as metformin are used to control postprandial hyperglycemia in patients with NIDDM. This has been attributed to increased glucose disposal by peripheral tissues, as observed in euglycemic clamp studies in rats and patients with NIDDM [10,11]. Direct stimulation of basal glucose transport, disposal, and metabolism in muscle and fat cells would explain increased glucose utilization. Therefore, cellular assays are used to determine the mechanism of action of natural or synthetic compounds from isolated rat diaphragms, as well as isolated and cultured rat 3T3 adipocytes. For this reason, it is highly desirable to find new antidiabetic agents from natural resources that stimulate glucose uptake/disposal by peripheral tissues such as adipose tissue or muscle cells.

Recent interests in the study of plant polyphenols have focused on their potential benefits to human health. The polyphenols are capable not only of reducing oxidative stress but also of inhibiting carbohydrate-hydrolyzing enzymes to prevent hyperglycemia [12,13]. *Senna surattensis* Burm. f./*Cassia surattensis* Burm. f. (syn: *C. glauca* Lam., Family: Caesalpiniaceae) is commonly known as *Glaucous cassia*. It is a small tree or a large shrub, distributed throughout India. The tender leaves are consumed as a vegetable with rice [14]. Bark and leaves are useful for treating diabetes and gonorrhea, and the aerial parts are used for treating diabetes [15,16]. The plant is described as a medication for diabetes, gonorrhea, and blennorrhea [17]. The beads made from its wood are worn around the neck to cure jaundice [18]. A phytoconstituent reported in this plant contains anthraquinone, flavonol glycosides, chrysophanol, kaempferol, and quercetin [19—21]. This plant has been used as traditional medicine for the treatment of diabetes, but scientific evaluation is still lacking in this regard. To clarify its mechanism of action, we evaluated the inhibitory effect of ethanolic extract of *S. surattensis* (EESS) on postprandial blood glucose levels *in vitro*. Therefore, this study was aimed at establishing the potential therapeutic value of EESS by evaluating its *in vitro* inhibitory activities against α-glucosidase, α-amylase, and by calculating the glucose uptake by the isolated rat hemidiaphragm.

2. Materials and methods

2.1. Plant materials

Fresh leaves of *S. surattensis* were collected from Tiruchirappalli (Tamil Nadu, India) in December 2006 and authenticated by the Botanical Survey of India (Coimbatore, Tamil Nadu, India; Ref. No.: BSI/SC/5/23/06-07/Tech-1638). An authentic voucher specimen was deposited in the Herbarium of Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

2.2. Preparation of plant extract

The collected leaves were air dried at room temperature without exposure to sunlight, coarsely powdered (300 g), and then extracted with ethanol (95%) in a Soxhlet apparatus. The solvent was then evaporated under reduced pressure in a rotary evaporator (Superfit, India) at $<40^{\circ}C$ to obtain a dry extract (yield 24.25% w/w) that was stored at $-20^{\circ}C$ in a refrigerator until further use.

2.3. Preliminary phytochemical analysis

The EESS was subjected to preliminary phytochemical analysis to detect the composition of the phytoconstituent using standard chemical tests [22].

2.4. Estimation of total phenol content

The total phenolic content of EESS was determined using Folin–Ciocalteu reagent [23]. In brief, the analysis is carried out as follows: 1 mL of extract solution containing 1 mg of extract was transferred into 100-mL Erlenmeyer flask and then the final volume was adjusted to 46 mL by adding distilled water. To this mixture, 1 mL of Folin–Ciocalteu reagent was added and after 3 minutes, 3 mL of Na_2CO_3 (2%) was also added. Subsequently, the mixture was shaken on a shaker for 2 hours at room temperature and then its absorbance was measured at 760 nm. The concentration of the total phenolic content was expressed as micrograms of pyrocatechol using an equation that was obtained from standard pyrocatechol graph. The equation used is as follows:

$$\text{Absorbance} = 0.001 \times \text{Pyrocatechol } (\mu g) + 0.0033.$$

2.5. Animal and ethical approval

Male and female Swiss albino rats and mice, weighing about 200–250 g and 20–25 g body weight, respectively, were used in this study. The animals were collected from a breeding colony and acclimatized to the laboratory condition for 2 weeks. They were housed in makrolon cages under standard laboratory conditions (light period: 7.00 AM to 7.00 PM, temperature: 21 ± 2°C; and relative humidity 55–70%). The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore, India) and had free access to water (*ad libitum*) during the experiments. The experiments conducted were in accordance with the rulings of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India (Registration No.: 0367/01/C/CPCSEA) and the study was permitted by the Institutional Animal Ethical Committee of the Jadavpur University, Kolkata, India.

2.6. α-Glucosidase inhibitory assay

This assay was carried out to investigate the *in vitro* inhibitory activity of EESS on sucrase and maltase (α-glucosidases). Although α-glucosidase isolated from yeast is extensively used as a screening material for α-glucosidase inhibitors, the results did not always agree with those obtained in mammals. Therefore, we used a small intestine homogenate of a mouse as α-glucosidase solution because we speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured by slightly modifying the method used by "Dahlqvist" [24]. After 20 hours of fasting, part of the animals' small intestine immediately below the duodenum and immediately above the cecum was cut, rinsed with ice-cold saline, and homogenized with 12 mL of maleate buffer (100 mM, pH 6). The homogenate was used as α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6), 2% (w/v) of each sugar substrate solution (100 μl), and the sample extract (20–640 μg/mL). The mixture was preincubated for 5 minutes at 37°C, and the reaction was initiated by adding crude α-glucosidase solution (50 μl), followed by incubating the mixture again for 10 minutes at 37°C. The amount of glucose released in this reaction was determined by a commercially available glucose estimation kit (Span Diagnostic Ltd., Mumbai, India). The rate of carbohydrate decomposition was calculated as a percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

Inhibition rate (%) = [{(amount of glucose produced by the positive control) − (amount of glucose produced by the addition of EESS) − (glucose production value in blank)/(amount of glucose produced by the positive control)}] × 100.

2.7. α-Amylase inhibitory assay

Test samples [EESS (6.25, 12.5, 25, 50, 100, 200 μg/mL) and nojirimycin (6.25–200 μg/mL)] of 500 μl concentration were added to 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/mL porcine pancreatic α-amylase solution (Sigma Chemical Co., St. Louis, MO, USA) and were incubated at 25°C for 10 minutes. After the preincubation, 500 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at prespecified intervals. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped by adding 1 mL of 3,5-dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled down to room temperature. The reaction mixture was then diluted after adding 10 mL of distilled water and absorbance was measured at 540 nm [25].

$$\%\ inhibition = \frac{Abscontrol\ (540) - Absextract\ (540)}{Abscontrol\ (540)}$$

2.8. Glucose uptake by isolated rat hemidiaphragm

Glucose uptake by rat hemidiaphragm was estimated according to earlier works [26,27], but with some modifications. Four groups, with each group containing six graduated test tubes ($n = 6$), were considered as follows:

Group 1: 2 mL of Tyrode solution with 2% glucose.
Group 2: 2 mL of Tyrode solution with 2% glucose and regular insulin solution (Novo Nordisk; 0.62 mL of 0.4 U/mL).
Group 3: 2 mL of Tyrode solution and 1.38 mL of EESS (0.1% v/v).
Group 4: 2 mL of Tyrode solution with 2% glucose and regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of EESS (0.1% v/v)

The volumes of all the test tubes were made up to 4 mL by adding distilled water to match the volume of the test tubes in Group 4. A total of 12 albino rats were fasted overnight and decapitated. The diaphragms were quickly dissected with minimal trauma and divided into two halves.

Two diaphragms from the same animal were not used for the same set of experiments. Six diaphragms were used for each group. The hemidiaphragms were placed in test tubes and incubated for 30 minutes at 37°C in an atmosphere of 100% oxygen and were shaken at a speed of 140 cycles/minute. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

2.9. Statistical analysis

The experimental results were presented as mean ± standard error of the mean of three parallel measurements. Statistical analysis was performed using one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. Values of $p < 0.01$ and $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Preliminary phytochemical analysis

Preliminary phytochemical analysis of EESS showed positive results for steroids, triterpenoids, and flavonoids. The total phenolic compounds of the ethanol extract were expressed as micrograms of pyrocatechol (phenolic content: 98 μg pyrocatechol/mg of extract) equivalent per milligram of *S. surattensis* leaves extract. A chromatographic technique on silica gel 60 with chloroform and methanol mixture as mobile phase in a saturated chamber is used for the baseline separation of the target compounds. The polyphenolic profile of *S. surattensis* can be visualized using fast blue salt B reagent. The major types of phenolic constituents identified in the leaves of *S. surattensis* are simple phenolic acids, flavonol, flavones, flavonones, and flavonoid glycosides. Phytochemical analysis of *S. surattensis* extract has shown the presence of the flavonoids quercetin, rutin, and kaempferol, quercetin 3-O-glucoside-7-O-rhamnoside, which are known for their antioxidant activities. Therefore, the presence of these chemicals may be one of the reasons why the EESS showed good *in vitro* antihyperglycemic activity.

3.2. α-Glucosidase and α-amylase inhibitory activities

In order to investigate the inhibitory effect of *S. surattensis* extract, an *in vitro* α-glucosidase inhibition test was performed. Although α-glucosidase isolated from yeast is extensively used as a screening material for α-glucosidase inhibitors, the results did not always agree with those obtained in mammals. The half maximal inhibitory concentration (IC_{50}) values of maltase and sucrase inhibitory activities were 209.15 and 366.44 μg/mL, respectively. The results in Figs. 1 and 2 show that EESS exhibited strong activity in a dose-dependent manner and is thus inferred to be an effective α-glucosidase inhibitor. In this study, EESS exhibited strong inhibitory activity against α-glucosidase and α-amylase, which is comparable with positive controls of acarbose and nojirimycin, respectively. In addition, EESS inhibited the activity of α-amylase with IC_{50} of 123.95 μg/mL, the result of which is shown in Fig. 3. However, EESS showed weak α-glucosidase and α-amylase inhibitory activities compared with acarbose and nojirimycin, respectively. These results confirm that EESS have α-glucosidase and α-amylase inhibitory properties.

3.3. Effect on peripheral glucose uptake

The estimation of glucose content in rat hemidiaphragm was used for the *in vitro* study of peripheral uptake of glucose. Table 1 shows glucose uptake (milligrams/gram tissue weight) in an isolated rat hemidiaphragm muscle in the presence of insulin (0.4 U/mL) and EESS (0.1% w/v). The results of this experiment indicate that the addition of EESS to the incubation media (Tyrode solution) caused significant increase in glucose uptake by the rat hemidiaphragm and was found to be less effective than insulin (240.876 ± 3.538). Moreover, EESS seemed to be more effective in enhancing peripheral glucose uptake in rat hemidiaphragm in the absence of insulin. Treatment with EESS (0.1% w/v) also elicited a significant increase ($p < 0.01$) in glucose uptake by the isolated rat hemidiaphragm (220.95 ± 5.443) when compared with the control groups (80.896 ± 3.406). These results show that treatment with insulin or EESS alone for 30 minutes produced a significant increase in glucose uptake by 2.98- and 2.73-fold, respectively; however, combined incubation with both insulin and EESS exerted the rate by 2.28-fold, an increase of glucose uptake in rat hemidiaphragm compared with untreated control groups. The addition of both EESS and insulin to the incubation media caused an increase of insulin binding to the rat hemidiaphragm as compared with the blank. The glucose uptake by rat hemidiaphragm was significantly more in all the groups tested when compared with the control group.

4. Discussion

Diabetes is characterized by high concentrations of blood sugar levels, which can cause serious complications, such as organ failures and/or destruction of the kidneys, eyes, and various cardiovascular diseases. Therefore, the treatment methods mainly focus on reducing fluctuations in blood sugar levels and their related complications. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase [28,29]. α-Glucosidases are enzymes that catalyze the absorption of digested glucose from dietary polysaccharides in the small intestine.

The α-glucosidase inhibition of EESS was evaluated by determining the α-glucosidase inhibitory activity using 4-Nitrophenyl-β-D-glucopyranosiduronic acid (pNPG) as the reaction substrate. The crude enzyme solution prepared from a mouse's small intestine was used as a source of α-glucosidases, sucrase, maltase, and isomaltase. α-Glucosidase catalyzes the final step in the digestion of carbohydrates and its inhibitors can retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia, which can be a useful

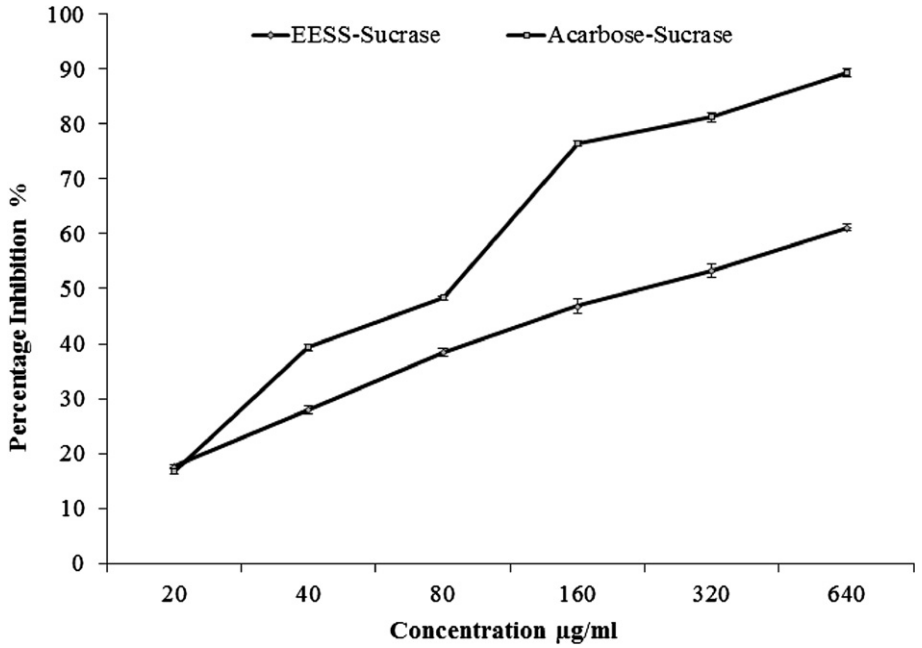

Figure 1 α-Glucosidase (sucrase) inhibitory activity of ethanolic extract of *Senna surattensis* (EESS). The values presented are expressed as mean ± standard error of the mean of triplicate experiments.

mechanism in the preparation of antidiabetic drugs [30]. However, it is unclear whether the mechanism of inhibition of α-amylase and α-glucosidase by EESS is due to competitive and noncompetitive method. The fact that α-amylase and α-glucosidase showed different inhibition kinetics seemed to be due to structural differences related to the origins of the enzymes [31]. However, the inhibition rate for α-glucosidase was close to that of acarbose, and the inhibition rate for α-amylase was obviously lower than that of acarbose. This indicated that EESS was a strong inhibitor for α-glucosidase with mild inhibitory activity against α-amylase. The inhibition of α-glucosidase, together with α-amylase by EESS, is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose [32].

Severe postprandial hyperglycemia commonly experienced by patients with diabetes could be prevented if the rate of glucose uptake from the intestine into the circulation could be reduced by inhibiting carbohydrate digestion

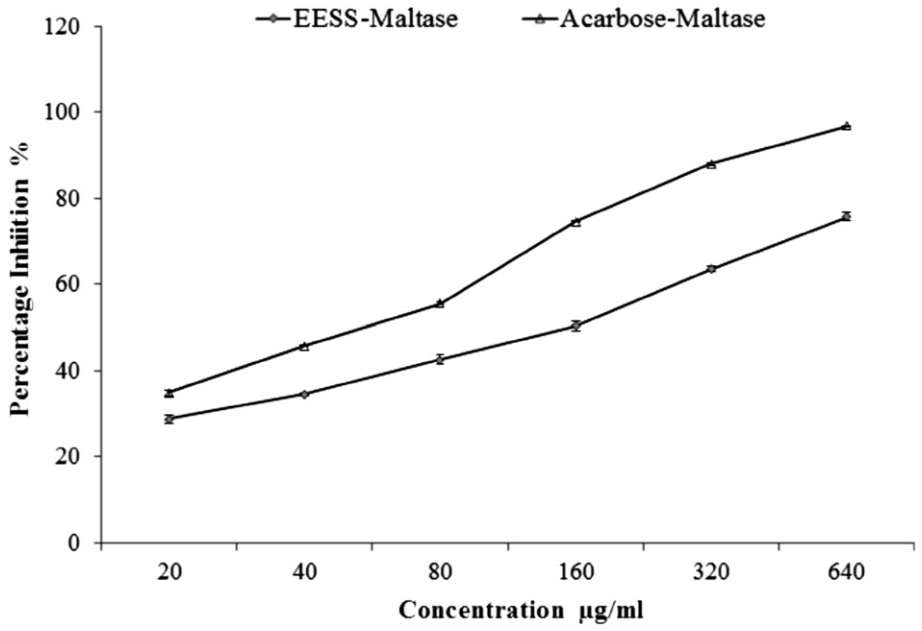

Figure 2 α-Glucosidase (maltase) inhibitory activity of ethanolic extract of *Senna surattensis* (EESS). The values presented are expressed as mean ± standard error of the mean of triplicate experiments.

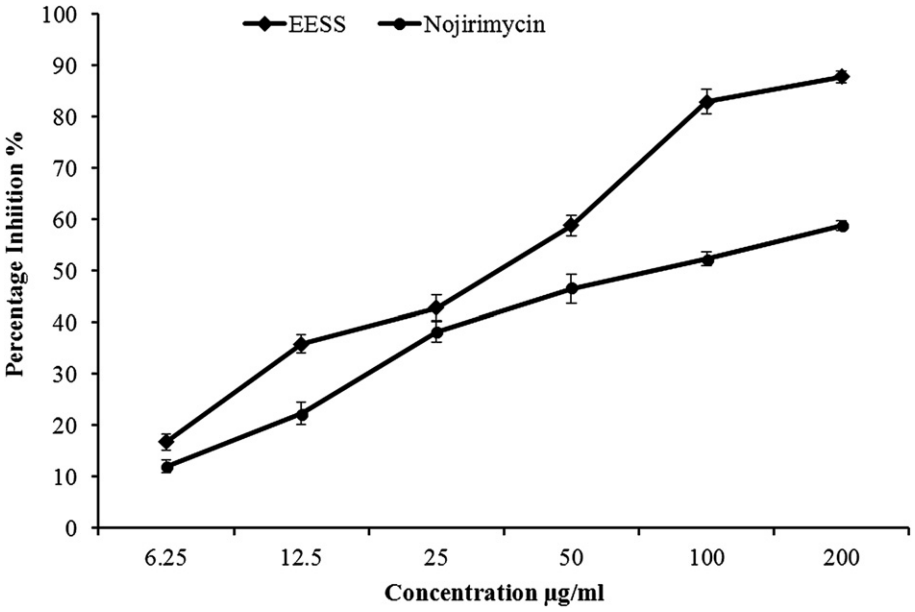

Figure 3 α-Maylase inhibitory activity of ethanolic extract of *Senna surattensis* (EESS). The values presented are expressed as mean ± standard error of the mean of triplicate experiments.

and absorption. Skeletal muscle represents 30—40% of the total body weight and seems to be one of the most important target tissues for the action of insulin and for the uptake of glucose at the peripheral level [33]. It is a well-known fact that insulin and antidiabetic drugs promote glucose uptake by peripheral cells and tissues [34]. Another important finding of this work is that EESS possesses considerable insulin-like properties, as evidenced by the enhancement of glucose uptake in the diaphragm, which represents muscle cells that are a major site of insulin-medicated glucose disposal. Pieces of hemidiaphragm were incubated with different concentrations of EESS and insulin for 30 minutes. The estimation of glucose content in rat hemidiaphragm is a commonly used and a reliable method for the *in vitro* study of peripheral uptake of glucose. In addition, EESS significantly enhances the uptake of glucose by isolated hemidiaphragm and is found to be less effective than insulin. It appears that EESS has direct peripheral action. The control value of glucose uptake by rat hemidiaphragm corresponds with those of earlier findings [27]. Uptake of glucose by the rat abdominal muscle (milligrams/gram tissue weight) was calculated as the clearing of glucose from the perfusion medium. Therefore, EESS acts directly on the uptake of glucose at the peripheral levels, either alone or as an insulin coadjuvant.

Table 1 Effect of EESS on glucose uptake by isolated rat hemidiaphragm.

Group	Glucose uptake (mg/g/30 min)
Control	80.896 ± 3.406
Insulin	240.876 ± 3.538*
EESS	220.95 ± 5.443*
EESS + insulin	184.94 ± 4.988*

Values are mean ± standard error of the mean ($n = 6$).
* $p < 0.01$ as compared with control.
EESS = ethanolic extract of the leaves of *Senna surattensis*.

A preliminary phytochemical analysis of EESS revealed the presence of polyphenols, flavonoids, flavonol, and flavonol glycosides, including rutin, quercetin, and kaempferol [35,36]. Polyphenolic compounds in plants have been shown to inhibit the activities of digestive enzymes due to their ability to bind with proteins [37]. Polyphenolic compounds of EESS may interfere with the activity of digestive enzymes in the brush border of the small intestine, could slow the liberation of D-glucose from oligosaccharide and disaccharides, resulting in delayed glucose absorption and decreasing postprandial glucose levels. Results from a number of studies suggest that these phenolic compounds isolated from a large number of medicinal plants [38,39] could have potential to treat Type II diabetes mellitus.

Polyphenols are a complex group of chemicals that are widely distributed throughout the plant kingdom and thus form an integral part of the human diet. Therefore, the inhibition of α-glucosidase found in our experiments could be due to the presence of polyphenolic compounds. Phenolic phytochemicals from EESS may potentially provide a natural source of α-glucosidase inhibitors. These polyphenolic compounds have been found to be responsible for blood glucose lowering activity and have also been reported to activate GLUT1-mediated glucose uptake [40,41]. Inhibition of carbohydrate digestive enzymes (α-glucosidase and α-amylase) and enhanced peripheral uptake of glucose by hemidiaphragm are the possible preliminary mechanisms involved in its hypoglycemic activity. Further investigation is needed to determine the individual polyphenol components present in *S. surattensis* that may be responsible for improvements in health conditions by regulating α-glucosidase and α-amylase inhibitory activities. Isolation of the active components is currently being pursued in our laboratory to better understand the mechanism of enzyme inhibition.

Acknowledgments

The authors are grateful to All India Council for Technical Education (New Delhi, India) for providing financial support through its Quality Improvement Scheme.

References

1. Iwamoto Y. Insulin sensitizer. *Horumon To Rinsho*. 1995;43: 167—173.
2. Kageyama S. Varieties and characteristics of drugs for diabetes. In: Ikeyama, ed. *How to Choose and Use Drugs for Diabetes*. Tokyo, Japan: Nankaido; 1996:23—44.
3. Yao Y, Sang W, Zhou M, Ren G. Antioxidant and α-glucosidase inhibitory activity of colored grains in China. *J Agric Food Chem*. 2010;58:770—774.
4. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. *Nutrition*. 2005;21:756—761.
5. Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drug*. 2005;65:385—411.
6. Watanabe J, Kawabata J, Kurihara H, Niki R. Isolation and identification of α-glucosidase inhibitors from tochu-cha (*Eucommia ulmoides*). *Biosci Biotechnol Biochem*. 1997;61:177—178.
7. Yoshikawa M, Murakami T, Yashiro K, Matsuda H. Kotalanol, a potent alpha-glucosidase inhibitor with thiosugar sulfonium sulfate structure, from antidiabetic ayurvedic medicine *Salacia reticulata*. *Chem Pharm Bull (Tokyo)*. 1998;46: 1339—1340.
8. Nishioka T, Kawabata J, Aoyama Y. Baicalein, an α-glucosidase inhibitor from *Scutellaria baicalensis*. *J Nat Prod*. 1998;61: l413—l415.
9. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest*. 2000;106:171—176.
10. Hirshman MF, Horton ES. Glyburide increases insulin sensitivity and responsiveness in peripheral tissues of the rat as determined by the glucose clamp technique. *Endocrinology*. 1990; 126:2407—2411.
11. Mandarino LJ, Gerich JE. Prolonged sulfonylurea administration decreases insulin resistance and increases insulin secretion in non-insulin-dependent diabetes mellitus: evidence for improved insulin action at a postreceptor site in hepatic as well as extrahepatic tissues. *Diabetes Care*. 1984;7:89—99.
12. de Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, et al. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-α-dirhamnoside from *Bauhinia forficata* leaves. *J Nat Prod*. 2004;67:829—832.
13. Hanamura T, Hagiwara T, Kawagishi H. Structural and functional characterization of polyphenols isolated from acerola (*Malpighia emarginata* DC.) fruit. *Biosci Biotechnol Biochem*. 2005;69:280—286.
14. The Wealth of India. *A Dictionary of Indian Raw Materials and Industrial Products (Ca—Ci alphabets)*. New Delhi, India: Council of Scientific Industrial Research (CSIR); 1992. 369—370.
15. Singh V. Monograph on Indian subtribe Cassiinae (Caesalpinaceae). *J Econ Taxon Bot*. 2001;18:215—219.
16. Seetharami Reddi TVV, Rama Rao Naidu BVA, Prasanthi S. Herbal Remedies for Diabetes. In: Khan IA, Khanum A, eds. *Ethnomedicine and Human Welfare*, vol. III. Hyderabad, India: Ukaaz Publications; 2005:53.
17. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Allahabad, India: Lalit Mohan Basu Publishers; 1935. 870—873.
18. Maheshwari JK, Kalakoti BS, Lal B. Ethnomedicine of Bhil tribe of Jhabua District, MP. *Anc Sci Life*. 1986;5:255—261.
19. Tiwari HI, Misra M. Phytochemical investigation of *Cassia glauca* bark. *J Indian Chem Soc*. 1993;70:653—659.
20. Rai KN, Kaushalendra K, Singh J. Chemical constituents from the pods of *Cassia glauca* Lam. *Asian J Chem*. 1997;9:558—560.
21. Hus KK, Hong WH. Isolation of quercetin from the flower of *Cassia glauca*. *Chem Abstr*. 1965;62:3433.
22. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. New York: Chapman and Hall; 1998.
23. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin—Ciocalteu reagent. *Methods Enzymol*. 1999; 299:152—178.
24. Dahlqvist A. Method for assay of intestinal disaccharidases. *Anal Biochem*. 1964;7:18—25.
25. Kwon GJ, Choi DS, Wang MH. Biological activities of hot water extracts from *Euonymus alatus* leaf. *J Korean Food Sci Technol*. 2007;39:569—574.
26. Walaas E, Walaas O. Effect of insulin on rat diaphragm under anaerobic conditions. *J Biol Chem*. 1952;195:367—373.
27. Chattopadhyay RR, Sarkar SK, Ganguly S, Banerjee RN, Basa TK. Effect of extract of leaves of *Vinca rosea* Linn. on glucose utilization and glycogen deposition by isolated rat hemidiaphragm. *Indian J Physiol Pharmacol*. 1992;36:137—138.
28. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chem*. 2008; 106:247—252.
29. Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. Glucosidase inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. *Naturwissenschaften*. 1977;64:536—537.
30. Toeller M. α-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. *Eur J Clin Invest*. 1994;24(Suppl. 3):31—35.
31. Chiba S. Molecular mechanism in α-glucosidase and glucoamylase. *Biosci Biotechnol Biochem*. 1997;61:1233—1239.
32. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. *Biosci Biotechnol Biochem*. 1993;57:123—124.
33. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes*. 1981;30:1000—1007.
34. Melander A. Glucose uptake by hemidiaphragm. *Diabet Med*. 1996;13:143.
35. El-Sawi SA, Sleem AA. Flavonoids and hepatoprotective activity of leaves of *Senna surattensis* (Burm.f.) in CCl4 induced hepatotoxicity in rats. *Aust J Basic Appl Sci*. 2010;4:1326—1333.
36. Chew YL, Chan EWL, Tan PL, Lim YY, Stanslas J, Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. *BMC Complement Altern Med*. 2011;11:12.
37. McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors*. 2005;23:189—195.
38. Nickavar B, Abolhasani L, Izadpanah H. α-Amylase inhibitory activities of six *Salvia* species. *Iran J Pharm Res*. 2008;7: 297—303.
39. Yoshida K, Hishida A, Iida O, Hosokawa K, Kawabata J. Flavonol caffeoylglycosides as α-glucosidase inhibitors from *Spiraea cantoniensis* flower. *J Agric Food Chem*. 2008;56:4367—4371.
40. Fang XK, Gao J, Zhu DN. Kaempferol and quercetin isolated from *Euonymus alatus* improve glucose uptake of 3T3-L1 cells without adipogenesis activity. *Life Sci*. 2008;82:615—622.
41. Eid HM, Martineau LC, Saleem A, Muhammad A, Vallerand D, Benhaddou-Andaloussi A, et al. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant *Vaccinium vitis-idaea*. *Mol Nutr Food Res*. 2010;54:991—1003.