Gymnemagenin a Promising Drug Candidate for Management of Hyperglycemia: *In-Vitro* and *In-Vivo* Study

Javed Ahamad [1], Saima Amin [2], Kamran J Naquvi [1], Showkat R Mir [3*]

Abstract

Background: Diabetes mellitus is a chronic metabolic disorder resulting from absolute or relative impairments in β-cell functions, insulin resistance, or both and has been exceptionally increasing worldwide. *Gymnema sylvestre* is an important traditional plant used for the treatment and management of diabetes. Gymnemagenin is an aglycone part of gymnemic acids which is a bioactive component of *G. sylvestre*. The objective of the present study was to undertake *in-vitro* and *in-vivo* studies to generate a stronger biochemical rationale for the management of diabetes mellitus. **Results:** The result of the preliminary *in-vitro* study of gymnemagenin suggested further evaluation of the inhibition of key enzymes related to carbohydrate metabolism. Gymnemagenin showed strong α-amylase (IC_{50} 1.17±0.02 mg/mL) and strong α-glucosidase (IC_{50} 2.04±0.17mg/mL) inhibitory activity. The positive in-vitro enzyme inhibition tests paved the way for confirmatory *in-vivo* studies. The *in-vivo* studies demonstrated that gymnemagenin (20 mg/kg, b.w.) given orally significantly (P<0.01) reduced area under curve (AUC) in mice when challenged with oral administration of starch and sucrose separately. The reduction in AUC by gymnemagenin was comparable to that of acarbose (10 mg/kg, b.w., p.o.). **Conclusion:** The research findings clearly supported the traditional use of *G. sylvestre* in the treatment of diabetes mellitus.

Keywords: *Gymnema sylvestre*, gymnemagenin, diabetes mellitus, α-amylase, α-glucosidase.

1. Introduction

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. The number of people in the world with diabetes has increased significantly over recent years (Ahamad et al., 2019a). The global prevalence of type II diabetes has risen considerably in the last five years; the International Diabetes Federation estimates that there are 425 million people with this disease, which is among the top 10 causes of death worldwide. Hence, new therapies for diabetes are very relevant nowadays (IDF, 2020). Metformin is the first-line medication for the treatment of type 2 DM, while the use of other well-established agents such as glibenclamide and sitagliptin varies in different regions (Flores-Bocanegra et al., 2017). In the management of type 2 DM, controlling postprandial hyperglycemia (PPHG) is also an important strategy. Acarbose, miglitol, and voglibose are the available choices for controlling PPHG. As these agents non-preferentially inhibit α-amylase and α-glucosidase enzymes located in the brush border of the small intestine, delaying the digestion of starch and sucrose and the consequent postprandial blood glucose excursions (Ghani, 2015; Scheen, 2003; Ahamad et al., 2011). Thus, the search for selective inhibitors of these

---

Significance | The study of Clove essential oil as an antioxidant drug.

---

*Correspondence: Dr. Showkat R Mir, Phyto-pharmaceuticals Research Laboratory, Department of Pharmacognosy,
School of Pharmaceutical Education and Research, Jamia Hamdard,
PO Hamdard Nagar, New Delhi-110 062 India,
Email: showkatrmir@gmail.com

enzymes is still on and more and more medicinal plants are being screened continuously. Screening of traditionally used plants for the drug discovery process is often advocated as the chances of success using this approach are more (Fabricant and Farnsworth, 2001, Javed 2023). Hence, as part of an effort to discover α-amylase and α-glucosidase inhibitor lead compounds from natural sources (Ahamad et al., 2016; Ahamad et al., 2019b; Ahamad et al., 2020a,b), we have now investigated gymnemagenin from *Gymnema sylvestre* R.Br. (Asclepiadaceae). This is native to the tropical forests of India and Sri Lanka, commonly known as Gurmar (Kirtikar and Basu, 1998). Traditionally *G. sylvestre* is used for the treatment of diabetes in the Indian subcontinent (Nadkarni, 2007). The major bioactive constituents of *G. sylvestre* are a group of oleanane-type triterpenoid saponins known as gymnemic acids (Wen-Cai et al., 2000; Ahamad et al., 2020c; Alfaqi et al., 2018). The gymnemagenin (Figure 1) is an aglycone moiety of gymnemic acid, which is reported to have several bioactivities (Alfaqi et al., 2019). The present study is aimed to establish the effectiveness of gymnemagenin in achieving glucose homeostasis *via* the inhibition of carbohydrate metabolizing enzymes by *in-vitro* and *in-vivo* studies.

2. Material and Methods

2.1. Chemicals and Regents

α-Amylase, α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (PNPG), and 3,5-dinitrosalicylic acid (DNS) were purchased from SRL, Bangalore, India. Gymnemagenin was purchased from Natural Remedies Pvt. Ltd., Bangalore, India. Acarbose was obtained as a gift sample from Medley Pharmaceutical Ltd. Jammu, India. All other solvents and chemicals were of analytical grade.

2.2. In-Vitro Enzyme Inhibition Assays

The α-amylase and α-glucosidase enzyme inhibitory activity were carried out as per the method published elsewhere by Ahamad et al., 2016. The enzyme inhibition assay was performed for concentrations ranging from 0.15 to 5 mg/mL for gymnemagenin and acarbose is used as a positive control in the study (Ahamad et al., 2021), and briefly described as; gymnemagenin (60 μL) in DMSO with concentrations ranging from 0.15 to 5 mg/mL and 0.1 M phosphate buffer (50 mL; pH 6.8) containing α-glucosidase solution (0.2 U/mL) was incubated at 37 °C for 20 min in 96 well plates. *p*-Nitrophenyl-α-*D*-glucopyranoside (50 mL; 5 mM) solution in a 0.1 M phosphate buffer (pH 6.8) was applied to each well after pre-incubation and further incubated for 20 min at 37 °C. Then the reaction was stopped by adding 160 mL of 0.2 M NaCO₃ into each well. Absorbance (A) was measured at 405 nm with the micro-plate reader and compared to a control which had 60 μL of buffer solution in place of the test sample. Acarbose was

used as a standard drug and evaluated the same way as the test sample.

2.3. In-Vivo Antidiabetic Studies

The oral carbohydrate challenge tests in normal and STZ-induced diabetic rats were carried out as per the methods published elsewhere (Ahamad et al., 2016), and briefly described below:

Wistar albino mice (30-40 g) were obtained from Central Animal Facility, Jamia Hamdard, and maintained under controlled conditions of illumination (12h light/12h darkness) and temperature (20-25 °C). They were housed under ideal laboratory conditions and maintained on a standard pellet diet (Lipton rat feed Ltd., Pune, India) and water *ad libitum* throughout the experimental period. Animals were acclimatized to the conditions before the start of the experiments. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India (Approval no. JH/CAHF/173/CPCSEA/2012/926).

Mice were fasted overnight for 12 h but had free access to water. The animals were randomly divided into seven groups consisting of six mice in each group (n = 6). Group I, served as normal control which received 1 mL/kg b.w. vehicle (0.5% CMC in distilled water). For the oral starch tolerance test, Group II served as starch challenge control that received starch (3 g/kg, b.w.). Group III received acarbose as a standard drug (10 mg/kg, b.w.) while as group IV was administered gymnemagenin (20 mg/kg, b.w.). Treatment groups III and IV were fed starch (3 g/kg, b.w.) after 20 min of treatment. For the oral sucrose tolerance test, group V served as the sucrose-challenged control that received sucrose (4 g/kg, b.w.). Treatment groups VI and VII received acarbose (10 mg/kg, b.w.) and gymnemagenin (20 mg/kg, b.w.), respectively followed by sucrose (4 g/kg, b.w.) after 20 min of the treatment. Blood was withdrawn from the tail vein at 0, 30, 60, 90, and 120 min after the carbohydrate challenge. Blood glucose level (BGL) was measured using a one-touch glucometer (my life Pura, Switzerland). The AUC was calculated using the Trapezoidal method (Purves, 1992).

2.4. Statistical analysis

Values are expressed as mean±SD. Statistical significance was calculated by using a one-way analysis of variance followed by Dunnett's t-test. The values were considered significantly different when $P<0.05$.

3. Results

3.1. In-Vitro Enzyme Inhibition by Gymnemagenin

The gymnemagenin showed concentration-dependent α-amylase inhibition that varied from 57.96±1.01 to 19.29±2.75 % for concentrations ranging from 5 to 0.15 mg/mL, respectively. Acarbose is used as positive control which showed also a concentration-dependent response that varied from 81.33±2.31 to

Table 1. Effect on blood glucose level of gymnemagenin and acarbose in carbohydrate-challenged mice

| Group	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
	Starch tolerance test				
NC	101.4±1.20	100.8±1.07	102±1.14	103±0.45	102±1.09
StC	100.6±1.54	170.4±1.86[##]	161.8±1.93[##]	154.6±1.07[##]	146±1.64[##]
Acar	98.4±0.24	130.8±2.27[**]	115.4±1.33[**]	109.8±0.73[**]	106.4±0.75[**]
Gymn	98.8±0.86	149.4±2.16[*]	140.6±1.29[*]	135.2±1.77[**]	125.6±1.8[**]
	Sucrose tolerance test				
SuC	102.8±0.97	180.6±2.25[##]	175.2±2.08[##]	160.6±2.76[##]	148.8±1.07[##]
Acar	102.2±1.2	143.8±1.66[**]	139.8±1.16[**]	128.8±1.56[**]	115.6±0.68[**]
Gymn	100±1.30	177.2±2.67[ns]	162.6±2.01[*]	139.8±2.17[**]	133.2±1.24[**]
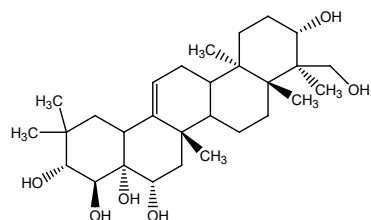
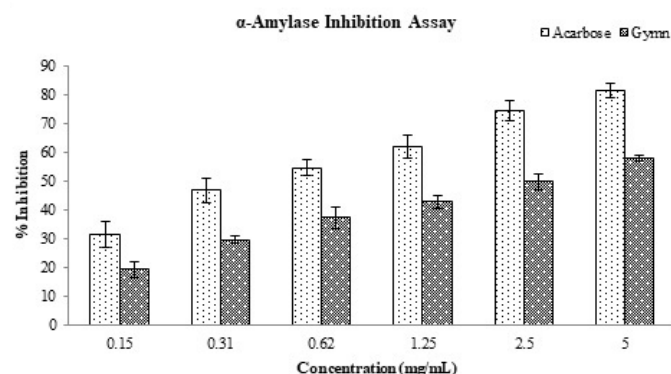**Figure 1.** Chemical structure of Gymnemagenin

Figure 2. Inhibitory activity of acarbose and gymnemagenin against α-amylase
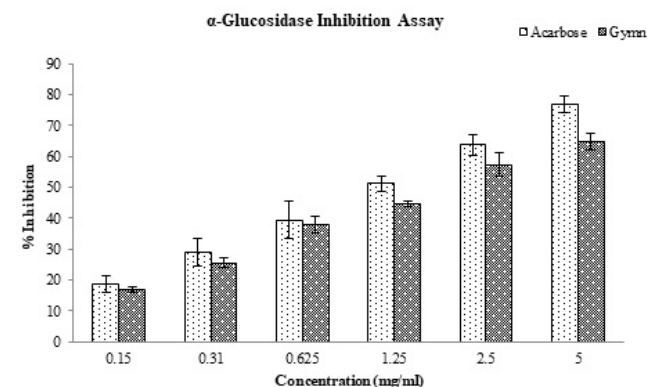(Data were presented as the mean of triplicate determinations ± SD)

Figure 3. Inhibitory activity of acarbose and gymnemagenin against α-glucosidase
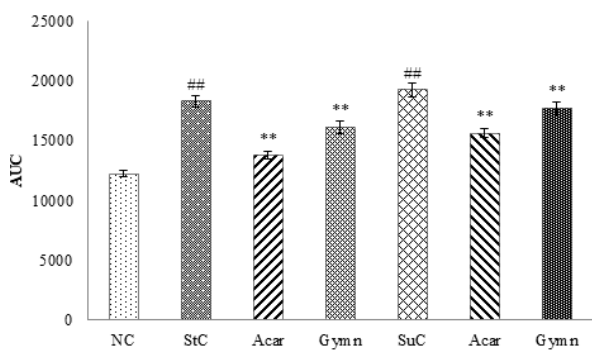(Data were presented as the mean of triplicate determinations ± SD)

Figure 4. AUC of carbohydrate-challenged mice after treatment with acarbose and gymnemagenin
Data were expressed as mean±SD, n=6, **$P<0.01$, test groups vs respective carbohydrate controls; ##$P<0.01$ carbohydrate controls vs normal control (NC). Treatment groups StC: starch control (3 g/kg, b.w.); SuC: sucrose control (4 g/kg, b.w.); Acar: acarbose (10 mg/kg, b.w.) and Gymn: gymnemagenin (20 mg/kg, b.w.)

31.29±4.35 % for 5 to 0.15 mg/mL, respectively. Figure 2 shows the percentage inhibition of α-amylase by gymnemagenin and acarbose. The IC$_{50}$ values for gymnemagenin and acarbose were found as 1.17±0.02 and 0.42±0.02 mg/mL, respectively.

The results of the *in-vitro* α-glucosidase inhibitory study are depicted in Figure 3. The gymnemagenin showed concentration-dependent α-glucosidase inhibition varying from 64.74±1.57 to 16.71±0.81 % for 5 to 0.15 mg/mL, respectively. Acarbose showed a concentration-dependent response that varied from76.82±4.48 to 16.81±2.68 % for 5 to 0.15 mg/mL, respectively. The IC$_{50}$ values for gymnemagenin and acarbose were found as 2.04±0.17 and 1.41±0.17 mg/mL, respectively.

Gymnemagenin is an aglycone of gymnemic acids, the main bioactive compound of Gurmar. Gymnemic acids and their derivatives are reported to have antidiabetic properties (Alkefai et al., 2018; Alkefai et al., 2019; Masayuki et al., 1997; Fushiki et al., 1992; Sugihara et al., 2000). The inhibitors of carbohydrate metabolizing enzymes such as acarbose significantly affect the activities of both these enzymes. The non-specificity of action of these inhibitors results in flatulence that is due to excessive inhibition of α-amylase leading to abnormal bacterial fermentation of undigested carbohydrates (Subramanian et al., 2008). Therefore, agents with comparatively more inhibitory activity against α-glucosidase than against α-amylase will be helpful to overcome this challenge (Ahamad et al., 2016). Our *in-vitro* results indicate that gymnemagenin showed almost similar inhibition against α-amylase (IC$_{50}$ 1.17±0.02 mg/mL) and α-glucosidase (IC$_{50}$ 2.04±0.17 mg/mL) enzymes. The inhibition for α-glucosidase and α-amylase by gymnemagenin was less than that of acarbose.

3.2. In-Vivo Antihyperglycemic Activity

In-vivo studies revealed that oral administration of starch (3 g/kg, b.w.) resulted in significant (*P*<0.01) increase in blood glucose levels in mice. Pre-treatment with gymnemagenin (20 mg/kg, b.w.) decreased BGL significantly (*P*<0.05) after 30 min of the starch challenge to mice. Acarbose (10 mg/kg, b.w.) in mice also produced a significant (*P*<0.01) blood glucose lowering response after 30 min (Table 1). The treatment with acarbose and gymnemagenin showed a significant (*P*<0.05) decrease in AUC in comparison to the starch control group (Figure 4). Oral administration of sucrose (4 g/kg, b.w.) resulted in a significant (*P*<0.01) increase in the BGL of mice. Pre-treatment with gymnemagenin (20 mg/kg, b.w.) decreased BGL non-significantly compared to sucrose challenge to mice after 30 min, but after 120 min gymnemagenin decreased BGL significantly (*P*<0.01) compared to sucrose challenge to mice. Acarbose (10 mg/kg, b.w.) also produced a significant (*P*<0.01) blood glucose lowering response after 30 min (Table 1). The treatment with acarbose and

gymnemagenin showed a significant (*P*<0.01) decrease in AUC in comparison to the sucrose control group (Figure 4).

In *in-vivo* experiments, pre-treatment with gymnemagenin restricted the blood glucose excursions and decreased both peak BGL and AUC in starch and sucrose-challenged mice and the effect was comparable to acarbose. Gymnemagenin seemed to inhibit the carbohydrate metabolizing enzymes in the brush border of the small intestine. It successfully delayed carbohydrate absorption. The retardation and delay of carbohydrate metabolism and absorption by gymnemagenin offer a prospective therapeutic approach for the management of PPHG for pre-diabetics or who have blood glucose levels only slightly above the level considered serious for management through oral hypoglycemics or insulin (Ahamad et al., 2016).

Data were expressed as mean±SD, n=6, **P*<0.05, ***P*<0.01, test groups *vs* respective carbohydrate controls; *\#\#P*<0.01 carbohydrate controls *vs* normal control (NC). Treatment groups StC: starch control (3 g/kg, b.w.); SuC: sucrose control (4 g/kg, b.w.); Acar: acarbose (10 mg/kg, b.w.) and Gymn: gymnemagenin (20 mg/kg, b.w.)

4. Discussion

Gymnemagenin is an aglycone of gymnemic acids, the main bioactive compound of Gurmar. Gymnemic acids and their derivatives are reported to have antidiabetic properties (Alkefai et al., 2018; Alkefai et al., 2019; Masayuki et al., 1997; Fushiki et al., 1992; Sugihara et al., 2000). The inhibitors of carbohydrate metabolizing enzymes such as acarbose significantly affect the activities of both these enzymes. The non-specificity of action of these inhibitors results in flatulence that is due to excessive inhibition of α-amylase leading to abnormal bacterial fermentation of undigested carbohydrates (Subramanian et al., 2008). Therefore, agents with comparatively more inhibitory activity against α-glucosidase than against α-amylase will help overcome this challenge (Ahamad et al., 2016). Our *in-vitro* results indicate that gymnemagenin showed almost similar inhibition against α-amylase (IC$_{50}$ 1.17±0.02 mg/mL) and α-glucosidase (IC$_{50}$ 2.04±0.17 mg/mL) enzymes. The inhibition for α-glucosidase and α-amylase by gymnemagenin was less than that of acarbose.

In *in-vivo* experiments, pre-treatment with gymnemagenin restricted the blood glucose excursions and decreased both peak BGL and AUC in starch and sucrose-challenged mice and the effect was comparable to acarbose. Gymnemagenin seemed to inhibit the carbohydrate metabolizing enzymes in the brush border of the small intestine. It successfully delayed carbohydrate absorption. The retardation and delay of carbohydrate metabolism and absorption by gymnemagenin offer a prospective therapeutic approach for the management of PPHG for pre-diabetics or who have blood glucose levels only slightly above the level considered

serious for management through oral hypoglycemics or insulin (Ahamad et al., 2016).

5. Conclusion

Natural products proved their role in the treatment and management of chronic diseases and disorders such as cancer, malarial, hypertension, diabetes mellitus, etc. *G. sylvestre* is known for its biological activities and it has been reported as a potential antidiabetic plant. The bioactive components of *G. sylvestre* specially gymnemic acids and their derivatives are responsible for the beneficiary activity. As gymnemagenin is an aglycone part of gymnemic acids and its derivatives, the present study demonstrated the role of gymnemagenin in controlling diabetes mellitus by inhibiting α-amylase and α-glucosidase enzymes in *in-vitro* and *in-vivo* studies. Gymnemagenin was found effective in achieving stricter glycemic control in carbohydrate-challenged mice through the inhibition of carbohydrate metabolizing enzymes. The present study proves scientifically the traditional claim of the beneficial role of *G. sylvestre* in diabetes mellitus.

Author Contributions

J.A. conceptualized, performed the experiments and revised the article. S.A. edited and revised; S.R.M. onceptualized and supervised the study.

Competing financial interests

The authors have no conflict of interest.

References

Ahamad, J., Alkefai, N.H.A., Amin, S., Mir, S.R. (2020b). Standardized extract from Enicostemma littorale ameliorates post-prandial hyperglycaemia in normal and diabetic rats. J. Biol. Active Prod. Nat., 10(1): 34-43.

Ahamad, J., Hasan, N., Amin, S., Mir, S.R. (2016). Swertiamarin contributes to glucose homeostasis via inhibition of carbohydrate metabolizing enzymes. J. Natural Remedies, 16(4), 125-130.

Ahamad, J., Kaskoos, R.A., Amin, S., Mir, S.R (2020c). Quantitative Analysis of Gymnemagenin in Gymnema sylvestre Leaves and in Herbal Formulation by a Validated HPTLC Method. J Biolog Active Prod Nat., 10:3, 211-219,

Ahamad, J., Naquvi, K.J., Amin, S., Mir, S.R. (2021). Gymnemic Acid-Rich Fraction from Gymnema sylvestre Leaves Ameliorates Post Prandial Hyperglycaemia in In-Vitro and In-Vivo Studies. J Biolog Active Prod Nat., 11(1), 32-41.

Ahamad, J., Naquvi, K.J., Mir, S.R., Ali, M., Shuaib, M. (2011). Review on role of natural alpha-glucosidase inhibitors for management of diabetes mellitus. Int. J. Biomed. Res., 6, 374-380.

Ahamad, J., Toufeeq, I., Khan, M. A., Ameen, M.S.M., Anwer, E.T., Uthirapathy, S., et al., (2019a). Oleuropein: A Natural Antioxidant Molecule in the Treatment of Metabolic Syndrome. Phytother. Res., 33(12), 3112-3128.

Ahamad, J., Uthirapathy, S., Ameen, M.S.M., Anwer, E.T. (2019b). Essential oil composition and antidiabetic, anticancer activity of Rosmarinus officinalis L. leaves from Erbil (Iraq). J. Essent. Oil Bear. Pl., 22(6), 1544-1553.

Ahamad, J., Uthirapathy, S., Ameen, M.S.M., Anwer, E.T., Mir, S.R. (2020a). Chemical composition and in-vitro antidiabetic effects of Olea europaea Linn. (Olive). Curr. Bioactive Comp., 16(8), 1157-1163.

Alkefai, N.H., Ahamad, J., Amin, S., Mir, S.R. (2018). Arylated gymnemic acids from Gymnema sylvestre R.Br. as potential α-glucosidase inhibitors. Phytochem Lett., 25: 196-202.

Alkefai, N.H., Sharma, M., Ahamad, J., Amin, S., Mir, S.R. (2019). New olean-15-ene type gymnemic acids from Gymnema sylvestre (Retz.) R.Br. and their antihyperglycemic activity through α-glucosidase inhibition. Phytochem. Lett., 32: 83-89.

Fabricant, D.S., and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect., 109(1), 69-75.

Flores-Bocanegra, L., Gonzalez-Andrade, M., Bye, R., Linares, E., Mata, R. (2017). New α-Glucosidase inhibitors from Salvia circinata. J. Nat. Prod., 80, 1584-1593.

Fushiki, T., Kojima, A., Imoto, T., Inoue, K., Sugimoto, E. (1992). Extract of Gymnema sylvestre leaves and purified gymnemic acid inhibits glucose-stimulated gastric inhibitory peptide secretion in rats. J. Nutr., 122, 2367-2373.

Ghani, U. (2015). Re-exploring promising α-glucosidase inhibitors for potential development into oral anti-diabetic drugs: Finding needle in the haystack. Eur. J. Med. Chem., 103, 133-162.

IDF Diabetes Atlas, 8th ed.; International Diabetes Federation. Available at http://www.diabetesatlas.org. Accessed June 27, (2023).

Javed Ahamad. (2023). Characterization of Essential Oil Composition of Syzygium aromaticum Linn. (Clove) by GC-MS and Evaluation of its Antioxidant Activity, Journal of Angiotherapy, 7(1), 1-5, 9358.

Kirtikar, K., Basu, B. (1998). Indian Medicinal Plants, International Book Distributors, Deharadun, India. Vol III, Pp. 1625.

Masayuki, Y., Toshiyuki, M., Masashi, K., Yuhao, L., Nubotoshi, M., Johji, Y., Hisash, M. (1997). Medicinal Foodstuffs (IX1) The inhibitors of glucose absorption from the leaves of Gymnema sylvestre R.Br. (Asclepiadaceae): structures of gymnemosides A and B. Chem. Pharm. Bull., 45(10), 1671-1676.

Nadkarni, K.M. (2007). Indian Materia Medica, Popular Prakashan, Bombay, India. Vol. I, p. 596-99.

Purves, R.D. (1992). Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the moment-curve (AUMC). J. Pharmacokinet Biopharm., 20, 211-227.

Scheen, A.J. (2003). Is there a role for α-glucosidase inhibitors in the prevention of type 2 diabetes mellitus? Drugs, 63, 933-951.

Subramanian, R., Asmawi, M.Z., Sadikun, A. (2008). In-vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim. Pol., 55(2), 391-398.

Sugihara, Y., Nojima, H., Matsuda, H., Murakami, T., Yoshikawa, M., Kimura, I. (2000). Antihyperglycemic effects of gymnemic acid IV, a compound derived from Gymnema sylvestre leaves in streptozotocin-diabetic mice. J. Asian Nat. Prod. Res., 2(4), 321-327.

Wen-Cai, Y., Zhang, Q.W., Liu, X., Che, C.T., Zhao, S.X. (2000). Oleanane saponins from Gymnema sylvestre. Phytochem., 53, 893-899.