Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 49- 54

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INVITRO α- AMYLASE AND α- GLUCOSIDASE INHIBITORY ACTIVITIES OF THE ETHANOLIC EXTRACT OF *DIOSCOREA VILLOSA* TUBERS.

ANITHA ROY [1*] AND R.V GEETHA[2]

1. Faculty of Pharmacology, Saveetha Dental College and Hospitals, Chennai, India
2. Faculty of Microbiology, Saveetha Dental College and Hospitals, Chennai, India

ABSTRACT

Objective: The objective of this study was to evaluate *α*- amylase inhibitory and α – glucosidase inhibitory activity of the ethanolic extract of *Dioscorea villosa* tubers *in vitro*.
Materials and Methods: Different concentrations of the plant extract (1.5, 3, 7, 15.30, 60, 125, 250, 500 and 1000 µg/ml) were prepared in ethanol and subjected to *α* amylase inhibitory and *α* Glucosidase inhibitory assay. The absorbance was read at 540nm and 546nm respectively using spectrophotometer. Using this method, the percentage of *α* -amylase inhibitory activity, *α* -glucosidase inhibitory activity and the IC_{50} values of each assay were calculated. Results: The ethanolic extract of *Dioscorea villosa* tubers exhibited appreciable *α*- amylase inhibitory activity with an IC_{50} values 72.44µg/ml when compared with acarbose, IC_{50} value 83.23 ± 0.39 µg/mL and α - glucosidase inhibitory activity with an IC_{50} value of 28.96 µg / ml when compared with acarbose IC_{50} value 35.03 ± 0.24 µg/mL
Conclusion: This study supports the possible use of *Dioscorea villosa t*ubers for diabetes because of its considerable α-amylase inhibitory activity as well as α - glucosidase inhibitory activity.

KEY WORDS **Diosgenin, *Dioscorea villosa,* extract, Antidiabetic activity.**

ANITHA ROY
Faculty of Pharmacology, Saveetha Dental College and Hospitals,
Chennai, India anitharoypeeter@yahoo.co.in

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins .Type 2 diabetes has developed into a major health problem and is responsible for early morbidities and mortality that affects over a billion people worldwide.[1] Studies and surveys show that its world prevalence in adults may reach 7.7 % by 2030 though it was 6.4% in 2010. Developing countries have a faster increase in number of adults with diabetes compared to developed countries.[2] Type 2 diabetes may be treated with many drugs like sulphonyl ureas, biguanides, thiazolidinediones, Meglitinides, acarbose and miglitol etc. Among these drugs, acarbose and miglitol are newer drugs with a different mechanism of action. They inhibit α-glucosidase enzymes responsible for the metabolism of carbohydrates. Inhibition of α-glucosidase and α-amylase helps to control the post prandial hyperglycemia. [3, 4] many plants have potential ingredients with α -glucosidase and α -amylase inhibitory activity and are used for the management of diabetes. *Dioscorea villosa* (wild yam) family *Dioscoraeceae* is a perennial, tuberous, twining vine with pale-brown, knotty, woody and cylindrical tubers. The plant consists of constituents like diosgenin, Dioscin, Protodioscin, Meprotodioscin , Perrisaponin and Progenin II etc; [5] Commercial diosgenin is reported to have different pharmacological activities like hypoglycaemic, antioxidant, hypolipidaemic, neuroprotective, vasodilating activity and has role in melanogenesis and cholesterol metabolism.[6] Many plants like Fenugreek[7] and other species of Dioscorea are reported to have diosgenin and have been evaluated for their anti-diabetic activity.[8] So, in the present study, an effort was made to explore the anti-diabetic activity of *Dioscorea villosa*, as the literature survey revealed that no scientific study has been carried out to evaluate the anti-diabetic activity of this particular species of wild yam. Inhibition of α -amylase and α - glucosidase enzymes can be an important strategy in management of postprandial blood glucose level in type 2 diabetes patients.[9] Hence , an *in vitro* α –amylase inhibitory activity and α – glucosidase inhibiting activity of the ethanolic extract of *D.villosa* was carried out.

MATERIALS AND METHODS

Plant material

The ethanolic extract of *Dioscorea villosa* tubers was obtained from Green Chem Herbal Extract & Formulations. Bangalore.

Preparation of extract

20 mg of extract was weighed and dissolved in 10 ml of ethanol and further dilution was carried out to obtain various concentrations (1.5µg / ml – 1000 µg / ml). From this 500 µl was used for estimation α-amylase and 200 µl was used for α – glucosidase activities.

In vitro α-Amylase Inhibitory Assay [10]

Invitro α-amylase inhibitory activity was evaluated by Miller 1959.[10] 500 µl of starch solution (0.2%), phosphate buffer (PO_4, 0.2M, pH 7), NaCl (1%) and extract was pre-incubated for 5 min with the test and control sample. The reaction was started by adding 200 µl of diastase (10µM) to the test alone and it was terminated after 15 min of incubation at 37^0C after adding NaOH (2M) and boiling for 1min. Then diastase enzyme was added to control tubes followed by DNSA (3, 5 dinitrosalicylic acid, colouring agent) to both control and test tubes. The tubes were boiled for 2 min, cooled and the OD was read at 540 nm. The assay procedures were done in triplicate and the standard error from the mean was taken. The inhibitory activity of the extract was calculated as follows and given in table 1.

% Inhibition= [(control-test)/control)]*100

Table 1
α - amylase inhibitory activity of the D.villosa extract

Concentration (µg/ml)	% inhibition
1.5	10.71±0.00
3	24.35±3.04
7	32.61±4.49
15	35.65±3.19
30	43.77±3.19
60	48.99±3.77
125	56.96±6.09
250	61.74±4.64
500	66.58±4.35
1000	71.88±3.77

Results are expressed in terms of mean ± SEM

In vitro α-glucosidase inhibitory assay [11]

In vitro α-glucosidase inhibitory activity was evaluated by Li *et al*., 2004.[11] 200 µl of α-glucosidase solution (0.6U / ml) was pre-incubated with the various concentrations of test samples and control sample for 5 min. The reaction was started by adding 200µl of sucrose (37 mM) and it was terminated after 15min incubation at 37^0C by heating in water bath. The liberated glucose was determined. The enzyme activity is directly proportional to the liberated glucose and the liberated glucose is measured by GOD-POD method at 546nm using semi auto analyzer. The assay procedures were done in triplicate and the standard error from the mean was taken. The inhibitory activity of the extract was calculated as follows and given in table 2. % Inhibition = [(control-test)/control)]*100

Table 2
α - glucosidase inhibitory activity of the D.villosa extract

Concentration (µg/ml)	%inhibition
1.5	14.65±1.26
3	25.13±0.33
7	37.19±0.45
15	44.06±0.69
30	51.24±2.05
60	58.32±1.25
125	63.21±1.24
250	70.71±1.47
500	74.97±1.65
1000	77.07±1.36

Results are expressed in terms of mean ± SEM

Calculation of 50% Inhibitory Concentration (IC_{50}) [12]

The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at ten different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, Where, Ac is the absorbance of the control and As is the absorbance of the sample. The IC_{50} value of the extract for both its α-glucosidase activity and α-amylase inhibitory activity were calculated and reported in the Table 3.

Table 3
IC_{50} for α-glucosidase and α-amylase inhibitory activitiesof the D. villosa extract

α - glucosidase inhibitory activity	28.96 µg / ml
α - amylase inhibitory activity	72.44 µg / ml

RESULTS AND DISCUSSION

In vitro tests can play a very important role in the evaluation of antidiabetic activity of drugs as initial screening tools, where the screening of large number of potential therapeutic candidates may be necessary. They might provide useful information on the mechanism of action of therapeutic agent .[13] Cochrane systematic review and meta-analysis revealed that fasting and postprandial blood glucose level were reduced by α-glucosidase inhibitors.[14]In the present study, the ethanolic extract of *D.villosa* showed a dose - dependent increase in the α - glucosidase inhibitory activity ranging from 14.65±1.26 to 77.07 ±1.36 percent with a concentration range of 1.5µg/ml - 1000µg/ml and the IC_{50} value was calculated as 28.96 µg / ml , when compared with acarbose IC_{50} value 35.03 ± 0.24 µg/mL. The α – amylase inhibitory activity also showed a dose - dependent increase ranging from 10.71 ± 0.00 to 71.88 ± 3.77 percent with a concentration range of 1.5µg/ml - 1000µg/ml and the IC_{50} value was calculated as 72.44 µg / ml when compared with acarbose, IC_{50} value 83.23 ± 0.39 µg/mL and is reported in Table 1, 2,3 and figure 1 and 2

Figure1
Linear graph for α - amylase inhibitory activity of the ethanolic extract of D. villosa

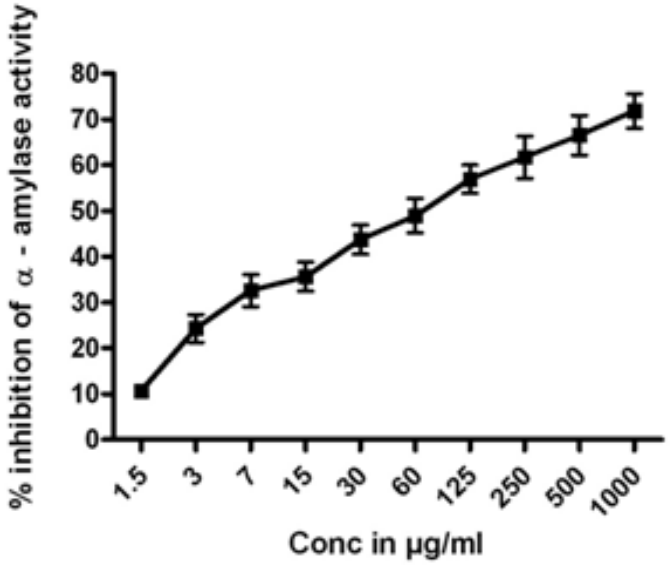
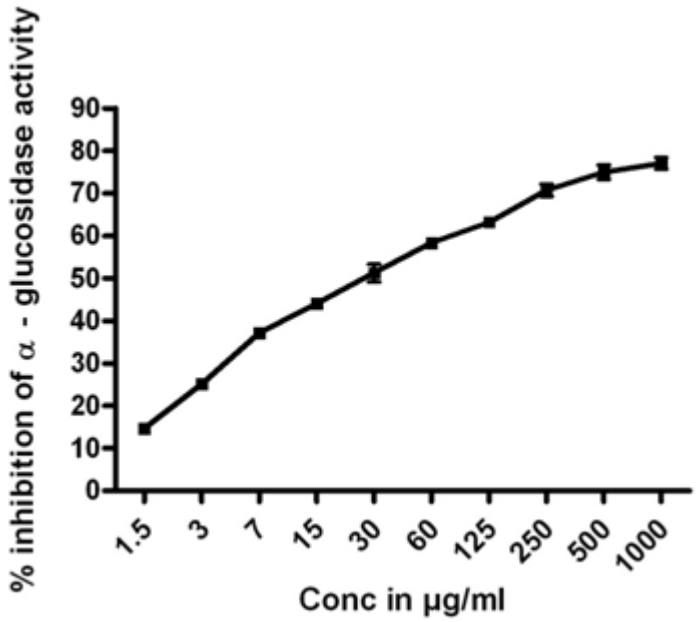


Figure 2
Linear graph for α glucosidase inhibitory activity of the ethanolic extract of D. villosa



CONCLUSION

The understanding of the activity of the α-amylase and α-glucosidase in the carbohydrate digestion and glucose absorption have lead to the development of many newer pharmacological agents.[12] Plants like *Sesbania grandiflora*. [15] and *Calotropis gigantean* [16] etc ; have studied for their *invitro* anti diabetic activity using such models. Definitely the newer mechanisms and new discoveries of drugs for the control of blood sugar will be highly promising as Diabetes is a major metabolic disorder faced by the society. Since the other species of Dioscorea have already explored, present study on *Discorea villosa* can bring novelty to this area of research.

ACKNOWLEDGMENT

The authors take opportunity to thank Mr R Rajendren, CEO, Green Chem, Bangalore for providing the extract for the research work. Authors are also thankful to the Principal, Dr.N.D Jayakumar, Saveetha Dental College and Hospital, for the constant motivation to carryout research work. Finally authors extend due thanks to R.P. Parameswari, Senior Research Fellow, Herbal and Indian Medicine Research Laboratory, Sri Ramachandra University, Porur for her timely help and interpretation

REFERENCES

1. Unwin N, Gan D, Whiting D,The IDF Diabetes Atlas: providing evidence, raising awareness and promoting action. Diabetes Res Clin Pract 2010; 87(1):2-3.
2. Shaw, J.E. , Sicree, R.A., Zimmet, P.Z. Global estimates of the prevalence of diabetes for 2010 and 2030 (Review) Diabetes Res Clin Pract 2010, 87 (1): 4-14.
3. Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Brunton LL, Lazo JS, Parker KL (Ed.), Goodman and Gilman's: The

pharmacological basis of therapeutics, 11th ed. (McGraw-Hill Medical Publication Division:New York 2001) 1706-1707.

4. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. Biological & Pharmaceutical Bulletin 2005; 28 (6): 1098-1102.
5. Saurabh Satija,Meenu Mehta , Vandna Kalsi, Wild Yam : A Review To Dioscorea villosa, Int.J.Pharma Res and Develop (IJPRD), 2011; Vol 3(5):117 – 121.
6. Mirunalini and Shahira, Novel effects of Diosgenin –a plant derived steroid-A review, Pharmacologyonline 1: 726-736 (2011) Newsletter.
7. Taylor WG, Elder JL.,Chang PR,Richards K.W, Microdetermination of diosgenin from fenugreek (Trigonella foenum- graecum) seeds. J. Agric Food Chem,2000; 48(11):5206 -5210.
8. Sougata Ghosh, Mehul Ahire, Sumersing Patil, et al., "Antidiabetic Activity of Gnidia glauca and Dioscorea bulbifera: Potent Amylase and Glucosidase Inhibitors," Evidence-Based Complementary and Alternative Medicine, Vol. 2012, Article ID 929051.10 pages (2012)
9. Ali H, Houghton PJ, Soumyanath A. alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus; J. Ethnopharmacol 2006; 107(3): 449–455.
10. G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar", Anal. Chem., 1959 (31): 426–429.
11. Li,Y.,Peng,G.,Li,Q.,Wen,S.,Huang,T.H.,Ro ufogalis,B.D.,Yama-hara, J., Salacia oblonga improves cardiac fibrosis and inhibits postprandial hyperglycemia in obese sucker rats. Life Sci; 2004 (75) :1735-1746.
12. M. B. Narkhede et al; In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract, Asian J. Plant Sci. Res., 2011, 1(2):101- 106 .
13. Krutika Thorat, Leena Patil, Dnyanesh Limaye and Vilasrao Kadam , Invitro Models for Antidiabetic Activity Assessment , Intl J. of Res. in Pharma. and Biomed. Sci ; 2012, 3 (2) :730-732.
14. Van de Laar FA, Lucassen PL, Akkermans RP, Van de Lisdonk EH, Rutten GE, Van Well C. Alpha-glucosidase inhibitors for patients with type 2 diabetes: Results from a Cochrane systematic review and meta-analysis. Diabetes Care 2005; 28:166-75.
15. Boonmee A, Reynolds CD, Sangvanich P. Alpha-glucosidase inhibitor proteins from Sesbania grandiflora flowers. Planta Med 2007; 73:1197-201.
16. Choudary NK, Jha AK, Sharma S, Goyal S, Dwivedi J. Anti-diabetic potential of chloroform extract of flowers of Calotropis gigantea: An in vitro and in vivo study. Intl J. of Green Pharmacy 2011; 5:293-301