*ejpmr, 2015,2(2),375-380*

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

# INHIBITORY EFFECT OF α - GLUCOSIDASE AND α - AMYLASE ENZYME ACTIVITIES OF *ADHATODA VASICA* NEES

***Vadivelan R[1], Dhanabal SP [2], Santilna KS [1], Shirisha S[1].**

[1]Department of Pharmacology, [2]Department of Phytopharmacy and Phytomedicine, JSS College of Pharmacy (JSS University, Mysore) Udhagamandalam, Nilgiris, Tamilnadu, India-643001.

**Article Received on 21/01/2015** **Article Revised on 11/02/2015** **Article Accepted on 02/03/2015**

***Correspondence for Author**
**Dr. R. Vadivelan**
Department of Pharmacology, JSS College of Pharmacy, (JSS University,Mysore) Udhagamandalam, Nilgiris, Tamilnadu, India-643001

## ABSTRACT

**Aim:** The present study is to evaluate the *in-vitro* antidiabetic activity of the *ethanolic* extract and fractions of *Adhatoda vasica Nees* by α-glucosidase and α -amylase inhibitory activity. **Methods:** The leaves of the *Adhatoda vasica Nees* was extracted using ethanol, and fractions by using chloroform and n-butanol. The extract and fractions were then used to study its α-glucosidase and α -amylase inhibitory activity **Results:** *Adhatoda vasica* ethanolic extract and fractions showed dose dependent inhibition of α-glucosidase and α -amylase enzyme and exhibited lower inhibitory activity than acarbose. **Conclusion:** The study revealed the antidiabetic potential and could be helpful to develop medicinal preparations and nutraceuticals and function foods for diabetes.

**KEYWORDS:** *Adhatoda vasica*, α- Glucosidase/ α - Amylase inhibitor, Diabetes, Nilgiris.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to the secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves.[1]

One of the therapeutic approaches is to decrease the postprandial hyperglycemia, by retarding the absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase.[2] From this point of view, many efforts have been made to search

for more effective and safe inhibitors of α-glucosidase and α-amylase from natural materials to develop physiological functional food to treat diabetes.[3]

Many traditional plants have been reported in India for diabetes, but only a small number of these have received scientific and medical evaluation to assess their efficacy. On the basis of ethno medical/tribal information *Adhatoda vasica has been* used to treat and prevent diabetes. *Adhatoda vasica* Nees possess a diverse number of pharmacological activities including antioxidant and radical scavenging activity,[4–6] anticholinesterase action[7,8] and anti-inflammatory property.

However, the studies on anti-diabetic effects of *Adhatoda vasica* were not focused on the enzyme inhibitory activity of the extract and fractions. The present study is designed to study the *in-vitro* antidiabetic activity of ethanolic extract of *Adhatoda vasica* and to understand how the extract and fractions acts against α-glucosidase and α-amylase.

## MATERIALS AND METHODS

### Plant Material

The entire plant of *Adhatoda vasica* was collected from the forests of Doddabetta in Nilgiris. The plant species was identified and authenticated by Botanist, Government Botanical garden,Ooty. The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, JSS College of Pharmacy, Ooty. The leaves of the plant were used in the study.

### Chemicals

Porcine pancreatic amylase, α-glucosidase from Bakers yeast, p-nitrophenyl-a-d-glucopyranoside and dinitrosalicylic acid were purchased from Sigma chemicals. All the chemicals used in the study are of analytical grade.

### Preparation of crude extract and fractions

The leaves were crushed in to powder after drying under shade. 100 g powdered sample were weighed and soaked in 250 ml of 95% ethanol in a separating funnel for 24 hours, with intermittent shaking. The plant extract were then collected and filtered through Whatman No. 1 filter paper. The extract were concentrated at 50 $^{0}$C using vaccum rotatory evaporator and then air-dried. The dried powder was stored at 40 $^{0}$C in an airtight bottle. The extract was fractioned with chloroform and n-butanol and all were used for enzyme studies.

**α – Amylase inhibitory activity**

The α-amylase inhibitory activity of the *Adhatoda vasica* ethanolic extract and fractions (chloroform and n-butanol) were determined.[9] 250 µl of sample and 125 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 6 mMNaCl) containing α - amylase solution (0.5 mg/ml) was incubated at 25 $^0$C for 10 min. After preincubation, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mMNaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 $^0$C for 10 min. The reaction was stopped with 0.5 ml of dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 5 ml of distilled water, and absorbance was measured at 540 nm. Acarbose was used as the positive control. The α-amylase inhibitory activity was calculated as follows:

**Inhibition (%) = (1- $A_s$ / $A_c$) × 100**

where $A_s$ and $A_c$ are the absorbance of the sample and the control respectively.

**α -Glucosidase inhibitory activity**

The α-glucosidase inhibitory of activity Adhatoda vasica ethanolic extracts and fractions (chloroform and n-butanol) were determined.[10] A mixture of 50 µl of sample and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α -glucosidase solution (1 U/ml) was incubated in 96 well plates at 25 $^0$C for 10 min. After preincubation, 50 µl of 5 mMpNPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 $^0$C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by microplate reader. Acarbose was used as the positive control. The α-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

**Inhibition (%) = (1- $A_s$ / $A_c$ ) × 100**

Where $A_s$ and $A_c$ are the absorbance of the sample and the control respectively.

**RESULTS**

The ethanolic extract and fractions (chloroform and n-butanol) of *Adhatoda vasica* showed dose dependent inhibition of the α-amylase enzyme ($IC_{50}$ =35-45µg/ml). The extract and fractions exhibited lower α-amylase inhibitory activity, compared with that of acarbose, which showed potent inhibition of α –amylase.

The ethanolic extract and fractions (chloroform and n-butanol) of *Adhatoda vasica* showed dose dependent inhibition of the α-glucosidase enzyme($IC_{50}$ =45-50µg/ml). The extract and fractions exhibited lower α -glucosidase inhibitory activity, compared with that of acarbose, which showed potent inhibition of α –glucosidase.

The enzyme inhibitory activities of extract and fractions of *Adhatoda vasica* on enzyme is given in **Table 1.**

**Table 1** α –**amylase and α –Glucosidase inhibitory activity of *Adhatoda Vasica***

| Groups | Treatment | α -amylase (IC 50) µg/ml | α -Glucosidase (IC 50) µg/ml |
|---|---|---|---|
| 1 | EEAV | 25.42±0.45 | 43.62±1.53 |
| 2 | CFAAV | 35.33±0.57 | 49.26±1.20 |
| 3 | BFAV | 42.45± 1.13 | 45.62±1.32 |
| 4 | ACARBOSE | 11.64±0.12 | 14.39±0.19 |

Values are expressed as Mean ± SEM (n=3)

EEMM – Ethanolic extract of *Adhatoda vasica*

CFMM – Chloroform fraction of *Adhatoda vasica*

BFMM – n- butanol fraction of *Adhatoda vasica*

## DISCUSSION

The treatment goal of diabetes patients is to maintain glycemic level in control, in both the fasting and post-prandial states. Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine.[11]

Pancreatic α- amylase is a key enzyme in the digestive system which catalyzes the initial step in the hydrolysis of starch, which is a principal source of glucose in the diet. α-Glucosidase, a key enzyme for carbohydrate digestion, has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 DM. α-amylase catalyzes the hydrolysis of α-1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α –glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes.[12] Therefore, effective and nontoxic inhibitors of a-amylase and a-glucosidase have long been sought.

In this study we have investigated the anti-diabetic potential of the *Adhatoda vasica*, which is used in traditional ayurvedic medicine for the treatment of several diseases.[13] This valuable herb was not previously investigated for its *in vitro* anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *Adhatoda vasica*, and revealed that the active principles responsible may be flavonoids, terpenes and phenolic compound.

Flavonoids, like anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. Although, in the present study, the enzyme inhibitory activity of these extract and fractions were assayed *in-vitro*, the results from this work should be relevant to the human body. In addition to α-amylase and α-glucosidase inhibitory activities, these phytoconstituents are also reported to have several other biological activities including anti-bacterial, anti-oxidative,anti-cancer etc.[14] This supportive evidence further increases the medicinal importance of this *Adhatoda vasica* indicating that this herb is not only beneficial for diabetes but also may be useful to a number of other human health complications.

## CONCLUSION

This study investigated the potential anti-diabetic activity of the *Adhatoda vasica Nees*, focusing on the inhibitory effects on α-glucosidase and α-amylase. Further isolation of active principles would be helpful to explain the pharmacological mechanism and also to develop medicinal preparations, nutraceuticals or functional foods for diabetes and related symptoms.

## ACKNOWLEDGEMENTS

The authors are grateful to the principal and management, JSS College of Pharmacy,(A constituent college of JSS University, Mysore) Udhagamandalam, Nilgiris, Tamilnadu, – 643001, India for providing the necessary infrastructure to carry out this research work in successful manner.

## REFERENCES

1. Matsui T, Tanaka T, Tamura , Toshima A, Miyata Y, Tanaka K., et al. Alpha-glucosidase inhibitory profile of catechins and theaflavins. J. Agric. Food Chem., 2007; 55: 99–105.
2. Bhandari M R, Nilubon JA, Gao , Kawabata J. α -Glucosidase and α amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergeniaciliata Haw.). Food Chem., 2008; 106: 247–52.

3. Kao Y H, Chang HH, Lee M J, Cheng C L. Tea, obesity and diabetes. Mol. Nutr. Food Res, 2006; 50(2):188–210.
4. Kumar A, Ram J, Samarth RM, Kumar M. Modulatory influence of Adhatoda vasica Nees leaf extract against gamma irradiation in Swiss albino mice. Phytomedicine, 2005;12:285–93
5. Samarth RM, Panwar M, Kumar M, Soni A, Kumar M, Kumar A. Evaluation of antioxidant and radical-scavenging activities of certain radioprotective plant extracts. Food Chem., 2008;106: 868–73.
6. Roja G, Vikrant BH, Sandur SK, Sharma A, Pushpa KK. Accumulation of vasicine and vasicinone in tissue cultures of Adhatoda vasica and evaluation of the free radical-scavenging activities of the various crude extracts. Food Chem., 2011; 126: 1033–8.
7. Gao H, Huang YN, Gao B, Li P, Inagaki C, Kawabata J. Inhibitory effect on α-glucosidase by Adhatoda vasica Nees. Food Chem., 2008; 108: 965–72.
8. Lahiri PK, Pradhan SN. Pharmacological investigation of vasicinolan alkaloid from Adhatoda vasica Nees. Indian J. Exp. Biol.., 1964; 2: 219–22.
9. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour. Technol., 2010; 101: 4676–89.
10. Apostolidis E, Lee C.In vitro potential of Ascophyllumnodosum phenolic antioxidant-mediated α -glucosidase and α amylase inhibition. J. Food Sci., 2010; 75: 97–102.
11. Hara Y, Honda M. The inhibition of α -amylase by tea polyphenols. Agric. Biol. Chem., 1990; 54(8): 1939–45.
12. He Q. Lv Y, Yao. K. Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. Food Chem., 2006; 101: 1178–82.
13. Wan SB, Chen D, Dou QP, Chan TH. Study of the green tea polyphenols catechin-3-gallate (CG) and epicatechin-3- gallate (ECG) as proteaseome inhibitors. Bioorg. Med. Chem., 2004; 12: 3521–7.
14. Rizvi SI, Anis MA, Zaid R, MishraN. Protective role of tea catechins against oxidation-induced damage of type 2 diabetic erythrocytes. Clin. Exp. Pharmacol Physiol, 2005; 32(1–2): 70–5.