



Research Article

EVALUATION OF *IN VITRO* ANTI-COAGULANT AND *IN VITRO* ANTI DIABETIC ACTIVITY OF *ALSTONIA SCHOLARIS* LEAVES

B. Nagaraju ^{*1}, A. Ramu ¹, S. Vidyadhara ¹, M. Yaso Deepika ²

¹Department of Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur-19, A.P, India

²Department of Pharmacology, Anurag group of institutions, Ghatkesar, Hyderabad, India

*Corresponding Author Email: bnrajupharma@gmail.com

Article Received on: 11/08/16 Revised on: 13/09/16 Approved for publication: 08/10/16

DOI: 10.7897/2230-8407.0710117

ABSTRACT

This study was conducted to evaluate in vitro anti-coagulant and in vitro anti diabetic activity of ethanolic extract of *Alstonia scholaris* leaves. For in vitro anti-coagulant activity we using blood samples, blood was centrifuged and plasma was used, in this method 0.2 ml of plasma is incubated with 0.1 ml of 200 and 400ug/ml plant extract and 0.3ml of 25mM cacl2 solution and time for coagulation is recorded and for in vitro antidiabetic activity we estimate the alpha amylase inhibition and alpha glucosidase inhibition methods and percentage of inhibition was calculated. Anti-coagulant activities of ethanolic extract of *Alstonia scholaris* were carried out, 400ug/ml solution delayed the coagulation time for 26.56 min. There was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 0.2 ml of plant extract showed a percentage inhibition 27.8% and for 1.0 ml plant extract showed inhibition of 96.3%. The *Alstonia scholaris* ethanol extract revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *Alstonia scholaris* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 89.4% - 31.7% for highest concentration to the lowest concentration. In this present study we evaluated in vitro anti-coagulant, in vitro alpha amylase and alpha glucosidase activity of crude methanol extract of *Alstonia scholaris* leaves. The plant showed significant inhibition activity.

Key words: In vitro, coagulant, alpha amylase, glucosidase

INTRODUCTION

Hemostasis is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel. It is the first stage of wound healing. This involves coagulation, blood changing from a liquid to a gel. Intact blood vessels are central to moderating blood's tendency to form clots. The endothelial cells of intact vessels prevent blood clotting with a heparin-like molecule, thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin. When endothelial injury occurs, the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von Will brand factor which initiate the maintenance of hemostasis after injury. Hemostasis has three major steps: 1) vasoconstriction, 2) temporary blockage of a break by a platelet plug, and 3) blood coagulation, or formation of a fibrin clot.

Phospholipids surfaces, calcium ions play an active role in the final development of the fibrin clot ¹. Most adult cardiovascular disorders involving cerebral hemorrhage, coronary thrombosis, arteriosclerosis and congestive heart failure are caused by problems in the blood circulatory system as blood clotting disorders which constitute a serious medical problem. A number of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin have been used as antithrombotic agents ²⁻⁵.

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycemia leading to acute and chronic complications. Apart from conventional diabetes therapy, several studies have shown that some plants used in traditional medicine have beneficial effects in diabetic

patients.⁶ Thus searching for a new class of compounds is essential to overcome diabetic problems there is continuous search for alternative drugs ⁷. Inhibition of alpha- amylase and alpha-glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient ⁸. Thus, objective of the present study is to investigate the in vitro anti-coagulant and in vitro antidiabetic activity of ethanolic extract of *Alstonia scholaris* leaves.

MATERIALS AND METHODS

Collection and identification

Alstonia scholaris leaves were collected from the local medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India. Authenticated by the Department of Botany, ANU, Guntur, voucher specimen are for future reference.

Drying and grinding

The collected plant part (leaves) was separated from undesirable material and the leaves were dried under shade at room temperature for two week. The dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in air tight container until the analysis was commenced.

Extraction

Coarsely powdered leaves (500 g) were successively extracted with petroleum ether for 7 days to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% ethanol to obtain ethanolic extract. The ethanolic extract was

evaporated under reduced pressure at low temperature to dryness and brownish yellow colour extracts of was obtained⁹.

Preliminary Phytochemical screening

Ethanol extract of *Alstonia scholaris* was subjected to preliminary phytochemical for the detection of various constituents¹⁰.

In vitro anticoagulant activity

Collection of blood samples

The samples of blood are obtained from the normal persons by using syringes, withdrawn from superior cubital vein of right arm of each person and transferred into the centrifuge tubes already coated with EDTA to prevent clotting. Now the blood was centrifuge at 3000 rpm for 15 minutes and collect the pure platelet plasma for determination of prothrombin time test^{11,12}.

Collection of blood and Plasma re-calcification

Take 0.2 ml of plasma, 0.1 ml of extract of different concentrations and 25 mM of CaCl₂ different volumes were mixed together in a clean tube and incubated at 37 °C in water bath. The control solution was prepared by extract solution is replaced by same volume of 0.9% saline solution. Now clotting time was recorded by using stopwatch by shaking the test tubes every 5 sec. This is called prothrombin time.^{13,14}

In vitro methods employed in antidiabetic studies

Inhibition of alpha-amylase enzyme

0.1% w/v of starch solution was prepared by using potato, this solution was prepared by take 100mg of potato starch in 16 mM of sodium acetate buffer 100 ml and stir the solution. The alpha amylase solution is prepared by mixing 27.5mg of alpha amylase powder in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25°C. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. This reaction is detectable at 540 nm¹⁵.

Inhibition of alpha-glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm¹⁶.

Calculation of 50% Inhibitory Concentration (IC₅₀) The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by $I\% = (Ac-As)/Ac \times 100$, (Shai et al., 2010).

Where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS

The primary phytochemical screening revealed the presence of alkaloid, steroid, flavonoid, tannin, glycoside etc. Results were show in Table 1.

In vitro anticoagulant activity

Anti-coagulant activity of ethanolic extract of *Alstonia scholaris* were carried out, the 400ug/ml solution delayed the coagulation time for 26.56 min. From the present study the extract have remarkable anti-coagulant activity than the control. Results were shown in table 2.

In vitro anti diabetic activity

There was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 0.2 ml of plant extract showed a percentage inhibition 27.8% and for 1.0 ml plant extract showed inhibition of 96.3% Results were shown in table 3.

The *Alstonia scholaris* ethanol extract revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *Alstonia scholaris* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 89.4% - 31.7% for highest concentration to the lowest concentration Results were shown in table 4.

DISCUSSION

Antithrombotic drugs are pivotal in the prevention and/or treatment of thrombotic disorders. Secondary metabolites from vegetal origin are a potential source of anticoagulant drugs The prevalence of atherosclerosis and coronary artery diseases has focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation process are of potential interest for primary prevention of cardiovascular diseases. Previous study showed that NSAIDs in small doses for an extended period of time inhibit platelet aggregation and thromboxane formation¹⁷. Anti-coagulant activity of ethanolic extract of *Alstonia scholaris* were carried out. From the present study it is proved that the extract have remarkable anti-coagulant activity than the control solution.

Many herbal extracts are used in Ayurveda for the treatment of diabetes and have been reported to have antidiabetic activity in the inhibition potential towards alpha amylase and glucosidase activity. Human bodies possess enzymatic and non- enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species. It was proposed that inhibition of the activity of such alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation¹⁸. In the present study, research has been carried out to evaluate the preliminary phytochemical investigation and the potential of ethanolic extract of *Alstonia scholaris* leaf in inhibiting alpha-glucosidase and alpha-amylase. The present finding reveals that *Alstonia scholaris* efficiently inhibits both alpha amylase and alpha-glucosidase enzymes in vitro in a dose dependent manner. The ethanol extract from *Alstonia scholaris* leaves both showed a dose dependent inhibitory effect on alpha-amylase activity¹⁹. The antidiabetic action of *Alstonia scholaris* can also be attributed to the intestinal alpha-amylase and alpha- glucosidase inhibitory activity. Further studies are required to elucidate whether *Alstonia scholaris* have antidiabetic potential by in vivo for validating the traditional claim of the plant.

Table 1: Phytochemical analysis of Ethanolic extract of *Alstonia scholaris*

Name of the Test	Results
Carbohydrates	++
Steroids	
Alkaloids	++
Glycosides	++
Tannins	++
Flavonoids	—
Saponins	++
Gums	

+ = Presence — = Absence.

Table 2: Determination of coagulation by using EEAS

Name	Amount of plasma (ml)	Amount of plant extract (ml)	Amount of calcium chloride (ml)	Time of coagulation
Control	0.2ml	0.1ml normal saline	0.3ml	70sec
EEAS 200ug/ml	0.2ml	0.1ml	0.3ml	9.42min
EEAS 400ug/ml	0.2ml	0.1ml	0.3ml	26.56min

Table 3: in vitro antidiabetic activity by Alpha Amylase method

S.no	Concentration of sample(ml)	% of inhibition
1	0.2	26.70
2	0.4	46.9
3	0.6	60.12
4	0.8	85.70
5	1.0	92.80

Table 4: In vitro antidiabetic activity by Alpha glucosidase method

S.no	Concentration of sample(ml)	% of inhibition
1	0.2	30.80
2	0.4	51.90
3	0.6	64.20
4	0.8	74.20
5	1.0	82.90

CONCLUSION

In this present study we evaluated in vitro anti-coagulant, in vitro alpha amylase and alpha glucosidase activity of crude methanol extract of *Alstonia scholaris* leaves. The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of anti-coagulant antidiabetic agent.

ACKNOWLEDGMENT

The authors are thankful to the Principal, and management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences for providing the facilities for conducting the study.

REFERENCES

1. M.S. Sirridge, R. Shannon, Hematology Principles and Procedures, Lea &Febiger, Philadelphia, 1993, 6th ed., 202-278.
2. Prasanta Dey. Evaluation of in-vitro anticoagulant activity of *Molineriarecurpata* leaf extract. Journal of Natural Product Plant Resoues., 2012, 2 (6):685-688.
3. A.L. Cerskus, M. Ali, J. Zamecnik, J.W.D. McDonald. Effects of indomethacin and sulfinpyrazone on *in vivo* formation of thromboxane B₂ and prostaglandin D₂ during arachidonate infusion in rabbits. Journal of Thrombosis Research. Volume 12, Issue 3, March 1978, Pages 549-553.
4. M Ali, JWD McDonald, Reversible and irreversible inhibition of platelet cyclooxygenase and serotonin release by nonsteroidal antiinflammatory drugs. Journal Thrombosis Research, Volume 13, Issue 6, December 1978, Pages 1057-1065.
5. A Bordia, SK Verma, AK Vyas, Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. Journal of Atherosclerosis, Volume 26, Issue 3, March 1977, Pages 379-386.
6. Wild, S., G.Roglic, A.Green, R.Sicree, and King, H. Journal of Global prevalence of Diabetes. 2004 May;27(5):1047-53.
7. Stalin.C, Vivekanandan.K & Bhavya.E. In Vitro Antidiabetic Activity of *Cardiospermum Halicacabum* leaves Extracts. Global Journal of Medical research Pharma, Drug Discovery, Toxicology and Medicine .Volume 13 Issue 7 Version 1.0 Year 2013.
8. Ali, H., P.J.Houghton, and Soumyanath, A. α -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. Journal of Ethnopharmacol. Volume 107: 449-455. 2006.
9. Kirtikar, K.R.; Basu, B.D.; Indian Medicinal Plants, Vol. II, P.565, 1975.
10. S.Sazada, A.Verma, A.A.Rather, F.Jabeen and M.K. Meghvansi. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Journal of Advances in Biological Research. Volume 3, 188-195 2009.
11. R.Biggs, R. McFarlane, Human Blood Coagulation and their disorders, Blackwell Scientific Publications, Oxford, 1962, 430-436.
12. Hull R, Delmore T, Carter C, Hirsh J, Genton E, Gent M, et al. Adjusted subcutaneous heparin versus warfarin sodium in the long-term treatment of venous thrombosis. New England Journal of Medicine 1982;306(4):189-94.

13. EABuLoeliger, AMHV Besselaar, Lewis, SM, Evaluation of in-vitro anticoagulant activity of *Molinieria recurvata* leaf extract. Journal of Internal Medicine, volume 53, 148-154, 1985.
14. R.W. Colman, J. Hirsh, V. J. Marder, Haemostasis and Thrombosis, Basic Principles and Clinical Practice, Lippincott Company, J.B., 1994, 759-762.
15. Malik, C.P., and Singh, M.B. 1980. Plant Enzymology and Histochemistry, Kalyani Publishers, New Delhi, p. 278.
16. M. Amin Mir, S. S. Sawhney, M. M. S. Jassal. In-vitro antidiabetic studies of various extracts of *Taraxacum officinale*. The Pharma Innovation Journal 2015; 4(1): 61-66.
17. 20. Manicam C, Abdullah JO, Rahayu E, Tohit M, Seman Z, Seio CC, Hamid M. In vitro anticoagulant activities of *Melastoma malabathricum* Linn. Aqueous leaf extract: A preliminary novel finding. Journal of Medicinal Plants Research; 08/2010; 4:1464-1472
18. RhabasoLhoret, R., and Chiasson, J.L., 2004. - Glucosidase inhibitors. In: Defronzo R.A, Ferrannini E, Keen H, Zimmet P. (Eds.), International Textbook of Diabetes Mellitus, Vol. 1, third ed. John Wiley and Sons Ltd., UK, pp. 901-914.
19. Karthic, K., K.S.Kirthiram, S. Sadasivam, and Thayumanavan, B. 2008. Identification of - amylase inhibitors from *Syzygium cumini* Linn seeds. Indian J. Exp. Biol. 6:677-680.

Cite this article as:

B. Nagaraju, A. Ramu, S. Vidyadhara, M. Yaso Deepika. Evaluation of in vitro anti-coagulant and in vitro anti diabetic activity of *Alstonia scholaris* leaves. Int. Res. J. Pharm. 2016;7(10):23-26 <http://dx.doi.org/10.7897/2230-8407.0710117>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.