



IN-VITRO AND IN-VIVO SCREENING METHODS FOR ELUCIDATING THROMBOLYTIC ACTIVITY OF BAUHINIA RACEMOSA (L)

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Article Received on 29/12/2017

Article Revised on 19/01/2018

Article Accepted on 09/02/2018

ABSTRACT

The plant *Bauhinia racemosa* (L). belongs to the Caesalpiniaceae family. It is popularly known as “Apta” in Marathi, “Kanchnal” in Hindi. Other common names include Mountain Ebony and Kachnar (India and Pakistan). The bark and leaves are known to cure skin diseases, throat troubles, tumours, chronic dysentery, headache and malaria. The aim of the present study is to elucidate the thrombolytic activity of *Bauhinia Racemosa* (L). Bleeding methods and clotting time assay were the methods employed to determine the thrombolytic activity of *Bauhinia Racemosa* (L). The effect of clot lysis was compared with streptokinase as a standard drug. Using in vitro clot lysis assay method, the ethanol extract showed an average clot lysis of $90.77 \pm 1.67\%$. The results obtained were significant. The in-vivo thrombolytic activity also showed significant clot lysis compared to that of standard drug. The extract showed activity close to that of the standard. Therefore this study was concluded that the *Bauhinia racemosa* extract is effective for the treatment of thrombosis and is an effective clot lyser.

KEYWORDS: *Bauhinia Racemosa*, Thrombolytic, Streptokinase, in-vivo, in-vitro.

INTRODUCTION

India with its biodiversity and knowledge of rich ancient traditional systems of medicine built a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people India.^[1] In addition, herbal medicines have some advantages such as fewer side effects, better tolerance, relatively less expensive and readily available. So, such plants should be investigated to better understand their properties, safety & efficiency and studies involving the use of plants as therapeutic agents should be emphasized. *Bauhinia* species, named as such after Jean Bauhin and Gaspard Bauhin are important ornamental, forest and medicinal plants, including climbers and trees. The plant *Bauhinia racemosa* (L). belongs to the Caesalpiniaceae family. It is popularly known as “Apta” in Marathi, “Kanchnal” in Hindi. Other common names include Mountain Ebony and Kachnar (India and Pakistan). The bark and leaves are known to cure skin diseases, throat troubles, tumours, chronic dysentery, headache and malaria.^[2] The review revealed the different medicinal uses of *Bauhinia racemosa*. Herbal medicines and phytochemicals have some critical drawbacks such as poor stability in highly acidic pH, pre systemic metabolism in liver, poor solubility associated with absorption problems, leads to very low concentration of

drug in the plasma which show low therapeutic effect on site of action.^[3] There are many plant actives such as glycosides, tannins, flavonoids, etc, are polar molecules and they have poor absorption capacity due to their large molecular size which minimizes the absorption efficiency via passive diffusion. These polar molecules also have poor lipid solubility efficiency which minimizes their efficiency to cross the lipid-rich biological membranes. Consequently such limitations lead to poor bioavailability on site of action deals with low therapeutic index of plant actives. The aim of the study is to elucidate the thrombolytic activity of *Bauhinia Racemosa* (L).^[4]

MATERIALS AND METHODS

Collection and identification of *Bauhinia racemosa*

The plant materials (leaves) were collected in and around Coimbatore, Tamilnadu during the month of December 2015. The taxonomic identification of the plant was authenticated by Dr.M.Palanisamy, Scientist “D” in-charge, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu. Vide Letter No. BSI/SRC/5/23/2016/Tech/585. Prior to all analysis, all the raw materials were cleaned to remove any foreign materials and dust. The samples were subjected to organoleptic, microscopic and phytochemical study so as

to generate inputs that can be considered for laying down standards in respect of this plant. The leaves were dried in shade, powdered in an electric grinder, passed through 100 mesh sieves and stored in an airtight container for physicochemical and phytochemical screening.

Extraction of leaves of *Bauhinia racemosa*^[5,6,7]

The leaves of *Bauhinia racemosa* were washed with water to remove the mud and other dust particles and then shade dried. These shade dried leaves were then coarsely powdered. Approximately 60 gm of drug powder was extracted with 500 ml ethanol using soxhlet apparatus for 8 hours by hot continuous method. The extract was then concentrated.

Determination of *in vitro* thrombolytic activity of the extract^[8-15]

The dry crude extract (1mg) was suspended in 1ml of DMSO. 1ml/tube of blood was added into pre-weighed sterile micro centrifuge tube and incubated at 37°C for 45 mins. After clot formation, the serum was completely removed without disturbing the clot and each tube having the clot was again weighed to determine the clot weight.

Clot weight = weight of clot containing tube – weight of tube alone

To each micro centrifuge tube containing the pre weighed clot, 100µl DMSO solutions of different partitionates along with the crude extract was added separately. As, a positive control 100µl of streptokinase (SK) and as a negative non thrombolytic control 100µl of distilled water and 100µl of DMSO were added separately to the different control tubes. All the tubes are then incubated at 37° C for 90 mins and observed for clot lysis. After incubation the released fluid was removed and the tubes were weighed again. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown.

% of clot lysis = (weight of released clot/ clot weight)* 100

Table 1: Effect of leaf extract/ drug on *in-vitro* clot lysis.

Extract/Drug	Percentage of clot lysis (Mean ± S.D)
Streptokinase	91.13 ± 1.29
Water	2.725 ± 0.98
Ethanol extract of <i>B.racemosa</i> leaves	90.77 ± 1.67
DMSO	1.130 ± 0.25

In vivo thrombolytic activity

The haematological parameters such as WBC, RBC, haemoglobin, packed cell volume and platelets were found. The results are given in Table no. 2.

Determination of *In vivo* thrombolytic activity^[16-20]

Male wistar rats of 8-10 weeks old weighing 200-250gm was used. Ferric chloride (35% w/v) was used to induce thrombus in tail injury model. The animal room temperature was maintained constantly at 24°C ± 2°C and a constant humidity of 50% ± 10% at light-dark cycle. Animals were divided into six groups with six animals in each group. Thrombus was induced by applying Whatmann filter paper saturated with ferric chloride on top of tail vein for 2 mins. Thrombus formation is continuously monitored and treated with standard drug and leaf extract. The animals were anesthetized using intraperitoneal injection and the blood sample was collected. It was centrifuged and haematological parameters were observed. The parameters were studied to find the significance using statistical analysis.

RESULTS AND DISCUSSION

Collection and identification of *Bauhinia racemosa*

The plant materials (leaves) were collected from Sullur, Tamilnadu during the month of December 2015. It was authenticated by Dr. M. Palanisamy, Scientist 'D', In charge Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu – 641003. Register No: BSI/SRC/5/23/2016/Tech./585.

Extraction of the leaves of *Bauhinia racemosa*

Extraction of the leaves of *Bauhinia racemosa* was carried out as per the procedure. The dried leaves powder approximately 60 gm was extracted with 500 ml of ethanol for 8 hours (hrs) by hot continuous extraction method by using soxhlet apparatus. The extract was concentrated. The yield was found to be 15.95% w/v.

Determination of *in vitro* thrombolytic activity of the extract

Determination of thrombolytic activity was carried out as per the procedure. Using *in vitro* clot lysis assay method, the ethanol extract showed an average clot lysis of 90.77 ± 1.67%. The results obtained were significant. The results are given in the Table No.1.

Table 2: *In vivo* thrombolytic activity.

Parameters	Control group	Group - 1	Group -2	Group-3
	(n=3)	(minimum dose) (n=3)	(medium dose) (n=3)	(maximum dose) (n=3)
Hb (g%)	14.32 ± 0.31	13.21 ± 0.25	13.73 ± 0.53	14.51 ± 0.37
WBC (10 ³ /cubic millimeter)	12.13 ± 0.4	12.27 ± 0.3	12.53 ± 0.35	12.42 ± 0.45
RBC	5.81 ± 0.17	5.53 ± 0.78	6.13 ± 0.40	6.24 ± 0.24
(10 ⁶ /cu mm) PCV (%)	41.28 ± 0.84	42.54 ± 0.45	41.63 ± 0.46	43.65 ± 0.73
Platelets (10 ³ /cu mm)	426.23 ± 11.67	403.12 ± 12.53	412.21 ± 12.74	432.54 ± 10.61

[The results are expressed as mean ± S.D]

CONCLUSION

The present study was a satisfactory attempt to elucidate the thrombolytic activity of *Bauhinia racemosa* leaf extract. The thrombolytic activity was carried out by *in-vitro* method and gave appreciable results. The extract showed activity close to that of the standard. The thrombolytic activity was carried out by *in-vivo* method after conducting the acute toxicity study and fixing the dose as per OECD guidelines. It gave appreciable results. The extract showed activity close to that of the standard. Therefore this study was concluded that the *Bauhinia racemosa* extract is effective for the treatment of thrombosis and is an effective clot lyser.

REFERENCES

1. The Wealth of India: Raw materials. Vol.II, Publication and Information Directorate, CSIR, New Delhi, 1988; 53-54.
2. Chopra R N, Nayar S L, and Chopra I C, Glossary of Indian Medicinal Plants. Publication and Information Directorate, CSIR, New Delhi, 1992; 35.
3. Nandkarni K M, Indian Materia Medica. Vol.I Popular Prakashan Pvt Ltd, Bombay, 1995; 182.
4. Asima Chatterjee, Satyesh Chandra Pakrashi, the Treatise of Indian Medicinal Plants. Vol.2, Publication and Information Directorate, CSIR, New Delhi, 1992; 16-21.
5. Gawhare Vikesh S. Study of physicochemical properties of Indrayava (*Holarrhena antidysenterica* wall.) and its antibacterial effect on Enteropathogenic e-coli (EPEC) (in vitro). *Int J Ayurvedic Med*, 2013; 4(2): 113-121.
6. BC Patel, NM Patel. Standardization of „Bhunimbadi Churna“-An Ayurvedic Polyherbal Formulation. *IJPSR*, 2013; 4(10): 4010-15.
7. Madhulika S, Ekta S. Preliminary Phytochemical Investigation of *Berberis aristata*, *Acacia catechu* and *Ficus benghalensis*- Important Medicinal Plants for Photoprotection. *Int J Biol Pharm Res*, 2013; 4(9): 614-617.
8. Hatim F Daginawala, Sweta Prasad, Rajpal S Kashyap, Jayant Y Deopujar, Hemant J Purohit, Girdhar M Taori. Development of an *in-vitro* model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal*, 2006; 4(14): 1-4.
9. Mohammed Aktar Sayeed, Mohammad Mamun Ur Rashid, Mohammad Fazlul Kabir, Rahedul Alam, Md. Saiful Islam, Rana Dhar, M Yusuf AT. *In-vitro* anti-arthritis and thrombolytic activities of methanolic extract of *Protium serratum* leaves. *Journal of Medicinal Plant Research*, 2014; 8(16): 615-618.
10. Mohammad Shahadat Hossain, Mohammad Ehasnul Hoque Chowdhury, Sumana Das, Imtiaz Uddin Chowdhury. In-vitro thrombolytic and anti-inflammatory activity of *Swertia chirata* ethanolic extract. *Journal of Pharmacognosy and Phytochemistry*, 2013; 1(4); 256-259.
11. Pushplata chougule, Vishal jain, Avinash suryavanshi, Ashih jain. Screening of thrombolytic activity of *Aegle marmelos* Linn leaves extract by in-vitro. *International Journal of Pharmaceutical sciences*, 2014; 3(3): 176-178.
12. Ramesh Lodonkar, Umesh M K, Sanjeev Kumar CB, Hanumantappa bherigi nayaka. In vitro thrombolytic activity of *Typha angustifolia*. *International journal of pharmacy and pharmaceutical sciences*, 2014; 6(5): 258-262.
13. Mohammad Sekender Ali, Mohammad Ruhul Amin, Chowdhury Mohammad Imtiaz Kamal, Mohammad Aslam Hossain. Thrombolytic activity of methanolic extract of the leaves of *Adiantum philippense*. *Asian Journal of Tropical Biomedicine*, 2013; 3(6): 464-469.
14. Md. Reyad-ul-Ferdous, Mohsina Mukti, Md. Naimul Islam, Md. Abul Barakat Fahad, Mohammad Mamun Ur Rashid. Thrombolytic activity of methanolic extract of *Bauhinia acuminata* leaves. *UK Journal of Pharmaceutical and Biosciences*, 2014; 3(6): 658-662.
15. Shah Md. Shahik, Mohd. Omar Faruk Sikder, Noman Ibna Amin Patwary, Md. Sohel, Md. Saiful Islam, Tasnim Faizah Nishi, Tasrin Sultana and Rinti Barua. In-vitro thrombolytic activity of *Mentha spicata*, *Mentha viridis*, *Mentha arvensis*. *IOSR Journal of Pharmacy and Biological Sciences*, 2014; 9(5): 97-102.
16. Anwar M.S, Khan I.N, Sarkar, M.M.I, Barua S, Kamal A.T.M.M and Hosen M.Z. Thrombolytic and cytotoxic effect of different herbal extracts. *International Journal of Pharmaceutical Sciences and Research*, 2011; 2(12): 3118-3121.

17. Cadroy Y and Stephen R.H. Effects of red blood cell concentration on hemostasis and thrombus formation in a primate model. *Blood*, 1990; 75(11): 2185-2193.
18. Hossain M.J, Khaleda L, Chowdhury A.M.M.A, Arifuzzaman M and Al-Forkan M. Phytochemical screening and evaluation of cytotoxicity and thrombolytic properties of *Achyranthes aspera* leaf extract. *IOSR Journal of Pharmacy*, 2012; 3(4): 1340-1353.
19. Islam M.A, Mahmud Z.A, Rahman S.M.A, Monirujjaman M and Saha S K. Evaluation of thrombolytic activity and Brine shrimp lethality bioassay of methanol extract of stems of *Tinospora crispa*. *International Journal of Pharmaceutical Sciences and Research*, 2013; 4(3): 1148-1153.
20. Kawsar H Md, Sikder Al. Md, Rana S Md, Nimmi I and Rashid A.M. Studies of thrombolytic antioxidant cytotoxic properties of two asteraceous plants of Bangladesh. *Bangladesh Pharmaceutical Journal*, 2011; 14(2): 103-106.