Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1):549-552

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antiangiogenic activity of *Strychnos nux vomica* leaf extract on chick chorioallantoic membrane model

A. L. Magdalin Joy*[1], M. Reginald Appavoo[1] and M. I. Glad Mohesh[2]

[1]*Department of Botany, Scott Christian College(Autonomous), Nagercoil, Kanyakumari District, India*
[2]*Department of Physiology, Shri Sathya Sai Medical College & RI, Ammapettai, Sembakkam Post, Kancheepuram District*

ABSTRACT

Strychnos nux vomica is a poisonous tree which is used to cure diseases like anemia, lumbago, asthma, bronchitis, paralysis, muscle weakness and appetite loss. The present study investigated the anti-angiogenic activity of Strychnos nux vomica leaf extract on a chick chorioallantoic membrane assay model(CAM Assay). Angiogenesis forms an important step in the development of cancer. If this process could be intervened there will be an indirect way to stop the cancer. Chorio allantoic membrane of chick embryos were used to evaluate the antiangiogenic activity of the methanolic extract of the Strychnos nux vomica leaves with different concentrations (5µg/ml,10µg/ml and 20µg/ml).The regression/absence of the blood vessels in the localised area in the CAM disk by visual observation was documented. In conclusion, results revealed the dose dependent antiangiogenic property of the Strychnos nux vomica leaf extract, by which it appears to be possessing anticancer phytochemicals which can be isolated and used as a anticancer drug.

Keywords: Anti-angiogenesis, Strychnos nux vomica, CAM assay, anticancer activity.

INTRODUCTION

Globally cancer has become the most threatening disease to the mankind. Cancer deaths account to about 0.3 million per year and stands as the second most common disease in India(1).There is a huge increase in the number of cancer patients every year in India. For a developing country like India, there need to be an urgent and special attention on this issue. Creating awareness among the public can help us to prevent its occurrence. However, various means to tackle this disease in terms of prevention, chemotherapy and radiation therapy should be made more vigorous. Cancer treatment facilities are mostly restricted to urban areas(2). Late diagnosis and inappropriate treatments are the worst scenario concerned with the cancer related deaths. Apart from the routine treatment available, the use of other complimentary or alternative therapies were also documented from other countries like China(3).

Anticancer activity of the medicinal plants is due to the presence of various antioxidants in large quantity which are natural and with no toxicity as compared to the modern allopathic drugs(4). Strychnos nux vomica is one such plant with a huge content of the alkaloids strychnine and brucine. Scientific evidence on this plants effective anti metastasis property on hepatocellular carcinoma is promising(5).The anti tumour activity of strychnos nux vomica on the human hepatoma cell line is also a progress on the evidence built on this plant as a anticancer one(6).

Angiogenesis refers to new blood vessel formation. Chemical signals in the body helps in the formation and repair of the blood vessels. However these signals are well balanced in such a way that blood vessels are formed only when they are needed. A separate and continuous blood supply is needed for the growth of tumours, without which they cannot grow beyond a certain size. There is continuous search for the angiogenesis inhibitors, called the antiangiogenic agents with an idea that these agents will prevent the growth and spread of cancer(7).

This study was aimed to investigate the antiangiogenic property of the plant Strychnos nux vomica through the Chick chorioallantoic membrane model (CAM assay) (8,9,10,11,12,13,14).

EXPERIMENTAL SECTION

1) Collection of plant material

Strychnos nux vomica leaves were collected from Ammapettai village near Tiruporur, Kancheepuram District, Tamilnadu state in the months of May-July, 2013. Botanical identification of the plant material was done by Prof. P.Jayaraman, Director, Plant Anatomy Research Centre, Medicinal plant research Unit, West Tambaram, Chennai.

2) Preparation of leaf extract

The leaves were carefully examined and removed from old, insect damaged, fungus infected leaves, twigs and flowers. Healthy leaves were shade dried in the laboratory at room temperature for 10 days. The leaves were then macerated and mixed in the solvent, methanol. The extract obtained were concentrated in a rotary evaporator at 40^0C and the residue was extracted twice again analogously there by obtaining the crude solvent extract.

Chicken Chorio Allantoic Membrane Assay (CAM)

Twenty five fertile white leghorn chicken eggs were obtained from a local hatchery. These eggs were weighed and cleaned with ethanol. These eggs were incubated in a vertical position at 37^0C in a custom built incubator for 48 hours with 70-80% humidification. The egg tray was automatically tilted for 45^0 angle every 30 minutes mimicking the natural process. On the third day of incubation, by the process of candling viable eggs with moving embryo was identified and the unfertilized eggs were removed. Eggs with the developing embryo were grouped into four. Group I (n=6) (Control 10µl of PBS), Group II(n=6) (5µg/ml), Group III(n=6)(10µg/ml) and Group IV(n=6) (20µg/ml). On the sixth day of incubation, a window on the broad end of the egg was cut in the shell using a sterile needle and a sterile forceps. Whatman's sterile filter paper discs containing 10uL of PBS and different concentrations of the methanolic extract 10µL of (5 µg/ml), (10 µg/ml) and (20 µg/ml) were implanted inside the eggs. PBS buffer served the control. The window made was closed with a porous plaster. These windows were then reopened after 48 hrs of incubation on the 8th day and close up photographs(Canon 350D with close up adapters) were taken to obtain the image of CAM. The images obtained were analysed for the changes that the drug has brought to the angiogenesis process in the CAM of the developing chick embryo.

RESULTS

Dose dependant changes with the angiogenic process of the Chick CAM assay model was noted. The antiangiogenic property was very much enunciated in a 48 hour observation with the process initiated even at a low dosage of the methanolic extract of the plant(5ug/ml).In all the three study groups we were able to see the antiangiogenic process happening in a proportionate fashion. All the chicks survived except for the angiogenic process being put on hold.

Table 1.1. Effect of Strychnos nux vomica flower extract on angiogenesis in chick chorioallantoic membrane

(n=6)	Angiogenesis	Antiangiogenesis
Group I (control) 10uL of PBS)	6	0
Group II (5µg/ml)	0	6
Group III (10µg/ml)	0	6
Group IV (20µg/ml)	0	6

Fig 1. Control 10µL of PBS

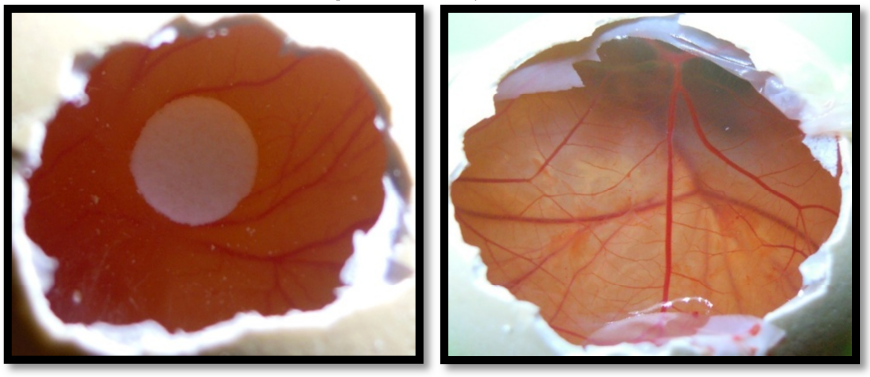


Fig 2 .10 µl of 5µg/ml concentration of the drug

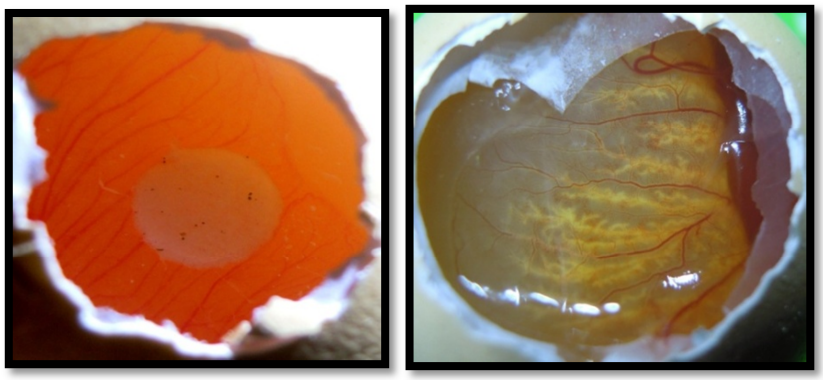


Fig 3. 10µl of 10µg/ml concentration of the drug

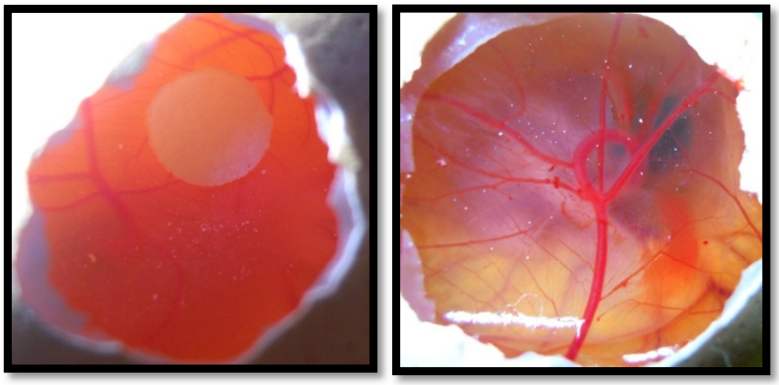
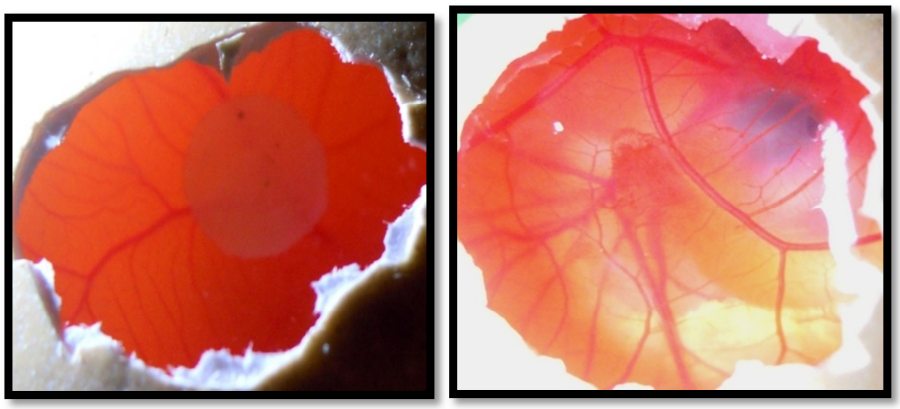


Fig 4. 10µl of 20 µg/ml of drug



DISCUSSION

World Health Organization (WHO) reports that 80% of the World's population primarily rely on plant medicines for their healthcare(15).Recent years have seen an increase in the incidence of cancer and so there is an equal increase in the search for anticancer drugs. In this present work the methanolic extract obtained from the leaves of the tree *Strychnos nux vomica* was tested for antiangiogenic property using the CAM assay model. CAM assay model has emerged as one of the important animal model to understand the anticancer property of herbal extracts(16,17,18,19). *Strychnos nux vomica* a tree used in traditional medicine has been emerging as a promising drug for anticancer activity because of its poisonous alkaloids like strychnine and brucine.

Earlier studies on mice revealed the regulatory function of strychnine on the main components of inflammatory angiogenesis, exposing the potential therapeutic of the plant extract(20).Another study indicated the role of the plants major alkaloid brucine on Hep G2 cells death via Caspase -3 and cyclooxygenase-2 pathways(21).

Our study on the indirect anticancer property of the plant is through the method of antiangiogenesis. With the drug in a quantity close to 5µg/ml bringing angiogenesis in the chick embryo to a halt without becoming lethal to the developing chick embryo itself is interesting and promising.

CONCLUSION

Strychnos nux vomica appears to be an important plant with anticancer property that could be explored further for its potential use as an anticancer drug. Studies done with positive control(VEGF) in larger animal models could definitely throw more light on the findings.

Acknowledgement
The Authors thank Dr. Menaga Magendran M.Sc., PhD., BioNeemTec India Pvt Ltd, (A Unit of Biotech park) Navalur, Chennai 603103 for her kind support and guidance in completing this research work.

REFERENCES

[1] Imran Ali, Waseem A. Wani, Kishwar Saleem. *Cancer Therapy* **2011**; 8 P: 56-70.
[2] Nair MK, Varghese C, Swaminathan A (2005). Cancer: Current scenario intervention strategies and projections for 2015. Burden of disease in India. Background papers. National Commission for Macroeconomics and health, Ministry of Health and Family Welfare, Government of India, New Delhi. pp218-25
[3] Hilal Zaid, Michael Silbermann, Eran Ben-Arye, and Bashar Saad, *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 349040, 13 pages, 2012. doi:10.1155/2012/349040.
[4] Prema R., Sekar S.D., Chandra Sekhar K B., *Int. J. Pharma & Ind*. Res., 2011, 1, 105.
[5] Shu G, Mi X, Cai J, Zhang X, Yin W, Yang X, Li Y, Chen L, Deng X. *Toxicol Lett*. 2013 Oct 24;222(2):91-101. doi: 10.1016/j.toxlet.2013.07.024.
[6] Deng XK, Yin W, Li WD, Yin FZ, Lu XY, Zhang XC, Hua ZC, Cai BC. *J Ethnopharmacol*. **2006** Jun 30;106(2):179-86.
[7] www.cancer.gov/about-cancer/treatment/types/immunotherapy/angiogenesis-inhibitors-fact-sheet. Accessed online of 08/11/2015.
[8] Domenical Ribatti,Beatrice Nico, Angelo Vacca, Marco Presta. *Nature protocols* **2006**;1(1):85-91.
[9] Dianna H Ausprunk, David R Knighton, Judah Folkman. *Developmental Biology* **1974**;38:237-248.
[10] Robert Auerbach, Rachel Lewis, Brenda Shinners, Louis Kubai, Nasim Akhtar. *Clinical Chemistry* **2003**; 49(1): 32– 40.
[11] Ribatti D, Vacca A, Roncali L, Dammacco F. *Int J Dev Biol*. **1996** ; 40(6):1189-97.
[12] Ponce ML, Kleinmann HK.. *Curr Protoc Cell Biol*. **2003** May;Chapter 19:Unit 19.5. doi: 10.1002/0471143030.cb1905s18.
[13] Ribatti D, Nico B, Vacca A, Roncali L, Burri PH, Djonov V. *Anat Rec*. **2001** Dec 1;264(4):317-24.
[14] West DC1, Thompson WD, Sells PG, Burbridge MF. *Methods Mol Med*. **2001**;46:107-29. doi: 10.1385/1-59259-143-4:107.
[15] Gurib-Fakim A: *Molecular Aspects of Medicine* **2006**; 27:1-93.
[16] K.Kalimuthu., R.Prabakaran.,M.Saraswathy. *Int. J. Curr. Microbiol. App. Sci* (**2014**)3(8)107-114.
[17] Lokman NA, Elder AS, Ricciardelli C, Oehler MK. *Int J Mol Sci.* **2012**;13(8):9959-70. doi: 10.3390/ijms13089959.
[18] Thamilarashi A.N, Mangalagowri A, Winkins Santosh. *Int J Pharm* **2014**; 4(3): 160-165.
[19] Anajwala CC, Patel RM, Dakhara SL, Jariwala JK. *J Adv Pharm Technol Res*. **2010** Apr;1(2):245-52.
[20] Saraswati S, Agarwal SS. *Microvasc Res.* **2013** May;87:7-13. doi: 10.1016/j.mvr.2013.01.003.
[21] Deng XK , Yin W, Li WD, Yin FZ, Lu XY, Zhang XC, Hua ZC, Cai BC. *J Ethnopharmacol*. **2006** Jun 30;106(2):179-86.