Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

EVALUATION OF ANTI-ANGIOGENIC PROPERTIES OF *TRIDAX PROCUMBENS* LEAVES EXTRACT, USING SHELL LESS CHICK EMBRYO CULTURE

Brahma JN and KT Dhumal*
Department of Zoology, HPT Arts and RYK Science College, Nasik, Maharashtra, India

Received for publication: December 3, 2013; **Revised**: December 14, 2013; **Accepted**: January 11, 2014

Abstract: *Tridax procumbens* is a perennial herb; belonging to order Compositae. It is commonly known as *Ghamra* in Hindi. It has been found to have significant medicinal properties. The present investigation deals with the analysis of antiangiogenic property of alcoholic extract of leaves of *Tridax procumbens*, using shell less culture of chick embryo. The results indicate that the ethanolic extract of *T. procumbens.* in a dose dependent manner inhibits angiogenesis *in vivo.*

Keywords: *Tridax procumbens,* Antiangiogenic, Shell Less Culture

INTRODUCTION

Angiogenesis is the physical process through which new blood vessels form from the pre-existing vessels. It is distinct from vasculogenesis, which is the de novo formation of endothelial cells from mesoderm cell precursors. The first blood vessels in the embryo form through vasculogenesis, after that most of the blood vessel that grow during development, form through angiogenesis. (Bruce R. Zetter, 1998).

Angiogenesis plays a critical role in a various physiological and pathological processes too, such as embryonic development, wound healing, chronic inflammation, tumor growth, and metastasis (Bruce R. Zetter, 1998).

Vegetative parts of various herbs have been checked for their angiogenic and anti-angiogenic properties. The anti-angiogenic property is particularly of importance because it can be used to check the angiogenesis in cancerous tissue and further prevent the growth of the tissue. *Tridax procumbens* is a common weed, a perennial, creeper growing widely in tropical areas. Chemical constituents (Sneha Mumdada, Ruchi Shivare, 2010) and various medicinal properties (V. K. Saxena and S. Albert, 2005) of this weed has been studied earlier In the present investigation, alcoholic extract of leaves of *Tridax procumbens* has been analysed for the antiangiogenic property, using shell less culture technique.

This study generally aims to evaluate the angiosuppresive property of the crude ethanol extract from *T. procumbens* through shell less chick embryo assay.

MATERIALS AND METHODS

Preparation of extract from Tridax procumbens leaves

Tridax procumbens plant material was collected from the local areas in Nashik. Leaves were plucked freshly and washed with water to remove the mud attached. The leaves were then dried on a filter paper for two days in open air and then in oven to make them crispy. The dried leaves were grinded into fine powder with the help of pestle and mortar. This powder was used to prepare alcoholic extract. Extract was prepared in 100% ethanol using the soxhlet apparatus The extract was then passed through rotar apparatus to evaporate the entire ethanol. Aliquots of different concentrations (10%, 20%, 40%, 60%, 80% and 100%) were prepared from this ethanol free extract and used for the assay.

Preparation of shell less culture of chick embryo: (Savita Datar, 2004)

Fertilized and unfertilized chick eggs were procured from the C & M hatcheries, Nashik. The eggs were washed with water and allowed to air dry. The incubator and laminar air flow were fumigated to maintain sterilized condition. They were further wiped with 70% ethanol before use. Eggs were surface sterilized by 70% ethanol and then kept in the incubator for 24 and 48 hours. Eggs were incubated for 24 hours, before treatment. All the glass wares and dissection equipment's were autoclaved. Further procedure was carried out under laminar hood. Thin albumen from unfertilized eggs was poured into a sterile bowl. This albumin acted as a shock absorber, and provided a cushion for the culture and prevented desiccation. The fertilized eggs were then cracked with the help of a scalpel approximately 3-3.5cm from the narrow end and the contents of the egg were gently released over the albumen cushion in the bowl. It is found that the chance of embryo survival was greater if the egg

***Corresponding Author:**
Dr. KT Dhumal,
Department of Zoology,
HPT Arts and RYK Science College,
Nasik, Maharashtra, India.

contents were transferred without damaging the yolk or the embryo, and if the embryo was kept in an upright position. Only embryos with the blastodisc positioned to the uppermost side of the yolk were used in the experiments.

The growing embryos were then treated with 50µl of the plant extract of different concentrations – 10%, 20%, 40%, 60%, 80% and 100% separately. The untreated embryos were kept as controls; i.e. 0%. Each shell less culture was covered with a sterile glass petri dish lid and incubated at 37.5°c with saturated humidity. The development was observed after every 24hrs and recorded by taking photographs.

RESULTS

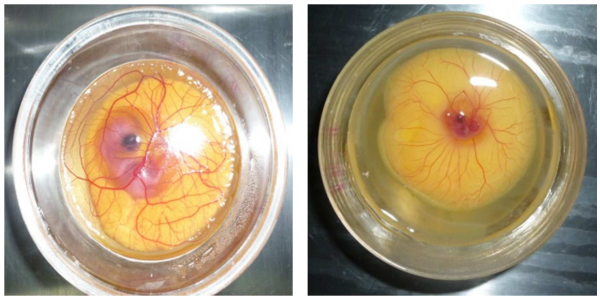

Figure 1: Control

Figure 2: Shell less embryos treated with 10% extract conc.

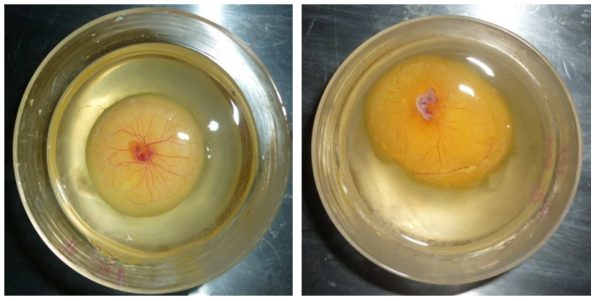

Figure 3: Shell less embryos treated with 20% extract conc

Figure 4: Shell less embryo treated with 40% extract conc.

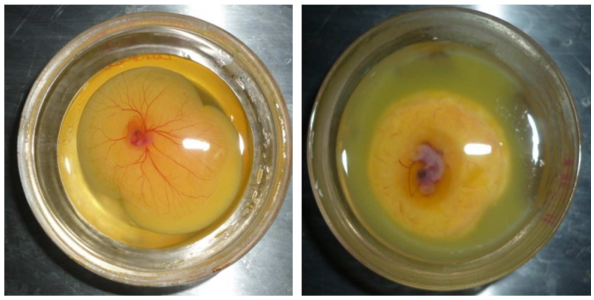

Figure 5: Shell less embryos treated with 60% extract conc.

Figure 6: Shell less embryo treated with 80% extract conc.

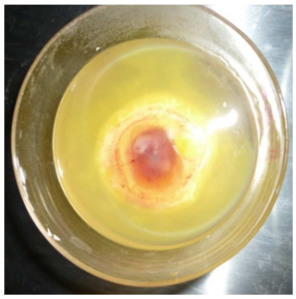

Figure 7: Shell less embryos treated with 100% Extract conc.

DISCUSSION

Over the recent years, more attention has been focused on the anti-angiogenic and antitumor effects of non-toxic compounds from natural products. Angiogenesis mainly depends on proper activation, proliferation, adhesion, migration and maturation of endothelial cells (Keshavarz M.*et al*., 2011). Inhibition of angiogenesis has been considered to be advantageous for prevention of tumor growth and metastatic activity (Rey G. Tantiado and Virgie P. Tan. 2012). Some antiangiogenic substances were identified to be effective in animal models of arthritis and several anti-rheumatic drugs such as methotrexate, contain antiangiogenic activity (Folkman J, 2006).

Some plant extracts contain many active ingredients. They are complex chemical cocktails with medicinal properties that affect tumor angiogenesis. A wide range of plants that contains compounds were identified and their phytochemicals were isolated and characterized [Fan TP, Yeh JC, Leung KW, Yue PYK and Wong RNS, 2006). In the present investigation, Shell less chick embryo assay was used for examining the anti-angiogenic activity of *T. procumbens*. The results indicated that *T. procumbens.* in a dose dependent manner inhibits angiogenesis *in vivo*.(Fig.1-7).

The study in the present investigation, clearly elucidated the antiangiogenic activity of *Tridax procumbens leaves* extract by performing *in vivo* anti - angiogenesis assay. It has been observed that the ethanolic leaves extract significantly inhibits the development of capillary networks in shell less chick embryo culture. The observation in this study suggests that *T. procumbens* extract exhibits a strong antiangiogenic activity. It may have the potential to be a useful deactivator of numerous serious diseases characterized by regulated angiogenesis.

ACKNOWLEDGEMENT

The authors are thankful to C and M Hatcheries, Nashik, for providing the fertilized eggs.

REFERENCES

1. Bruce Zetter: Angiogenesis and Tumour metastasis; Annual Review of Medicine. 1998, 49,407-424.

2. Domenico Ribatti, The Chick Embryo Chorioallantoic Membrane as an In Vivo Assay to study Anti-angiogenesis; Pharmaceuticals, 2010, 3, 482-513.

3. Fan TP, Yeh JC, Leung KW, Yue PYK and Wong RNS "Angiogenesis: from plants to blood vessels", Trends in Pharmacological Science., 2006, 27, (6), 297-309.

4. Folkman J, Angiogenesis in cancer vascular rheumatoid and other diseases. Nature Med. 1995, **1:** 27-31.

5. Folkman J, "Angiogenesis", Annual Review of Medicine, 2006 57, 1-8.

6. Keshavarz M, Bidmeshkipour A, Mostafaie A, Mansouri K and M-Motiagh HR, Anti-tumor activity of *Salvia officinalis* is due to its anti-angiogenic, anti-migratory and anti-proliferative effects, Cell Journal, 2011,12, (4), 477.

7. Rey G, Tantiado and Virgie P, Tan.: Evaluation of the Angiosuppresive Activity of *Tinospora rumphii* Boerl. Stem Extract Using the Chorioallantoic Membrane Assay in *Anas platyrhynchos* Embryos. International Journal of Bio-Science and Bio-Technology 2012, 4 (2), 93-102.

8. Savita Datar and Ramesh Bhonde : Shell less chick Embryo Culture as an alternative In Vitro Model To investigate Glucose–Induced Malformations in Mammalian Embryos; Journal of the Society for Biomedical Diabetes Research,2004:,2,221-227.

9. Saxena V K and Sosanna Albert, May *b*-Sitosterol-3-O-*b*-D-xylopyranoside from the flowers of *Tridax procumbens* Linn. Journal of Chemical Sciences, 2005:117, No. 3, pp. 263–266. Indian Academy of Sciences.

10. Sneha Mundada and Ruchi Shivare: Pharmacology of *Tridax procumbens* a weed: review; International Journal of Pharm Tech Research, 2010,2, 1391-3194.

Source of support: Nil
Conflict of interest: None Declared