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Preliminary investigation of Angiogenic property of Ethanolic leaf extract of *Acyranthus Aspera* using chorioallantoic membrane model

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Abstract

The present study is an attempt to investigate the angiogenic property of ethanolic leaf extract of *Achyranthus aspera* by *in vitro,* Hen's Egg Chorioallantoic Membrane method (HET-CAM). Ethanolic leaf extract of *Achyranthus aspera* treated CAM showed increased density of new blood capillaries as compared with control group treated with 0.9% Nacl. The results obtained in this study suggest that the *Achyranthes aspera* leaf extract revealed a significant scope to develop a novel broad spectrum of herbal formulations for wound healing and different herbal formulations.

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INTRODUCTION

Angiogenesis, the formation of new blood vessels which, is a highly regulated process essential to reproduction and wound healing. In the normal response to injury, several factors act to initiate the budding of vasculature from the existing blood vessels. This newly formed vasculature is highly branched. Controlled angiogenesis is needed in wound healing and may be useful in minimizing

tissue damage following ischemic tissue damage due to injury or heart arrest.

Achyranthes aspera (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts, which are used by traditional healers for the treatment of fever, dysentery and diabetes [1-3]. The present article is an attempt to compare the *in vitro* angiogenic potential of ethanolic leaf extract of *Achyranthus aspera* on hen's egg chorioallantoic membrane.

MATERIAL AND METHODS

Plant material & Preparation of ethanolic leaf extract

The leaves of *Achyranthes aspera* were freshly collected during January-March in and around Yenugonda village (Mahabubnagar District, Andhra Pradesh, India) and were cleaned with distilled water and shade dried at room temperature. The dried leaves were powdered (100g) and were extracted separately to exhaustion in a soxhlet apparatus using ethanol solvent system. The extract were filtered through Whatman filter paper No.1 and then concentrated by evaporating at low temperature (40-50°C) to get 2.23g yield from ethanol fraction. The extract was preserved in airtight containers at 4 ± 2°C until further use.

Qualitative analysis of phytochemicals

A preliminary qualitative phytochemical analysis of ethanolic leaf extract of *Achyranthes aspera* was carried out.

A small quantity of the extract was treated with sodium hydroxide solution; Formation of yellow color indicates the presence of Flavonoids

Foam Test: The extract is diluted with 20ml of distilled water and agitated in graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins.

Haemolysis Test: About 2ml of blood was taken in two test tubes separately. To one of the test tubes, equal quantity of water was added. To the other test tube, an equal quantity of ethanolic extract dissolved in water was added. A clear red liquid was formed in the first tube, which indicates that red blood corpuscles were haemolysed. The extract in the second test tube also haemolysed. It indicates the presence of saponin[4].

Test system

The Hen's Egg CAM model is used as an *in vitro* model to assess the angiogenic activity[5-7] of *Achyranthes aspera* leaf aqueous and ethanolic extracts. The fertile, clean eggs were selected for the study. The eggs were candeled, incubated to nine days and viable eggs were selected for the study. At broad end of egg, a small window was opened by breaking eggshell and eggshell membrane. Then, a sterile disc with the extract (40 µg/disc) was placed at the junction of two large blood vessels. The window was resealed with parafilm and adhesive medi tape and the eggs were further incubated at 37 ± 1 °C in a well-humidified chamber for 72 hours. After completion of 72 hours of incubation, the tape was opened and new blood vessel formation was observed and compared with the control eggs containing discs without the extract. In this study, three eggs per group were used.

RESULT AND DISCUSSION

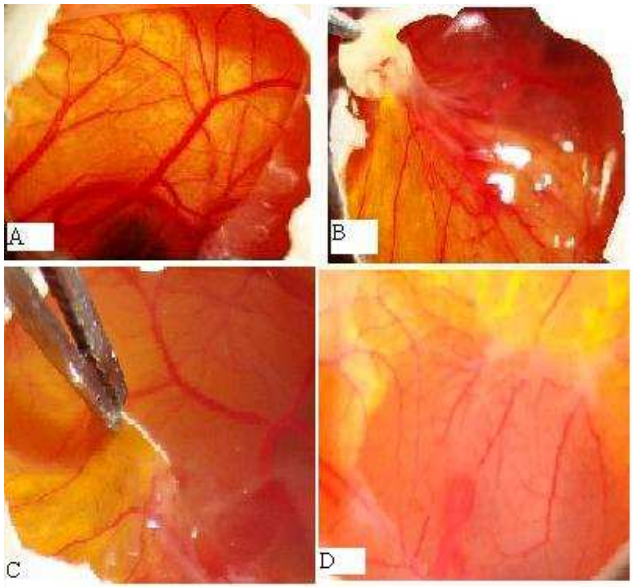
In this study, we have investigated the angiogenic property of ethanolic leaf extract of *Achyranthes aspera* using hens egg test CAM model.

The ethanolic leaf extract of *Achyranthes aspera* was subjected to preliminary qualitative phytochemical analysis and found it is rich with flavonoids and saponins. The ethanolic leaf extract showed an increased density of new blood capillaries (neovascularization) and formation of microblood vessels on the treated membrane surface as compared to negative control. Out of three eggs, two

eggs from ethanolic extract showed the attachment of disc with the new developing, budding blood capillaries with good density of new blood vessels surrounding the disc area such finding was absent from control group.

This observation indicates that the ethanolic leaf extract of *Achyranthes aspera* has good angiogenic property (Figure 1).

Figure 1: Photographs of Chorioallantoic membranes after treatment.



Key: A = CAM treated with 0.9% Nacl (Control), B & C = CAM Treated with ethanolic extract showing blood vessels surrounded and attached to the disc. D = CAM Treated with ethanolic extract showing neovacularization from pre - existing blood vessels.

CONCLUSION

From present study, it can be concluded that the ethanolic leaf extract of *Achyranthes aspera* has a positive angiogenic potential, which could be beneficial in conducting wound healing studies and are a subject for future studies.

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