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SCREENING OF DRUG TARGETING ANGIOGENESIS

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ABSTRACT

Angiogenesis is the process of formation of new blood vessels from existing one during embryonic development and continues into adult life. Many diseases arise due to altered tissue vascularization and oxygen availability. Insufficient vascular growth contributes to diseases like coronary artery, while excess angiogenesis leads to tumor formation. Thus molecules targeting angiogenesis hold greater potential for treating various diseases. Many herbs from natural origin are currently being investigated to understand their effect on angiogenesis. White Leghorn chick embryo Chorioallontoic membrane (CAM) assay is widely used to study angiogenesis. The aim of present study was to screen various plants for anti-angiogenic and pro angiogenic activity. The plants selected were *Parkia biglandulosa* (Mimosaceae), *Cissus quadrangularis* (vitaceae), *Solanum* surattense (Solanaceae), *Bombax ceiba* (Bombaceae) and *Erythrina variegata* (Fabaceae). The chick embryos on embroynal day 8 were exposed to different doses of ethanolic extracts of all plants. On embroynal day 12; visual inspection of CAM showed inhibition of blood vessels formation in ethanolic extracts of *Parkia biglandulosa*, *Solanum* surattense and *Erythrina variegata* treated group indicating its anti-angiogenic activity. While ethanolic extracts of *Cissus quadrangularis* and *Bombax ceiba* treated group showed decreased density of blood vessels indicating its pro-angiogenic activity.

KEYWORDS: Angiogenesis, Chorioallantonic Membrane Assay, *Parkia Biglandulosa*, *Cissus Quadrangularis*, *Solanum* Surattense, *Bombax Ceiba*, *Erythrina Variegate*.

INTRODUCTION

Angiogenesis plays a critical role in many normal physiological processes as well as in tumor neovascularization, wound healing etc. Angiogenesis occurs during embryonic development and is associated with cancer progression. In angiogenesis formation of new blood vessels takes place from the existing ones. During normal physiological condition, the balance between pro- and anti-angiogenic factors is essential for regulating angiogenesis. VEGF and its tyrosine kinase receptors (VEGFRs) are the key regulators in angiogenesis and are highly conserved across vertebrate species. Among them, VEGF-A, the most important member of VEGF, binds and activates the VEGFR2 (KDR), subsequently activate the main signaling pathway. To form a new blood vessel, the endothelial cells need to receive the stimulatory signals and secretion of matrix metalloproteinase (MMPs) and heparanase. This causes the decomposition of the extra cellular matrix. The tight junction between the endothelial cells is then altered, and the cells project throughout the newly created space. The newly formed endothelial cells organize into fresh capillary tubes, allowing the sprouting vessels to progress towards the source of a fresh blood supply. Angiogenesis processes can be described as developmental or disease-associated, although both types share many mechanistic features, the differences might only be related to their regulatory control. Tissue repair is a self-limiting process that occurs due to hypoxia near the site of tissue injury, while progressive tumor growth creates ongoing hypoxia and acidosis that do not regress as normally occurs after injury. Agents that stimulate or suppress angiogenesis usually do so by interfering with the critical steps. Possible targets for therapeutic intervention in angiogenesis include broad categories of therapeutic strategies for anti-angiogenesis like inhibition of extra cellular cells (ECs) activation, inhibition of ECs proliferation, inhibition of ECs migration, disruption of the organization of a three-dimensional structure. The three dimensional structure includes formation of capillary tubules and loops, interference with the biosynthesis and remodeling of basement membrane and extra cellular matrix. Thus inhibition of proteases secreted by the extra cellular cells ECs, Induction of ECs apoptosis and direct killing of ECs can be the approaches for newer therapy the chick embryo chorio allantoic membrane assay is commonly used as an experimental *in vivo* assay to study both angiogenesis and anti-angiogenesis in response to tissues or cells.

MATERIAL AND METHODS

Collection and authentication of plant material: The fresh leaves of the plant *Parkia biglandulosa* for this study were collected from Veermata Jijabai Bhosle Udyan, Byculla,

Mumbai and the crude powder of *Cissus quadrangularis* stem, *Solanum surattense* whole plant, *Bombax ceiba* bark and *Erythrina variegata* leaves were collected from the Rajesh chemicals Mumbai in the month of August 2014. The plant material was authenticated by Dr. Harshad Pandit, Department of Botany, Guru Nanak Khalsa College, Matunga Mumbai-400019. The voucher specimen (specimen # : ss)P 14101415, *Cissus quadrangularis*- vdj p 1060430, *Solanum* surattense- vdj p 1060431, *Bombax ceiba*- vdj p 1060432, *Erythrina variegata*- vdj p 1060429) has been preserved for future reference.

Preparation of ethanolic extract of different crude powders: These five different powders were passed through 40 mesh sieve and macerated for 8 hours using the solvent petroleum ether for defatting of the material. Crude extract was obtained by placing 20-30 gm of each powder in the soxhlet extractor using ethanol at 40°c. The crude extract that is Ethanolic extract of *Parkia biglandulosa* (EEPB), Ethanolic extract of *Cissus quadrangularis* (EECQ), Ethanolic extract of *Solanum* surattense (EESS), Ethanolic extract of *Bombax ceiba* (EEBC) and Ethanolic extract of *Erythrina variegata* (EEEV) was further evaporated in the Rotavac evaporator and dried to get the free flowing powder. The powders were stored in airtight container.

Experimental procedure

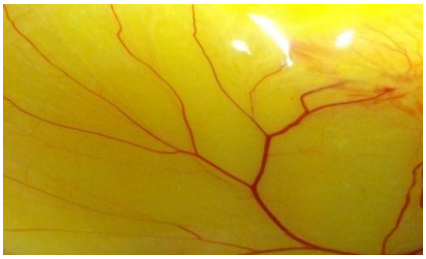
Chick chorioallantoic membrane (CAM) assay

On Day 1, embryonal eggs were brought from the central poultry organization, Mumbai. They were kept for acclimatization in incubators at 37.5˚C +/- 0.3°C. They were cleaned with sterilized using 70% alcohol. They were kept in an egg incubator at 37.5°C +/- 0.3°C. On day 8, when the embryo is still small, eggs were prepared to be used for the procedure by cleaning them with 70% ethanol to minimize contamination. On the day of experiment, the eggs were viewed by candling in order to locate the CAM as well as to check their viability. The eggs were maintained in vertical position. All these procedure was carried out in an aseptic area between the burners. A small hole was made with a scalpel on the wide end of the egg, where the air sac is located. The sterile filter disc soaked in the drug solution was implanted on the CAM by making a small window on day 8 of embryonic development inside the sterile conditions that is between burners. 10 µl of vehicle (negative control), standard (Positive control) or test compound solutions of suitable concentrations (disc soaked into suitable concentrations) were implanted into the cavity through the opening. The openings were sealed with adhesive tape and the eggs were re-incubated in egg incubator at

37°C. Then eggs were opened on day 12 of embryonic development, CAM was removed and observations were made. A cut was made at the point of inoculation and CAM was lifted with the help of forceps. This part of CAM was put on the eggshell and the albumin was drained, embryo and the rest of the material. CAM had seemed to be attached on the inner side of the eggshell. Once, the entire material is drained, shell had broken and the CAM was removed with help of forceps from shell and fixed in 10% formalin in Phosphate Buffered Saline (PBS) pH 7.4 solution.

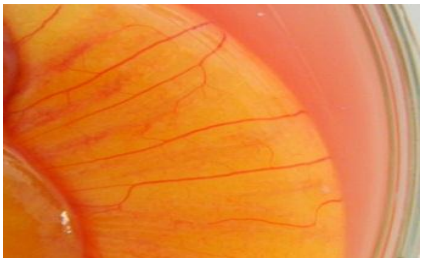
RESULT AND DISCUSSION

Vehicle control



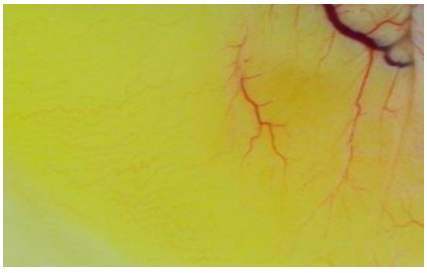
EEPB: 8 mg/ ml

EEPB: 4 mg/ml

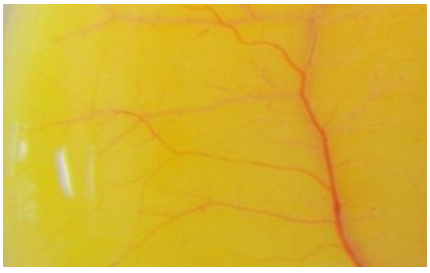


EECQ: 6mg/ml

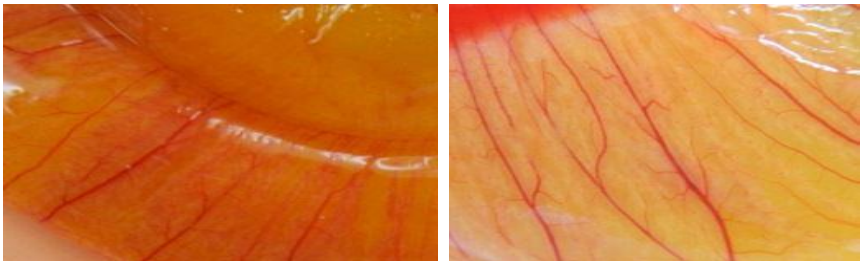
EECQ: 3mg/ml



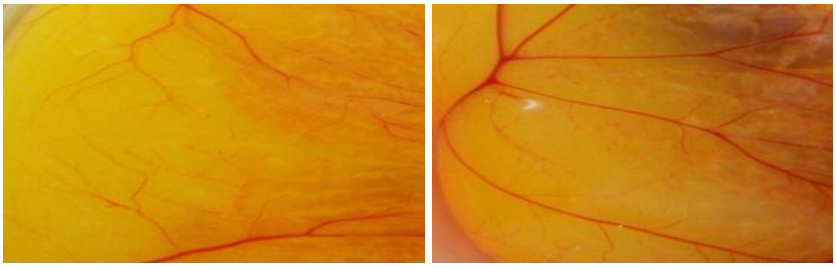
EESS: 6mg/ml



EESS: 3mg/ml



EEBC: 6mg/ml **EEBC: 3mg/ml**



EEEV: 6mg/ml **EEEV: 3mg/ml**

Figure No. 1: Chick chorioallantonic membrane on embryonal day 12.

In this study the pharmacological activity as pro and anti-angiogenic activity of EEPB, EECQ, EESS, EEBC and EEEV was carried out using chick embryo chorioallantoic membrane assay (CAM). Visual inspection of CAM showed some inhibition of blood vessels in groups treated with that EEPB, EESS and EEEV as compared to vehicle control group whereas the density of blood vessels in EECQ and EEBC treated group was more than that of vehicle control group. The reason behind this effect might be due to activation or inhibition of the angiogenetic or pro angiogenic factors. *Parkia biglandulosa, Cissus quadrangularis, Solanum surattense, Bombax ceiba and Erythrina variegate* appears to be a vital plants which can be further explored in future to determine their pro or anti-angiogenic mechanisms and for development of new molecules from their chemical constituents those are important in its pharmacological activity.

CONCLUSION

From the CAM assay we can conclude that EEPB, EESS and EEEV has potential anti angiogenic activity as compared to vehicle group where as EECQ and EEBC treated group had more density of blood vessels than the vehicle control group indicating its pro-angiogenic activity.

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