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Review Article

ANTIANGIOGENIC ACTIVITY OF *TRIANTHEMA PORTULACASTRUM* LINN EXTRACT USING CHICK CHORIOALLANTOIC MEMBRANE (CAM) MODEL

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ABSTRACT

The present study aimed to assess the Anti-angiogenic activity of methanolic extract of *Trianthema Portulacastrum* Linn extract using Chick Chorioallantoic Membrane (CAM) model. Inseminated chicken eggs were incubated at 37 °C with 80% relative humidity and on day 2 of post development, 3 mL of albumin were withdrawn to minimize adhesion of the shell membrane with CAM. On 8th day, these eggs were divided into 5 groups of 10 each and treated with phosphate buffered saline and methanol extract of *T. portulacastrum* (METP) at concentrations of 50, 100, 200 and 300 µg/mL. After 48 h of incubation, CAM was cut around the disk and photos were taken and analyzed for numbers of vessel branch points contained in a circular region (equal to the area of each filter disk) were counted manually for the ability to inhibition angiogenesis. In the presence of METP, the number of blood vessels branches is reduced from 14 to 12, 9, 7 and 5, respectively to a concentration of 50, 100, 200 and 300 µg/mL and correspondingly an ability to inhibition angiogenesis to 14.29%, 35.71%, 50.00% and 64.29% with IC50 = 200 µg/mL. Based on these results, it was found that the methanolic extract of *T. portulacastrum* is capable of inhibiting the angiogenesis in Chick Chorioallantoic Membrane (CAM) Model.

Keywords: *Trianthema Portulacastrum Linn; Chick Chorioallantoic Membrane; CAM; Angiogenesis; Anti-angiogenesis activity; METP; Chick.*

INTRODUCTION

Angiogenesis is the process of forming novel blood vessels and is critically required for tissue growth and [1] which is valuable stage for many processes including embryogenesis and wound healing. Regulation of angiogenesis is complex and is upheld by the balance amid endogenous stimulators (e.g., vascular endothelial progress factor (VEGF), platelet derived growth factors (PDGFs) and hypoxia-inducible factors (HIFs)), and inhibitors (e.g., angiostatin and endostatin). Additional body conditions also donate to the regulation of angiogenesis under physiological conditions. For example, certain metabolic demands such as the essential for more oxygen can persuade VEGF secretion and angiogenesis in heart and intelligence tissues[2]. Since angiogenesis touches many organs and tissues in the body, an imbalance in its directive has been associated with dissimilar pathologies [3]. For example, cancer, rheumatoid arthritis and diabetic retinopathy feature an upregulation of proangiogenic factors. Conversely, if antiangiogenic factors were upregulated, some cardiovascular diseases are more likely to happen [4].

Trianthema portulacastrum Linn is plant of the personal Aizoaceae, found almost throughout India as a weed in cultivated and wastelands. The shrub is bitter and used as analgesic, stomachic, laxative, and helps as alterative cure for bronchitis, heart disease, blood anemia and inflammation. The root practical to the eye to treat corneal ulcers, itching, dimness of sight and night blindness [5]. The plant has a remarkable defense against the chemical induced hepatotoxicity [6] and hepatocarcinogenesis [7]. Previously, we reported the hypoglycemic and hypolipidemic activities of *Trianthema portulacastrum* (TP) in normal and alloxan induced diabetic rats[8]. In this present study, we aimed to evaluate the Anti-Angiogenic Activity of *Trianthema Portulacastrum* Linn extract using Chick Chorioallantoic Membrane (CAM) model

MATERIALS AND METHODS

Extraction of plant material: The collection of plant *Trianthema portulacastrum L,* and the extraction process was reported earlier by us (Anreddy et al., 2010). The whole plant remained dried under shade and ground to a fine powder in a mechanical blender. The powder of the vegetable was initially extracted in a *Soxhlet apparatus* with petroleum benzene (60°-80°C) to confiscate the chlorophyll shadowed by methanol by the technique of continuous hot removal to get the methanol extract of *Trianthema portulacastrum* (METP). This methanolic extract remained stored at 2-8°C and used for subsequent experiments.

Phytochemical screening: The methanolic excerpt was screened for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, glycosides, flavonoids, phenolic compounds and carbohydrates [9,10]..

Antiangiogenic activity: Many researchers successfully employed this Chick Chorioallantoic Membrane (CAM) as a suitable model to study about the angiogenesis [11,12,13]. In this study, the antiangiogenic activity of methanol extract of *T. portulacastrum* was also evaluated using this CAM Model as reported by Ngoua-Meye-Misso *et al* [14]. and Ribatti, (2008) with minor alterations. Briefly, fertilized chicken eggs were acquired from a local poultry farms in Karimnagar and were incubated at 37 °C with 80% relative humidity in a incubator after sterilized with 70° ethanol. Happening day 2 of post incubation, 3 mL of albumin were solitary to minimalize adhesion of the shell membrane with CAM. A square window of 1 cm^2 was undid in the egg shell at the contrary to blunt edge and sealed with an adhesive adhesive tape. The eggs were kept in incubator for further incubation.

***Drug administration**:* On 8th day, the experimental groups were divided into 5 of each containing 10 eggs. Group 1 preserved with phosphate buffered saline (PBS) alone as bad control, a newspaper disc Whatman N°1 soaked of 10 µL PBS at pH 7.4 remained placed on the CAM of egg; whereas Group-2, 3 and 4 were treated with methanol extract of *T. portulacastrum* (METP). Sterile discs (diameter: 10 mm) of Whatman N°1 saturated of 10 µL of the METP at attentions of 50, 100, 200 and 300 µg/mL was applied to the CAM in group 2-5 respectively. The treated CAM examples were hatched for 48 h.

Angiogenesis quantification: After 48 h of development at 37 °C and 80% relative humidity, a capacity of 10 µL of formaldehyde at 4% was practical to the CAM. 5 min later, the CAM was cut around the floppy and all (disc and CAM) remained placed in a Petri. Then the photos were occupied with a Nikon numerical camera and the images were subsequently analyzed with the software Image J. The numbers of vessel branch points limited in a round region (equal to the area of each filter disk) were counted physically. The fraction of vascularization (density) is measured relative to a normal control vascularization.

The aptitude to inhibition angiogenesis was calculated by the following equation:

$\%AIA = [(NBV_{control} - NBV_{sample})/NBV_{sample}] \times 100$ *whereas % AIA-percentage of ability of inhibition of angiogenesis; NBV= number of blood vessel branch points.*

The IC50 (concentration providing 50% inhibition) of excerpts was determinate using regression curves in the linear range of concentrations. The experiments have been recurrent at least four times, and the results were reproducible

RESULTS AND DISCUSSION

Preliminary phytochemical broadcast of the methanolic extract of *T. portulacastrum* discloses the presence of alkaloids, flavonoids, saponins, phenolic compounds then terpenoids. Different doses of methanolic excerpt were screened for their oral toxicity. No mortality was recorded till 4000 mg/kg with methanolic extract, hence the excerpts were found to be safe up to the amount levels of 4000 mg/kg.

The antiangiogenic potential of the extracts remained evaluated in vivo with the chicken chorioallantoic skin (CAM) the eighth developing day. The fertilized eggs were treated with methanolic extract of *T. portulacastrum* (100, 200 and 300 µg/mL). The degree of vessel branches formation on CAM was scored 48 h later. The vessel density is the percentage of blood supply to the analysis area. It is inversely relative to the degree of inhibition. The results of Antiangiogenic activity of the methanolic extract of *T. portulacastrum* were showed in Figure-1 and 2.

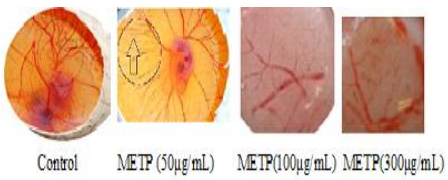

Fig.1: Angiogenesis in control and METP treated eggs

In the attendance of phosphate buffered saline (PBS) used as a bad control, the target area has a vascularization percentage of 100%, consistent to a normal vasculature with a amount of containers branches equal to 14. The inhibitory possible of extracts was expressed through their inhibitory attentiveness fifty (IC50).

In the presence of METP, the number of blood vessels branches is reduced to 12, 9, 7 and 5, respectively to a concentration of 50, 100, 200 and 300 µg/mL and respectively an ability to inhibition angiogenesis to 14.29%, 35.71%, 50.00% and 64.29% with IC50 = 200 µg/mL.

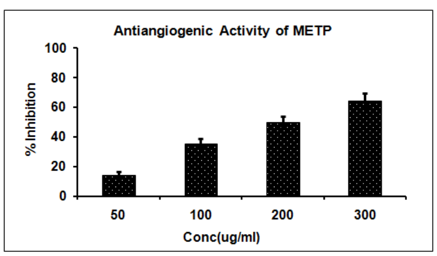

Fig.2: Antiangiogenic effect of methanolic extract of T. portulacastrum

In support of our present study results about the Antiangiogenic property of METP, Narendra et al., (2020) reported the antitumor activity of hydroalcoholic extract of Trianthema portulacastrum L (TPE) on NMU-induced mammary tumors in rats and it was proven that TPE treatment slowed down the tumor growth rate, reduced the tumor frequency and size and showed more intratumoral necrosis and less malignancy in comparison with the NMU control, which could be attributed to its antiproliferative, antiangiogenic, apoptosis-inducing and metastasis-inhibiting properties. These findings suggest that TPE is a promising cancer therapeutic agent, owing to its non-toxicity in the host. Animesh and Anupam, (2015) also reported the antitumor activity of the extract of Trianthema portulacastrum Linn in rats.

Based on results presented here, it was found that the methanolic extract of *T. portulacastrum* is capable of inhibiting the angiogenesis in Chick Chorioallantoic Membrane (CAM) Model. Though, further investigations are necessary to identify the detailed molecular mechanisms accountable for its anti- angiogenesis activity and a comprehensive phytochemical analysis is required.

REFERENCES

1. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307. doi: 10.1038/nature10144, PMID 21593862.
2. Diniz C, Suliburska J, Ferreira IMPLVO. New insights into the antiangiogenic and proangiogenic properties of dietary polyphenols. Mol Nutr Food Res. 2017;61(6). doi: 10.1002/mnfr.201600912, PMID 27981783.
3. Shibuya M. VEGF-VEGFR system as a target for suppressing inflammation and other diseases. Endocr Metab Immune Disord Drug Targets. 2015;15(2):135-44. doi: 10.2174/1871530315666150316121956, PMID 25772179
4. Gacche RN, Meshram RJ. Angiogenic factors as potential drug target: efficacy and limitations of anti-angiogenic therapy. Biochim Biophys Acta. 2014;1846(1):161-79. doi: 10.1016/j.bbcan.2014.05.002, PMID 24836679.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal Plants. New Delhi: Council of Scientific and Industrial Research; 1956.
6. Sarkar A, Pradhan S, Mukhopadhyay I, Bose SK, Roy S, Chatterjee M. Inhibition of early DNA-damage and chromosomal aberrations by Trianthema portulacastrum in carbon tetrachloride-induced mouse liver damage. Cell Biol Int. 1999;23(10):703-8. doi: 10.1006/cbir.1999.0439, PMID 10736194.
7. Bhattacharya S, Chatterjee M. Inhibitory effect of Trianthema portulacastrum L. diethylnitroso-amine-induced phenobarbital promoted hepatocarcinogenesis. Neoplasma. 1999;46(2):105-11. PMID 10466434.
8. Reddy ARNR, Mahender P, Reddy YN, Krishna RK. Hypoglycemic and hypolipidemic activities of Trianthema portulacastrum plant in normal and alloxan induced diabetic rats. Int J Pharmacol. 2010;6(2):129-33.
9. Kokate CK. Practical pharmacognosy, Vallabh Prakashan, New Delhi; 1994.
10. Harborne JB. Phytochemical methods. London: Chapman & Hall; 1998.
11. Ribatti D. The chick embryo chorioallantoic membrane (CAM). A multifaceted experimental model. Mech Dev. 2016;141:70-7. doi: 10.1016/j.mod.2016.05.003, PMID 27178379.
12. Ribatti D. Chick embryo chorioallantoic membrane as a useful tool to study angiogenesis. Int Rev Cell Mol Biol. 2008;270:181-224. doi: 10.1016/S1937-6448(08)01405-6, PMID 19081537.
13. Tufan AC, Satiroglu-Tufan NL. The chick embryo chorioallantoic membrane as a model system for the study of tumor angiogenesis, invasion and development of anti-angiogenic agents. Curr Cancer Drug Targets. 2005;5(4):249-66. doi: 10.2174/1568009054064624, PMID 15975046.
14. Ndong JDLC, Obiang CS, Ondo JP, Ndong-Atome GR, Abessolo FO, Obame-Engoga LC. Ngoua-Meye-Misso RL. Clin Phytosci. 2018. Phytochemical studies, Antiangiogenic, anti-inflammatory and antioxidant activities of Scyphocephalium ochocoa Warb. (Myristicaceae) Medicinal plant from Gabon;4:15-9.
15. Mandal A, Bishayee A. Trianthema portulacastrum Linn. Displays anti-inflammatory responses during chemically induced rat mammary tumorigenesis through simultaneous and differential regulation of NF-κB and Nrf2 signaling pathways. Int J Mol Sci. 2015;16(2):2426-45. doi: 10.3390/ijms16022426, PMID 25622256.
16. Narendra K, Rajendra S, Saminathan M, Singh KP, Dhama K, Arun APM, Kurade NP. Therapeutic effect of hydroethanolic extract of Trianthema portulacastrum L. against N-nitroso-N-Methylurea-induced mammary tumors in Wistar rats. Ind J Trad Knowl. 2020;19(2):406-15..