

**EVALUATION OF *IN VITRO* ANTIANGIOGENESIS ACTIVITY ON METHANOLIC EXTRACT OF *CLEMATIS BUCHANIANA* PLANT**MANOJ B<sup>1</sup>, MAHAK A<sup>1</sup>, VARSHA T<sup>1</sup>, MANOJ B<sup>1</sup>, ABHISHEK T<sup>2\*</sup><sup>1</sup>Devsthali Vidyapeeth College of Pharmacy, Lalpur, Rudrapur - 263 148, Uttarakhand, India. <sup>2</sup>Devsthali Vidyapeeth Institute of Pharmacy, Lalpur, Rudrapur - 263 148, Uttarakhand, India. Email: abhishekt1983@gmail.com

Received: 24 September 2016, Revised and Accepted: 25 October 2016

**ABSTRACT**

**Objective:** Angiogenesis plays an important role in embryonic development and various physiological processes. However, excessive angiogenesis is associated with several pathological conditions including cancer. *Clematis* is a genus of about 300 species within the buttercup family Ranunculaceae. It is native to the India, where has been used as folk medicine for the treatment of various ailments. This work is aimed to evaluate the antiangiogenesis activity in the crude methanolic extract of *Clematis buchaniana* plant.

**Methods:** The entire aerial parts of *C. buchaniana* were extracted by soxhletation in methanol. Then, the solvent was evaporated to dryness to yield the dried crude extract of *C. buchaniana*. Then, the extract was subjected to preliminary phytochemical screening to determine the active constituents for effective pharmacological activity. The *in-vitro* antiangiogenesis effects were later evaluated using chorioallantoic membrane model carried out by incubation in fresh chicken's eggs.

**Results:** The crude methanolic extract of *C. buchaniana* was found to have slight ability to inhibit angiogenesis that was evaluated by visualization.

**Conclusion:** *C. buchaniana* plant extract inhibits angiogenesis by blocking normal vascularization in chick embryo. The ability of inhibiting angiogenic process in eggs by this extract can provide us an herbal anticancer agent in future for further scrutiny.

**Keywords:** Antiangiogenesis, Chorioallantoic membrane, Incubation, Angiogenesis, *Clematis buchaniana*, Methanolic extract.

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**INTRODUCTION**

Angiogenesis, the formation of new blood vessels is a biological process that plays a fundamental role in embryonic development [1]. It plays a key role in a various physiological and pathological process such as embryonic development, wound healing, chronic inflammation, tumor growth, and metastasis [2,3]. Angiogenesis blockade has been shown to be an effective strategy in inhibiting tumor growth and metastasis [4]. Endothelial cells in tumor bed tend to be more susceptible to cytotoxic agents due to their high proliferation rate. In addition, endothelial cells, on the contrary to cancerous cells, are genetically stable as they do not undergo mutations, and hence more sensitive to apoptotic effects of the cytotoxic agents. Thus, these features of endothelial cells make them a compelling target for antiangiogenesis treatment [5]. Consequently, cytotoxic agents pose as candidates as antiangiogenic agents on top of their potent activity in causing the death of cancerous cells. Extensive studies have been conducted to assess the role of oxidative stress, and hence the use of antioxidants in the prevention of many diseases such as cancer, inflammation, and atherosclerosis [6]. The use of traditional herbs for medicine has been a common practice from stone age. From years ago man has always scrutinized his biotic world for medicines which mainly contains plants as the most precious store for medicines. Keeping it in view familiarity increased with the various species of *Clematis* (Ranunculaceae) which made it an essential garden plant in today's period. *Clematis* is a genus of about 300 species within the buttercup family Ranunculaceae. Their garden hybrids have always been popular among gardeners, beginning with *Clematis jackmanii*, a garden since 1862; more hybrid cultivars are being produced constantly. They are mainly obtained from Chinese and Japanese origin. Some other species of *Clematis* has been evaluated for its anti-inflammatory, cytotoxic, and antimicrobial effects but *Clematis buchaniana* has not been studied much. The whole plant of *C. buchaniana* was traditionally

used in Nepalese medicinal plants for stronger immunity, cooling effect, and asthma [7]. Due to its traditional uses and other established pharmacological activities on the different species of *Clematis* and minimal pharmacological data on *C. buchaniana* made it an intense area for studying antiangiogenic activity. The main purpose of this antiangiogenic activity on *C. buchaniana* was actually to determine the ability in the extract to inhibit angiogenesis in a growing and well incubated fresh chicken's egg at 37°C [8].

**METHODS****Collection and authentication of plant material**

The entire plant of *C. buchaniana* was collected from the village of Kotma Kalimath (Kedarnath region) hilly areas of Garhwal and then further identified and authenticated by the Botanical Survey of India, Dehradun. The voucher specimen no. 115904 was deposited in herbarium. Further, the identified plant was washed to remove any dust and other earthy matter, further was shade, dried, and powdered with laboratory mill. The crushed plant was subjected for extraction.

**Chemicals used**

Various chemicals used in this activity are crude dried extract, ethanol (for sterilization), water for injection as the solvent used in dosing of eggs,  $\beta$ 1,4-galactan sulfate (standard) pellets.

**Instruments used**

The various instruments used in performing this activity are given in Table 1.

**Preparation of crude plant extract**

About 200 g of the dried plant was kept in the Soxhlet apparatus (Borosil), and then, it was subjected to Soxhletation using methanol as



**Fig. 1: Effect of *Clematis buchaniana* methanolic extract on angiogenesis inhibition of blood vessels, (a) Control group: Formation of blood vessels and (b) Test group: Slight inhibition of angiogenesis after addition of test drug (10 µg/0.5 ml)**

the menstruum to obtain methanolic extract of the entire crude plant *C. buchaniana*. The obtained methanolic extract was filtered, and the excessive solvent was evaporated using vacuum rotator evaporator under reduced pressure. After evaporation of the solvent, the crude extract was placed in the desiccator for removal of remaining moisture from the extract to dry it completely. Then, the percentage yield of the dried crude extract was evaluated using the given formula that yielded to about 52 g of crude dry extract.

$$\% \text{ Yield} = \frac{\text{Weight of extract (g)} \times 100}{\text{Weight of dry powder (g)}}$$

#### Antiangiogenesis activity

The antiangiogenic activity was performed as follows using chorioallantoic membrane (CAM) assay method:

#### CAM assay

Fresh fertile eggs were bought from the local poultry farm of Lalpur and were kept for incubation at 37.5°C in humidified incubator (humidity 55-60%). Ethanol (70%) was used for sterilizing the surface of eggs. After 3 days of incubation with the help of sterile syringe, 5-8 ml of albumin was sucked and removed from each egg by making a window of 1 cm in diameter at the blunt end of the eggs or by directly pricking the needle through egg shell (this is done to get better quantification of CAM vasculature). The egg was sealed up with the help of sterile plastic film tape and again incubated for 24 hrs.

#### Procedure of preparation and administration of sample solutions

Sterile water for injection was used as vehicle in the preparation of sample solutions. Sample solution of varying concentrations was prepared after calculating effective dose 50% from lethal dose 50% that were found to be 200 and 2000 mg/kg. Then, the eggs were weighed individually, and average weight was calculated accordingly the dose of the sample was found as 10 mg/kg for which a concentration of 20 mg/ml was prepared. The pH of the solution was maintained between 6.5 and 7.5 by the addition of diluted HCl and NaOH solutions. 0.5 ml of above concentration of sample solution was applied with the help of syringe from the window in all groups of growing embryos on the 5<sup>th</sup> day of their incubation. Again kept for incubation for next 24 hrs. The pictures were clicked at every stage after incubation of all three groups (control, test, and standard). The  $\beta$ 1, 4-galactan sulfate was taken as standard, and similar concentration was prepared as that of the sample solution. The extent of degeneration of CAM vasculature was noted on the 7<sup>th</sup> day of incubation in all three groups [9-15].

**Table 1: List of Instrument used in Antiangiogenic activity**

|                             |           |
|-----------------------------|-----------|
| Electronic weighing machine | Citizen   |
| Ultrasonic bath sonicator   | Scientech |
| Vacuum rotatory evaporator  | Scientech |
| Hot air oven                | Scientech |
| Water bath                  | Scientech |
| BOD incubator               | Scientech |

**Table 2: Antiangiogenic activity of methanolic extracts of *Clematis buchaniana* extract**

| Sample                                   | Concentration | Antiangiogenic score $\pm$ SD |
|--|---------------|-------------------------------|
| Test sample                              | 10 µg/0.5 ml  | 0.6 $\pm$ 1                   |
| $\beta$ 1, 4-galactan sulfate (standard) | 50 µg/pallet  | 1.4 $\pm$ 1                   |

SD: Standard deviation

## RESULTS AND DISCUSSION

### Antiangiogenic activity

Angiogenesis, tightly modulated through a balance of positive and negative regulatory factors, is to operate by proangiogenic growth factors such as vascular endothelial growth factor, which in turn induce activation of their respective receptors on the surface of endothelial cells, resulting in angiogenesis, therefore, identification of new agents that inhibit the growth in endothelial cell could have potential to inhibit tumor angiogenesis and subsequently repress tumor growth. After 3-5 days of treatment, untreated control eggs gave branching pattern of tube-like capillaries. In contrast, capillary tube formation was slightly suppressed in eggs, which was treated with *C. buchaniana* (10 µg/0.5 ml) [16].

Fig 1 shows the effect of *Clematis buchaniana* methanolic extract on angiogenesis inhibition of blood vessels in control and test group.

The result of antiangiogenic activity is shown in Table 2.

## CONCLUSION

This study has been subjected to following aspects of an important herbal medicinal plant *C. buchaniana* (family Ranunculaceae), the study included antiangiogenic activity via CAM assay method. Angiogenesis is an important initiative process in case of many diseases such as tumor, cataract, and psoriasis. The methanolic extract of *C. buchaniana* shows slight inhibitory activity against this process. These herbs may have promising antitumor activity if given as an adjuvant in chemotherapy or in targeting other angiogenesis-related diseases.

The anti-inflammatory and antiangiogenic activity of *C. buchaniana* need further more detailed study in the path of isolation and authentication of screened phytochemicals responsible for above activities of this medicinal drug [17-21].

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