RESEARCH ARTICLE

Evaluation of Antiangiogenic Potential of Ethanolic Extract of *Bauhinia variegata* Leaves in Mice

Namrata S. Kathole[1], Vikas V. Karande[1]*, Babasaheb C. Ghumare[1], Chandrashekhar S. Mote[2], Vijaysinh D. Lonkar[3], Samir N. Jadhav[4], Pooja P. Bachapalle[1]

Abstract

The present study was planned to evaluate the antiangiogenic potential of ethanolic extract of *Bauhinia variegata* using the sponge implantation method in mice. Swiss albino adult male mice (n=30) weighing 18-22 g were divided into three groups comprising of ten animals in each. Groups I, II, and III were used as negative control, positive (SU5416) control, and treatment (extract of *Bauhinia variegata*) groups, respectively. Sponges were implanted subcutaneously and the mice were sacrificed after 14th day. The implanted sponges were then segregated and processed for haemoglobin concentration, vascular endothelial growth factor (VEGF) estimation, and histopathological evaluation. In treatment groups I to III, the mean (±SE) haemoglobin content was found to be 1.919±0.048, 0.410±0.049, and 1.604±0.101 µg/mg weight of sponge, VEGF values 2.462±0.181, 0.529±0.044, and 1.609±0.069 pg/mg weight of sponge, and the mean vessels density (±SE) per field (microscopically under 40x) was 14.9±1.25, 1.3±0.3, and 10.6±0.92, respectively. The extract of *Bauhinia variegata* was found to have inhibitory potential on haemoglobin concentration, VEGF concentration, and mean vascular density.

Key words: Angiogenesis, *Bauhinia variegata,* VEGF, MVD, Sponge implant.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.5.14

Introduction

Tumor cells in cancer cause metastatic proliferation of the tissues, which is important for the blooming of vasculature. This blooming of vasculature includes both lymphangiogenesis and angiogenesis. Without vascular support, tumors may become necrotic or even apoptotic (Nishida *et al*., 2006). Angiogenesis is developing new capillaries (blood vessels without a completely established tunica media) from pre-existing blood vessels, either by sprouting or intussusception (Kolte *et al*., 2016). Angiogenesis allows the delivery of oxygen and nutrients to the body tissues and removes waste products (Hoff and Machado, 2012). It is a vital function required for growth and development as well as wound healing.

Pro-angiogenic factors expressed by tumor cells include vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), as well as enzymes such as cyclooxygenase-2 (COX-2) and protein kinase-A (PKA) and signaling molecules like integrins (Sun *et al.,* 2015). Being a powerful inducer of angiogenesis, vascular endothelial growth factor (VEGF) stimulates the growth and proliferation of endothelial cells (ECs), preventing apoptosis of endothelial cells (Yadav *et al*., 2015). SU5416 (Semaxanib) is a small organic compound that inhibits the VEGF-mediated auto-phosphorylation of the VEGFR receptor needed for its activation (Kieran *et al.,* 2009).

Bauhinia variegata is locally known as 'Kachnar'. *B. Variegata* has different pharmacological properties such as anti-inflammatory (Mohamed *et al*., 2009), anticancer (Agrawal and Pandey, 2009), nephroprotective (Pani *et al*., 2011), antimicrobial (Mishra *et al*., 2013), anthelmintic (Bairagi *et al*., 2012), hepatoprotective, and anti-diabetic (Gurjar *et al*., 2018) activities. Various compounds, such as

[1]Department of Veterinary Pharmacology and Toxicology, KNP College of Veterinary Science, Shirwal, Dist: Satara-412801, Maharashtra, India

[2]Department of Veterinary Pathology, KNP College of Veterinary Science, Shirwal, Dist: Satara-412801, Maharashtra, India

[3]Department of Poultry Science, KNP College of Veterinary Science, Shirwal, Dist: Satara-412801, Maharashtra, India

[4]Department of Veterinary Biochemistry, KNP College of Veterinary Science, Shirwal, Dist: Satara-412801, Maharashtra, India

Corresponding Author: Vikas V. Karande, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, KNP College of Veterinary Science, Shirwal, Dist: Satara-412801, E-mail: drvikaskarande1@gmail.com

How to cite this article: Kathole, N. S., Karande, V. V., Ghumare, B. C., Mote, C. S., Lonkar, V. D., Jadhav, S. N., & Bachapalle, P. P. (2023). Evaluation of Antiangiogenic Potential of Ethanolic Extract of *Bauhinia variegata* Leaves in Mice. Ind J Vet Sci and Biotech. 19(5), 70-73.

Source of support: Nil

Conflict of interest: The author(s) declare(s) that there is no conflict of interest.

Submitted:12/07/2023**Accepted:** 02/08/2023 **Published:** 10/09/2023

cyanidin glucoside, malvidin glucoside, peonidin glucoside, and kaempferol galactoside, isolated from *B. variegata* are believed to inhibit the growth and spread of various cancers of the lung, liver, oral cavity, larynx, and malignant ascites (Gautam, 2012). In view of all these facts, *B. Variegata* plant was selected to investigate its antiangiogenic potential in the mouse model.

Materials and Methods

The present study was conducted at the Department of Veterinary Pharmacology and Toxicology, KNP College of Veterinary Science, Shirwal, Satara, Maharashtra, India during October 2022 to March 2023 using adult male mice following approval of the protocol by the institutional animal ethics committee of the institute (IAEC approval No.: IAEC/21/KNPCVS/2022).

Experimental Design:

The Swiss albino male mice (n=30) weighing 18-22 g were procured from the National Institute of Biosciences, Pune. All the selected animals were kept under acclimatization for seven days before the start of the experiment. Mice were maintained at an ambient temperature of 22-25°C, relative humidity of 40-70%, and 12 h light and dark cycle was maintained. Pelleted mice feed (supplied by Amrut Feed, Sangli, Maharashtra) and purified potable water in glass bottles were provided *ad-libitum*. SU5416 is the standard angiogenic inhibitor procured from Sigma-Aldrich. The mice were divided into three equal groups comprising ten animals in each, Group I treated with saline-soaked blank sponges (negative control), group II treated with SU5416, standard VEGF inhibitor (positive control), and Group III treated with the leaves extract of *Bauhinia variegata* (treatment group).

The leaves of *B. variegata* were collected from the surrounding area of Shirwal and identified by the botanist. Leaves were cleaned and dried in the shade, and mechanically fine powder was prepared. Ethanolic extract of *B. variegata* was prepared using Soxhlet's apparatus with solvent laboratory-grade ethanol (70% v/v) at 60-70°C till colorless ethanol was obtained from a side tube which required 40-50 cycles. Ethanol was removed by rotary evaporator to obtain ethanol-free extract. The extract was then stored in Eppendorf tubes at refrigerated temperature (8°C) till further use.

Sponge Preparation and Implantation:

To study the antiangiogenic potential of *B. variegata,* the *in vivo* angiogenesis assay comprising subcutaneous implantation of a gel foam sponge treated with concentrations of test extracts along with positive control and untreated control sponge in mice was performed as specified by McCarty *et al*. (2002). Sterile absorbable gel foam sponges (Goodwill Lifesciences Private Limited, Gujrat) were cut (4×4×4 mm), soaked in sterile PBS in a Petri dish, and kept overnight. About half an hour prior to implantation, sponges were soaked in separate Petri dishes containing saline, SU5416 (1 mg/mL), and an extract of *B. variegata* (50 mg/mL) solutions for 10-15 min and subsequently dipped in 0.4% agarose for 10 min. for solidification. After solidification sponges treated with saline, SU5416, and extract of *B. variegata* were implanted to the animals of group I, II, and III, respectively.

Mice were anaesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg b. wt.) with the intraperitoneal route. An incision (1 cm long) was made vertically on the sterile dorsolateral portion of the back. The sponges were inserted aseptically on both the sides of incision in the pouch made subcutaneously, in the respective groups. The incision was closed aseptically with a horizontal mattress suture. Animals were kept for 14 days under observations in normal laboratory conditions. On day 14^{th}, all the animals were sacrificed, and implanted sponges were segregated and processed for haemoglobin estimation, VEGF determination, and histopathological evaluation.

Haemoglobin Estimation:

The haemoglobin (Hb) level in the sponges was measured using standard Drabkin's method in practice to assess the extent of the vascularization of the sponge implants. The sponge implants were homogenized in Drabkin's reagent (2 mL) and centrifuged at $8x10^3$g for six minutes. The supernatants were filtered through a 0.22 µm filter. The haemoglobin concentration from supernatant samples was assessed calorimetrically at absorbance 540 nm using a spectrophotometer.

VEGF Determination:

On 14^{th} day the gel foam sponges from the skin site were removed and homogenized manually in 1.0 mL PBS containing 0.05% Tween 20 followed by centrifugation at 4°C for 10 min at 10^3 *g*. in a cooling microcentrifuge (Eppendorf). VEGF concentration was assessed using the Mouse VEGF ELISA kits procured from Ray Biotech, life Inc (USA). The cytokines (proinflammatory cytokines such as interleukins) in the 50 µL of supernatant from each implant were measured using Immunoassay ELISA kits as per the manufacturer's protocol.

Histopathological Evaluation:

To know the extent of neovascularization in control and treated groups in the mice, histopathological study was performed. Sponges were harvested, fixed in formalin stained with H&E, and the number of vessels was counted in 15 consecutive fields using a 40 X objective under a microscope, and the mean vascular density (MVD) per microscopic field was calculated.

The numerical data were expressed as mean (±SE) and analyzed by one-way analysis of variance. A P-value of less than 0.05 was considered significant.

Results and Discussion

The sponge implantation method to study angiogenesis has been adopted in several studies involving angiogenesis (Gosavi *et al*., 2019; Rathod *et al*., 2022). In the present study, the antiangiogenic activity of the ethanolic extract of *Bauhinia variegata* was evaluated using a mouse model by sponge implantation method.

Results of mean haemoglobin (µg Hb/mg sponge), mean VEGF (pg/mg sponge), and mean vascular density (MVD) per microscopic field are depicted in Table 1.

Haemoglobin Concentration:

The mean haemoglobin concentration of the sponges from group I (control) was significantly highest than in group II (SU5416) and III (*B. variegata*). Thus, treatment with blank sponges in group I indicated normal progression of angiogenesis. The mean Hb concentration (µg/mg of sponge) from group II was found to be the lowest than group I and III, and the latter two groups also differed significantly from each other.

Rajkapoor *et al*. (2003) reported a reduction in the haemoglobin content of RBC while evaluating the antitumor activity of the ethanolic extract of *B. variegata* against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Kolhe *et al*. (2015) evaluated the cytotoxic effect of *B. variegata* in albino rats and reported decreased values of Hb due to the high amount of tannin content. Tannin affects the absorption and metabolism of iron, possibly one of the reasons behind the reduced Hb values. However, it is unclear whether the reduced Hb values in their study were due to the tannin effect or due to reduced vascularization in the sponge. Therefore, more intensive study is essential to find out the exact mechanism of effect on haemoglobin levels.

VEGF Concentration:

Group I had significantly ($p \leq 0.05$) highest mean VEGF concentration as compared to other groups. The mean VEGF value of group II (SU5416) was the lowest among the other groups. Group III (*B. variegata*) showed lower VEGF concentration than control Group I. Thus *B. variegata* was able to reduce VEGF concentration to a certain level. Singh and Kale (2010) reported the cancer prevention potential of *Bauhinia variegata* bark against DMBA-induced skin papilloma genesis in mice.

Mean Vascular Density:

Group I had the highest MVD value followed by Group III and II (Table 1, Fig. 1-3). The MVD concentration was the lowest in group II, meaning standard drug SU5416 has excellent potential to inhibit the development of blood vasculature. The group receiving an ethanolic extract of *B. variegata* varied significantly ($p<0.05$) from groups I and II in MVD values. Pandey (2017) evaluated the antitumor activity of *B. variegata* against the B16F10 melanoma tumor model in C57BL mice and observed a reduction in the tumor growth by the *B. variegata* extract which may be related to a reduction in host angiogenesis. The tumor control group showed a prominent and dense microvasculature.

The literature citing antiangiogenic or similar kind of experiments are scanty, which limits the in depth analysis of present results.

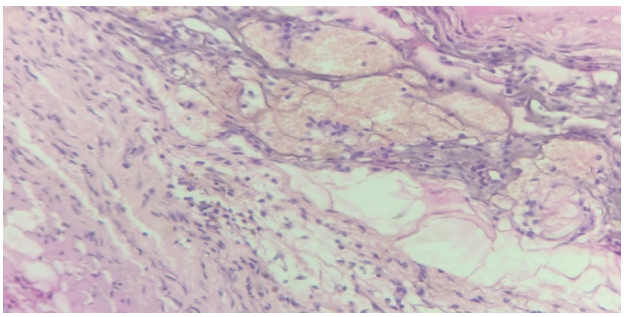

Fig. 1: Microphotograph of saline-treated sponge (Group I) showing moderate angiogenesis with newly formed blood vessels filled with RBCs and fibrous tissue proliferation in the sponge implant. HE X400

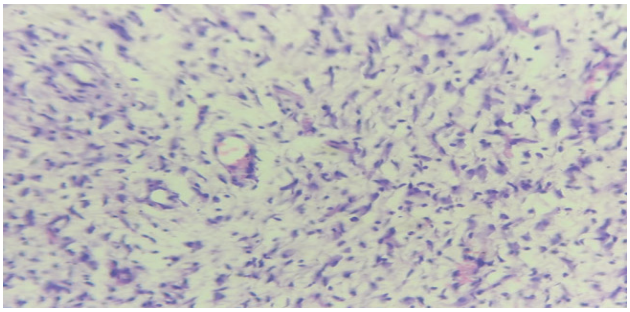

Fig. 2: Microphotograph of sponge treated with SU5416 (Group II) showing minimal angiogenesis with few newly formed blood vessels filled with RBCs and fibrous tissue proliferation in the sponge implant. HE X 400

Table 1: Mean (±SE) concentration of haemoglobin, VEGF and mean vascular density (MVD) from processed sponges of different groups of mice

Group No.	Treatment group	Hb concentration (µg/mg wt of sponge)	VEGF concentration (pg/mg sponge)	MVD (per microscopic Field, 40X)
I	Negative Control	1.919 ± 0.048^{c}	2.462 ± 0.181^{c}	14.9 ± 1.25^{c}
II	Positive Control	0.410 ± 0.049^{a}	0.529 ± 0.044^{a}	1.3 ± 0.31^{a}
III	*Bauhinia variegata* extract	1.604 ± 0.101^{b}	1.609 ± 0.069^{b}	10.6 ± 0.92^{b}

Means bearing different superscripts within the column differ significantly ($p \leq 0.05$).

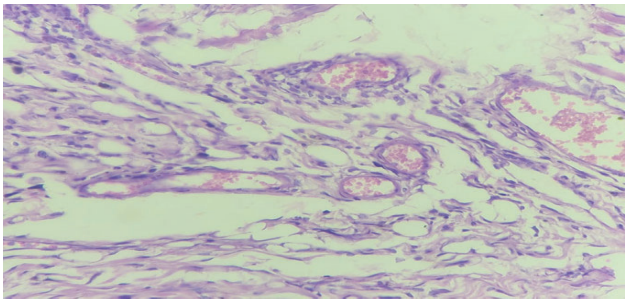

Fig. 3: Microphotograph of sponge treated with extract of *B.variegata* (Group III) showing moderate angiogenesis with newly formed blood vessels filled with RBCs and fibrous tissue proliferation in the sponge implant. HE X400

Conclusions

B. variegata showed a potential of inhibiting angiogenesis when compared with the untreated group. However, studies need to be conducted to find the exact mechanism of preventing angiogenesis.

Acknowledgments

The authors are grateful to the Associate Dean of KNP College of Veterinary Science and Authorities of MAFSU, Nagpur, for extending help in the form of funds and facilities.

References

Agrawal, R.C., & Pandey, S. (2009). Evaluation of the anticarcinogenic and antimutagenic potential of *Bauhinia variegata* extract in Swiss albino mice. *Asian Pacific Journal of Cancer Prevention, 10*(5), 913-916.

Bairagi, S.M., Aher, A.A., & Nimase, P.K. (2012). *In vitro* anthelmintic activity of *Bauhinia variegata* bark (Leguminosae). *International Journal of Pharmacy and Pharmaceutical Sciences, 4*(3), 672-674.

Gautam, S. (2012). *Bauhinia variegata* Linn: All-purpose utility and medicinal tree. *Forestry Bulletin, 12*(2), 61-64.

Gosavi, S.C., Karande, V.V., Ghumare, B C., Mote, C.S., Mhase, P.P., & Mehere, P.V. (2019). Evaluation of the antiangiogenic activity of *Nigella sativa* extract by sponge implantation method in a mouse model. *Indian Journal of Pure and Applied Biosciences*, *7*(5), 25-29.

Gurjar, H., Pandey, H., Verma, A., Irchhaiya, R., & Singh, P.P. (2018). Antidiabetic activity of *Bauhinia variegata* extracts in alloxan-induced diabetic rats. *Journal of Drug Delivery and Therapeutics, 8*(1), 29-32.

Hoff, P.M., & Machado, K.K. (2012). Role of angiogenesis in the pathogenesis of cancer. *Cancer Treatment Reviews, 38*(7), 825-833.

Kieran, M.W., Supko, J.G., Wallace, D., Fruscio, R., Poussaint, T.Y., Phillips, P., & Pediatric Brain Tumor Consortium. (2009). Phase I study of SU5416, a small molecule inhibitor of the vascular endothelial growth factor receptor (VEGFR) in refractory pediatric central nervous system tumors. *Pediatric Blood & Cancer, 52*(2), 169-176.

Kolhe, R., Acharya, R.N. and Ravishankar, B. (2015). Chronic toxicity study of stem bark powder of Kanchanara (*Bauhinia variegata* L.) in albino rats. *Indian Journal of Natural Products and Resources*, *5*(3), 244-248.

Kolte, D., McClung, J.A., & Aronow, W.S. (2016). Vasculogenesis and angiogenesis. In: *Translational Research in Coronary Artery Disease.* Academic Press, pp. 49-65.

McCarty, M.F., Cheryl, H., Corazon, B., Bucana, D., & Fidler, I.J. (2002). Quantitative and qualitative *in vivo* angiogenesis assay. *International Journal of Oncology 21*, 5-10.

Mishra, A., Sharma, A.K., Kumar, S., Saxena, A.K., & Pandey, A.K. (2013). *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. *BioMed Research International, 2013*.

Mohamed, M.A., Mammoud, M.R., & Hayen, H. (2009). Evaluation of antinociceptive and anti-inflammatory activities of a new triterpene saponin from *Bauhinia variegata* leaves. *Zeitschrift für Naturforschung C, 64*(11-12), 798-808.

Nishida, N., Yano, H., Nishida, T., Kamura, T., & Kojiro, M. (2006). Angiogenesis in cancer. *Vascular Health and Risk Management, 2*(3), 213.

Pandey, S. (2017). *In vivo* antitumor potential of extracts from different parts of *Bauhinia variegata* Linn. Against b16f10 melanoma tumor model in c57bl/6 mice. *Applied Cancer Research, 37*(1), 1-14.

Pani, S.R., Mishra, S., Sahoo, S., & Panda, P.K. (2011). Nephroprotective effect of *Bauhinia variegata* (Linn.) whole stem extract against cisplatin-induced nephropathy in rats. *Indian Journal of Pharmacology, 43*(2), 200.

Rajkapoor, B., Jayakar, B., & Murugesh, N. (2003). Antitumour activity of *Bauhinia variegata* on Dalton's ascitic lymphoma. *Journal of Ethnopharmacology, 89*(1), 107-109.

Rathod, R.V., Karande, V.V., Ghumare, B.C., Rangnekar, M., Mote, C.S., Lonkar, V.D., & Tulavi, S.M. (2022). Evaluation of the antiangiogenic activity of *Justicia adhatoda* extract by sponge implantation method in mouse. *Journal of Veterinary Pharmacology and Toxicology, 21*(1), 9-11.

Singh, M., & Kale, R.K. (2010). Abstract A64: Chemopreventive potential of *Bauhinia variegata* against DMBA-induced skin papilloma genesis in Swiss albino mice. *Cancer Prevention Research, 3*(1_Supplement), A64-A64.

Sun, Q., Heilmann, J., & König, B. (2015). Natural phenolic metabolites with antiangiogenic properties – A review from the chemical point of view. *Beilstein Journal of Organic Chemistry, 11*(1), 249-264.

Yadav, L., Puri, N., Rastogi, V., Satpute, P., & Sharma, V. (2015). Tumour angiogenesis and angiogenic inhibitors: A review. *Journal of Clinical and Diagnostic Research, 9*(6), XE01.