

Anti-Angiogenic Activity of *Ficus carica* Latex Extract on Human Umbilical Vein Endothelial Cells

Ali Mostafaie, Ph.D.*, Kamran Mansouri, M.Sc., Amir-Hossein Norooznezhad, B.Sc., Hamid-Reza Mohammadi-Motlagh, M.Sc.

Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

* Corresponding Address: P.O.Box: 1568, Medical Biology Research Center, , Sorkheh LigeH, Kermanshah, Iran
Email: amostafaie@kums.ac.ir

Received: 10/May/2009, Accepted: 13/Jan/2010

Abstract

Angiogenesis, the formation of new blood vessels, is a key process in cancer development and metastasis. In this study, the anti-angiogenic and anti-proliferative potentials of *Ficus carica* latex extract have been investigated using human umbilical vein endothelial cells (HUVECs).

Different doses of latex extract were prepared and added to a three-dimensional culture of HUVEC in a collagen matrix. After 3-5 days of treatment, the anti-angiogenic effects of the extracts were monitored microscopically. For the anti-proliferation assay, different doses of the extracts were examined on HUVECs.

The results clearly indicated that latex extract could inhibit proliferation and capillary tube formation of HUVECs in a dose-dependent manner at the range of 100-400 µg/ml. In addition, the extract was not cytotoxic up to 450 µg/ml as assessed by trypan blue and lactate dehydrogenase (LDH) cytotoxicity assays.

It is concluded that latex extracts of *F. carica* contain strong anti-angiogenic and anti-proliferative activities. Our data indicates that latex extract could be a candidate as a potential agent for the prevention of angiogenesis in cancer and other chronic disorders.

Keywords: Anti-Angiogenesis, Anti-Proliferative, Umbilical Cord, Endothelial Cell

Cell Journal(Yakhteh), Vol 12, No 4, Winter 2011, Pages: 525-528

Angiogenesis, the formation of new blood vessels, is linked to many pathological conditions such as diabetic retinopathy, atherosclerosis, tumor growth and metastasis (1). Angiogenesis can be separated in to several main steps, including: digestion of basement membrane and extracellular matrix, migration, proliferation and rearrangement of endothelial cells to form new blood vessels (2, 3). Inhibition of angiogenesis has been considered to be advantageous for the prevention of neoplastic growth and chronic inflammatory diseases (1, 4).

Latex from the fig (*Ficus carica*) tree is used in Iranian traditional medicine for the treatment of papillomatosis (5). Furthermore, it has been reported that latex extract has different therapeutic effects such as hypoglycemic induction (6), cancer suppression (7) and anti-helminthic effect (8). Since angiogenesis plays a role in the development of some diseases, including tumor growth and metastasis; therefore, the discovery of anti-angiogenic agents is very important. In this study, we have addressed the anti-angiogenic and anti-proliferative activities of latex extract on three-

dimensional cultures of human umbilical vein endothelial cells (HUVECs).

This work was approved by the Research Ethics Committee of Kermanshah University of Medical Sciences.

The used materials in this study were including: latex from the *F.carica* tree (Kermanshah, western Iran, harvested May-June 2008), rat tail collagen (Sigma, St. Louis, MO, USA) at 2 mg/ml in 0.5 M acetic acid, 10x minimum essential medium (10× MEM), MCD131 medium, Dulbecco's modified eagle's medium (DMEM), RPMI1640, fetal bovine serum (FBS; Gibco, NY, USA), 1.4 % (w/v) sodium bicarbonate solution, sodium hydroxide solution (1 M) (Sigma, St. Louis, MO, USA), dextran-coated cytodex 3-microcarriers (Amersham Pharmacia Biotech), HUVEC were obtained from the human umbilical veins of newborns by the actinidin digestion method (9), trypan blue 0.4%, and LDH cytotoxicity assay kit (Roche Chemical Co.).

After harvesting *F.carica* latex (Kermanshah, May-June 2008), the mixture was centrifuged at 20000×g for 30 minutes and the

resulted supernatant freeze dried. The powder was weighed and dissolved in culture medium DMEM.

Umbilical cords were obtained from a local hospital in Kermanshah, Iran. HUVECs were isolated according to standard methods with actinidin treatment (9). Endothelial cells were identified by immunostaining for the presence of Von Willebrand factor with the use of rabbit anti-Von Willebrand factor and goat anti-rabbit IgG horseradish peroxidase (HRP) conjugated. Cells were cultured in MCDB131 medium that contained 20% FBS. Passages 3 to 5 were used for subsequent experiments.

Cytotoxic concentrations of latex extract which reduced the viability of HUVECs were determined. Cells were grown in medium that consisted of different concentrations (10-700 µg/ml) of the extracts. After a 48 hours incubation period, cell viability was determined by trypan blue exclusion and LDH assays. The absorbance of converted dye in the LDH assay was measured at a wave length of 490 nm with background subtraction at 630 nm (10).

HUVECs were grown in MCDB131 medium supplemented with 10% FBS at 37°C and 5% CO₂. For an *in vitro* system of angiogenesis, the cells were cultured in a standard microcarrier-based model in collagen gel (11, 12). The cells were mixed with cytodex 3-microcarriers and shaken gently every 20 minutes for 4 hours. Mixtures were transferred to a 24-well tissue culture plate and left for 12-16 hours in MCDB131. The following day, beads coated with cells were mixed in type 1 collagen solution. Then, 100 µl of col-

lagen/bead mixture was added to each well of a 96 well tissue culture plate.

After the collagen/bead mixture was gelled, MCDB131 medium was added to each well and after 8-12 hours, different doses of the extracts were added. After 3-5 days of treatment, the anti-angiogenic effects of the extracts were monitored microscopically.

The inhibitory effect of latex on HUVEC proliferation was also performed. At first, HUVECs were cultured in a 96 well culture microplate containing MCDB131 medium supplemented with 10% FBS for 24 hours. Then, the cells were treated with different doses of the latex extract (0-500 µg/ml) for 72 hours. Finally, the cells were counted against control samples by a Coulter counter (KX-21, Sysmex Co).

All experiments were performed in triplicate unless otherwise noted. Results were expressed as mean ± standard deviation. Values of $p < 0.05$ were considered significant.

The results indicated that latex extract was not cytotoxic up to 450 µg/ml as assessed by trypan blue and LDH cytotoxicity assays. The extract induced a significant decrease in the proliferation of HUVEC which was dose dependent (Fig 1).

In addition, the extract was able to inhibit angiogenesis in HUVEC capillary tube formation model at 100-400 µg/ml (Fig 2).

The aim of this study was to evaluate anti-angiogenic activity of latex extract on HUVEC capillary tube formation in three-dimensional collagen matrix and the anti-proliferative effect of latex extract on HUVEC.

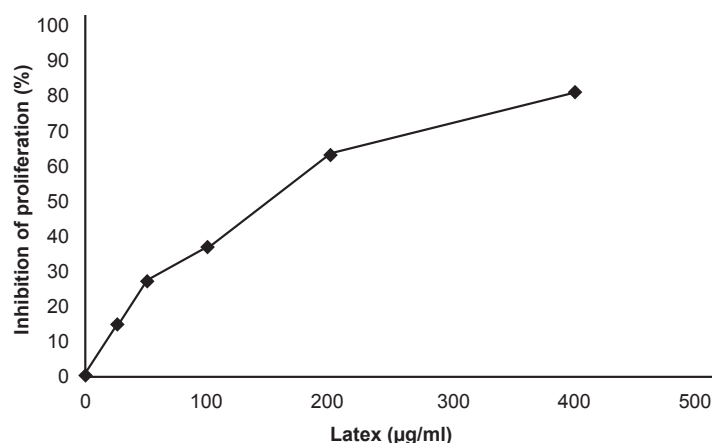


Fig 1: Dose-dependent inhibition of HUVECs by *F. carica* latex extract. Cells were cultured with the extract at various concentrations for 72 hours and the numbers of cells were counted using a Coulter counter. Data are means of three independent experiments, expressed as the percentage of test to control wells.

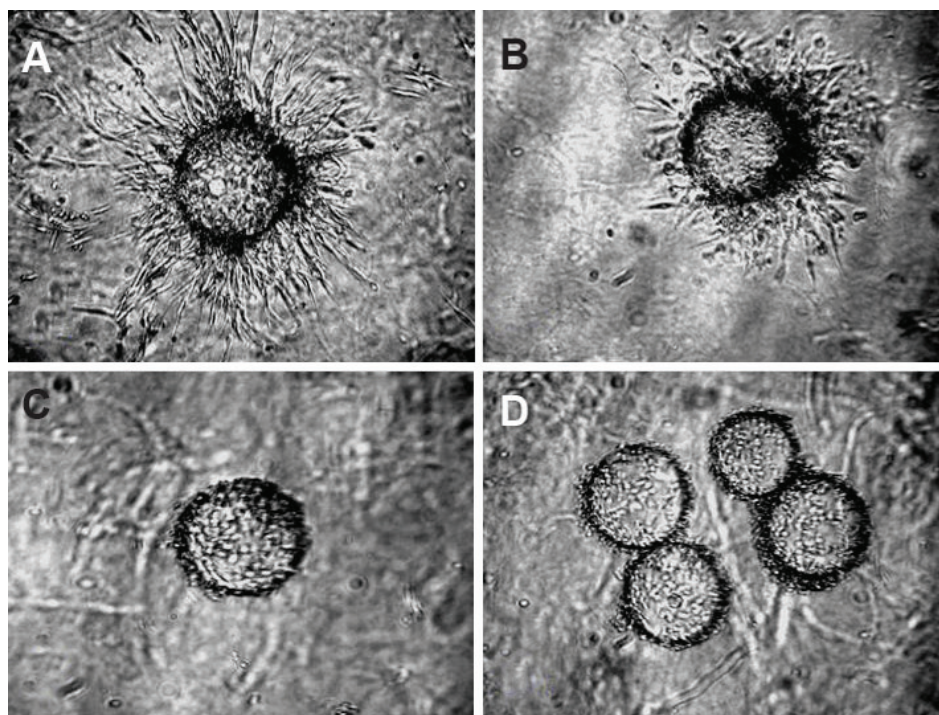


Fig 2: Anti-angiogenic activity of *F.carica* latex extract on HUVEC capillary tube formation in a three-dimensional collagen matrix. The sprouting of HUVECs was inhibited in the presence of *F.carica* latex extract at concentrations of 50 µg/ml (B), 100 µg/ml (C), and 200 µg/ml (D). Panel (A) is a control which did not receive extract.

Angiogenesis, the process of new vessel formation, is very important for the development and promotion of pathogenic processes of a variety of disorders, including tumor growth and metastasis (13, 14). Therefore, inhibition of angiogenesis could be useful in tumor treatment. In previous studies, we have reported that certain natural products, such as: aqueous extract of shallot (15), soybean trypsin inhibitor (16), a peptide from shark cartilage (17) and green tea extract (18) could inhibit angiogenesis on HUVEC three-dimensional model. In the present study, for the first time, we have shown that latex extracts of *F. carica* contain strong anti-angiogenic and anti-proliferative activities.

Therefore, we suggest that latex be considered as a new anti-angiogenic agent. However, further investigations are required to elucidate the responsible component(s) and mechanisms of action of this extract.

Acknowledgments

We are appreciative of Dr. Yadollah Shakiba and Miss Maryam Keshavarz for their valuable assistance. This work is supported partly by a grant from the Medical Biology Research Center, Kermanshah University of Medical Sciences. There is no conflict of interest in this article.

References

1. Folkman J. Angiogenesis. *Annu Rev Med.* 2006; 57: 1-18.
2. Plank MJ, Sleeman BD. Tumor - induced angiogenesis. *J Theor Med.* 2004; 5(3-4): 137-153.
3. D'Amore PA, Thompson RW. Mechanism of angiogenesis. *Annu Rev Physiol.* 1987; 49: 453-464.
4. Murry JC. Angiogenesis Protocols. In: Murry JC, editor, *Methods in Molecular Medicine*. Vol 46. Totowa: Humana Press Scientific and Medical Publishers. 2001; 185-204.
5. Hemmatzadeh F, Fatemi A, Amini F. Therapeutic Effects of Fig Tree Latex on Bovine Papillomatosis. *J Vet Med B Infect Dis Vet Public Health.* 2003; 50(10): 473-476.
6. Serracarla A, Hawkins F, Perez C, Dominguez E, Campillo JE, Torres MD. Hypoglycemic action of an oral fig-leaf decoction in type-I diabetic patients. *Diabetes Res Clin Pract.* 1998; 39(1): 19-22.
7. Rubnov S, Kashman Y, Rabinowitz R, Schlesinger M, Mechoulam R. Suppressors of cancer cell proliferation from fig (*Ficus carica*) resin: isolation and structure elucidation. *J Nat Prod.* 2000; 64(7): 993-996.
8. de-Amorin A, Borba HR, Carauta JP, Lopes D, Kaplan MA. Anthelmintic activity of the latex of ficus species. *J Ethnopharmacol.* 1999; 64(3): 255-258.
9. Mostafaie A, Bidmeshkipour A, Shirvani Z, Mansouri K, Chalabi M. Kiwifruit actinidin: a proper new collagenase for isolation of cells from different tissues. *Appl Biochem Biotechnol.* 2008; 144(2): 123-131.
10. Spiridonov NA, Kononov DA, Arkhipov VV. Cytotoxicity of some Russian ethnomedicinal plants and plant compounds. *Phytother Res.* 2005; 19(5): 428-432.
11. Auerbach R, Lewis R, Shinnors B, Dubai L, Akhtar N. Angiogenesis assays: a critical overview. *Clin Chem.* 2003; 49(1): 32-40.
12. Nehls V, Dreckhahn D. A novel, microcarrier-based in

Anti-Angiogenic Activity of *F.carica*

- vitro assay for rapid and reliable quantification of three-dimensional cell migration and angiogenesis. *Microvasc Res.* 1995; 50(3): 311-322.
13. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med.* 1995; 1(1): 27-31.
 14. Leikens S, Clercq ED, Neyts J. Angiogenesis: regulators and clinical application. *Biochem Pharmacol.* 2001; 61(3): 253-270.
 15. Mohammadi Motlagh HR, Mansouri K, Shakiba Y, Keshevarz M, Khodarahmi R, Siami A, et al. Anti-angiogenic effect of aqueous extract of shallot (*Allium ascalonicum*) bulbs in rat aorta ring model. *Yakhteh Medical Journal.* 2009; 11(2): 184-189.
 16. Shakiba Y, Mansouri K, Mostafaie A. Anti-angiogenic effect soybean kunitz trypsin inhibitor on human umbilical vein endothelial cells. *Fitoterapia* 2007; 78(7-8): 587-589.
 17. Hassan ZM, Feyzi R, Sheikhan A, Bargahi A, Mostafaie A, Mansouri K, et al. Low molecular weight fraction of shark cartilage can modulate immune responses and abolish angiogenesis. *Int Immunopharmacol.* 2005; 5(6): 961-970.
 18. Shakiba Y, Mostafaie A. Inhibition of corneal neovascularization with a nutrient mixture containing lysine, proline, ascorbic acid, and green tea extract. *Arch Med Res.* 2007; 38(7): 789-791.
-