Original Article

Access this article online
Quick Response Code:

Website:
www.carcinogenesis.com
DOI:
10.4103/jcar.jcar_23_01_13

The Antiangiogenic Effect of Eriodictyol (C15H12O6) in Ex Vivo and in Vivo Animal Study

Dalya Nadhum Muaeh Al-Shilib[1], Hayder B Sahib[2]

Abstract

Background: The Angiogenesis is defined as a process of vascularization from preexistent blood vessels, in particular capillaries. Its critical in many physiological and pathological activities and controlled by a complex balance between anti- and pro-antigenic factors. Eriodictyol is a flavonoid that is part of subclasses of flavanones and is found greatly in citrus fruit, vegetable, and medicinally essential herbs. Objective: This study was aimed at the investigation of the effect of Eriodictyol upon angiogenesis process. Material and Method: twelve- to fourteen-week-OLD male albinorats are used in this study. Eriodictyolwas dissolved in DMSO and then it was seriallydiluted into six concentrations. The rat-aortic-ring assay was mainly used to explore the antiangiogenic effect of Eriodictyol exvivo. The use of Chorioallantoic membrane (CAM) assay in vivo is to measure the inhibition zone of the small capillaries by Eriodictyol. The zone of inhibition was calculated as mean inhibition zone of a blood vessels in mm ± standard deviation. Regarding MTT assay, it was used for the assessment of proliferation inhibition of human umbilical vein endothelial cells (HUVEC) cell line. The data obtained were analyzed statistically. Results: results revealed that Eriodictyol has significant dose dependent inhibiting effect on grow of blood vessels by 96.70% ± 3.5 with the concentration 200μg/ milliliter compared with -ve control. It also showed that Eriodictyol is a toxic antiangiogenic compound through the MTT assay through the logarithmic and linear regression equations with a value of 5.34. Conclusion: findings suggested that the activity of Eriodictyol can hugely diminish blood vessel growth in the assay of rat aorta rings, CAM and MTT. Eriodictyol has a repressive activity on tumorgenesis which could take an advantage like an anti-angiogenic antiprolifrative compound.

Keywords:

Antiangiogenesis- CAM- rat aorta rings- Eriodictyol- MTT.

Introduction

Angiogenesis, the formation of new blood vessels from preexisting ones, it plays a great role in physiological and pathological processes such in wound healing, placenta formation, embryonic growth, tumor development and metastasis [1]. Angiogenesis is precipitated by hypoxia that induces hypoxia inducing factor HIF which causes upregulation of pro-angiogenic factors expression [2]. Such process is enhanced only under a certain condition whenever there's a physiological demand for increment in the blood supply as in wound healing or implantation of the fertilized eggs in the endometrium [3].

Strict regulation of this system is CRITICAL for human beings, cause both excessive and insufficient develop of blood vessels could be leading to serious disease [4]. Normally angiogenesis is only restricted to maybe a few days or several weeks. However, in case of disease, angiogenesis last for months/years [5].

Angiogenesis process can straight forwardly be described as multiple steps. Firstly, antigenic stimulation that enhances endothelial cells permeability, cells proliferation and elongation of capillaries sprout processes. Secondly, the activation of matrix metalloproteinase (MMPs) which leads to proteolysis of the components of the basement membrane. Thirdly, the step of migration of the endothelial cells away from the vessel wall and their proliferation which

[1]*Department of pharmacology, college of Medicine, Al-Nahrain University, Baghdad, Iraq,* [2]*College of pharmacy, Al-Nahrain University, Baghdad, Iraq*

Address for correspondence:
Hayder B Sahib, College of pharmacy, Al-Nahrain University, Baghdad, Iraq,
E-mail: haider_bahaa@yahoo.com

Submitted: 22-April-2023
Revised: 24-Oct-2023
Accepted: 26-Dec-2023
Published: 07-Jan-2024

How to cite this article: Al-Shilib D N M, Sahib H B. The Antiangiogenic Effect of Eriodictyol (C15H12O6) in Ex Vivo and in Vivo Animal Study. J Carcinog 2024; 23(1):99-106.

Precipitate formation of tube. Lastly, constructing of the basement membrane and adherent junctions which cause a capillary to stabilize [6]. Eriodictyol is a type of flavanone extracted majorly in yerba Santa Clause (Eriodictyon californium), which is a herb found in North America [7].

Eriodictyol manage a significant number of pathways concerning cellular signaling to treat many diseases owing it to its many healing activities. It is an essential part of diet and processes a massive antioxidant effects beneficial in decreasing the risks of many health problems [8]. The objective of the study is to explore the probable anti-antigenic and ant proliferative effect of Eriodictyol.

Material and Method

The study was accomplished in tissue culture labs of Al-Nahrain University College of Medicine Pharmacology Department. study began on August 2022 ended on March 2023. Experiments were conducted after the revision and approval of the Ethics Committee of Al-Nahraine University, College of Pharmacy (Letter No. SY/3/2/1012, Dated in 24 November 2020).

Rat aorta ring antiangiogenic exvivo assay

This assay was performed following the standard protocol that is conducted by Brown and co-workers [9] with a few slight changes. Twelve to fourteen weeks old albino male rats were obtained from the animal house of Al-Nahraine Pharmacy College. They were sacrificed humanly through cervical dislocation after being anesthetized with chloroform[10]. The aortic tube is cut off, rinsed with media, cleansed from fibro adipose residuals then lastly cross-cut into very thin rings of a 1 millimeter thick. Forty-eight well-tissue culture plates were used for the assay. 300µll of a 3mg./ml fibrinogen in growth medium then was put on every well with a 5mg/ml of Aprotinin. The aortic ring tissue was embedded in the middle of the wells. After that 10microliter of thrombin which was made out of 50NIH U/milliliter in 0.15M sodium chloride then was put on to every well then incubated for thirty minutes in 5% CO2 incubator which was humidified and temperature at thirty-seven Celsius degrees to solidify resulting in forming a fibringel. After so, every single well receives 300µl of medium(M199) together with 20% heat inactivated fetal bovine serum (HIFBS), 0.1 percent E-amino-caproicacid, 1 percent Lglutamine plus 0.6 percent gentamycin antibiotic. Eriodictyol was made by dissolving in DMSO and diluted in (M199) medium in order to prepare the end DMSO conc. 0.1%, added to the growth medium at many varying conc. Each concentration was performed in 3 replicates. Culture plates were placed in a 5% CO2 humidified incubator at thirty-seven Celsius degrees for (five) consecutive days. The upper layer medium was removed and replaced with new fresh medium on day four. The rings tissue that received just one percent dimethyl sulfoxide are known as negative control (-ve control). Inhibition of vessel grow was evaluated as mean% of inhibition to the negative control ±standard deviation[11]. The level of capillaries growth inhibition was determined in accordance with technique that is developed by Nicosia [12] and it was quantified manually under 40X magnification by using an inverted microscope on day 5 of the procedure with the use of a camera and a software package. Percentage of capillaries inhibition is determined in accordance with the following formula:

Blood vessels inhibition % = 1- (A0/A) ×100

A0= distance of blood-vessels grows of the tested material in millimeters; A= distance of blood-vessels grows of the negative control in millimeters.

Dose-response study of Eriodictyol with rat aorta assay

The test substance was dissolved in dimethylsulfoxide to prepare stock-solution of 0.1% conc. And then diluted in (M199) medium in order to prepare serial-dilutions of those concentrations: 200, 100, 50, 25, 12.25, 6.25 µg/ ml. The wells that are without tested sample received only medium plus 0.1% dimethylsulfoxide and considered a negative control. Our data analysis was represented as mean standard deviation. Concentration that inhibits fifty precent capillaries growth (IC50) was determent through using the logarithmic-equation.

Y= %inhibition

X= concentration.

Chorioallantoic membrane CAM in vivo

Fertilized chicken and eggs obtained from by a local hatchery and in Baghdad and all dirt was cleaned by using seventy alcohols (ethanol 70%) and then incubated for about 72 hrs. at room temperature with a relative humidity sixty percent. Eggs are placed horizontally and rotated many times. 72 hrs. later, 2 milliliter of albumin was then aspirated mainly through a small hole punctured on the side of the egg and closed to majorly allow the CAM to detach perfectly from the shells, then eggs were, incubated for another 24hrs. Moreover, a relatively tiny window-like hole, three to four centimeter in diameter of the shell was cut and the tested sample that was immersed on previous time in filter-paper disc was settled on the CAM and then window was completely shielded with a sterile surgical-tape, embryos will be incubated one more time for 48 hrs. at 37°C. Last the inhibition zones was photographed and then calculated. The test sample which is Eriodictyol was prepared as 10mg./ml put on the filters paper-discs and let for drying before transferring to the eggs chorioallantoic membrane. This whole assay was performed in a very aseptic conditions [13].

Quantification and Imaging of CAM

Responses were graded as + (3 - 6 mm), ++ (6 - 9mm) and +++ (> 10mm). results were presented as Mean

±Standard-errrors of means—quantification of zone of inhibition was done using image-analyzer (BIOCOMVisiolab-TM2000) [14].

Statistical Analysis

The design of the experiment used is Randomized Complete Block Design((RCBD)). Results were presented also as means ±SD. The Statistical Packages of Social Sciences.–. SPSS (2016) program used for the detection of the effect of various factors in the study parameter. The Least significant difference –LSD test (Analysis of variation-ANOVA one way)) was used to significantly compare between means in this study and it is considered significant at P value < 0.05. The concentration that inhibits 50% of blood vessel (IC50)) was calculated by using the logarithmic equation.

Assessment of proliferation inhibition of human umbilical vein endothelial cells (HUVEC) cell line

The (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) MTT assay was used for the identification of cellline proliferation ability in accordance to the Mosmann method. All of those cells were between passage 4-7. Cells were then treated with a good amount of Eriodictyol for 48 hours. Then MTT was prepared through the addition of 5mg/ml in PBS ((phosphate buffer saline)).

Twenty µl of MTT then was used per each well and then the plates were incubated at 37°C, in 5% CO2 for five hours. Plates then were taken out the incubator and the supernatant layer was removed. (200µl) of DMSO solvent was added to all wells. Then plates were shaken vigorously for 1 min. at room temp. to dissolve the blue crystals. Absorbance was taken at 570nm by using enzyme-linked immune sorbent assay ((ELISA)). Absorbance of cells cultured in the control media was taken to be represented as 100% viability. Viability of treated cells was determined as percentage of un treated control and every conc. was tested on quadruplicate, then the experiment was redone twice. The concentration of cells in each well is 1x104, the percent of cell line inhibition was also determined as mean ± SD by using the following equations:

1-(A0-A1)// (A2-A1)
A0 = the absorbance of the sample
A1 = the absorbance of the blank
A2 = the absorbance of the control

The IC50 values were calculated through the linear and logarithmic correlating equation

Statistical analysis

Design of the experiment that was used for this study is Rationalized Complete Block Design (RCBD). Results are similarly represented as means±SD (Standard Deviation). level of toxicity was calculated using logarithmic and linear-regression equations.

Results

Rat aorta ring assay

Comparing Eriodictyol and -ve control

Aortic-rings which were treated with the test compound of 200µg/milliliter of Eriodictyol then immersed in M199 complete-growth medium have appeared to have a significant decline in blood vessels growth by 96.70% ±3.5µg/milliliter at P-value < 0.05 at day five in the experiment comparing to negative control rings that were treated with 1% DMSO only. Shown in Table 1.

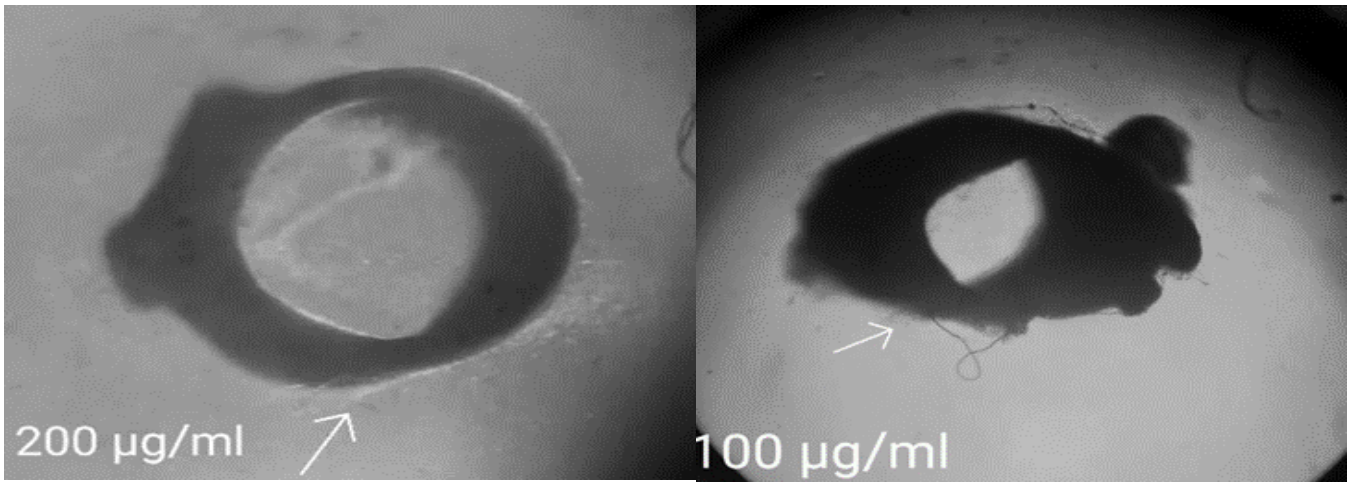
Table 1. Percentage of inhibition of Blood Vessel-Growth Produced by Eriodictyol and Negative Control

Tested-Compound	Inhibition-percent±SD
Eriodictyol	96.70 ± 3.5
DMSO 1%	0

The effect of dose response of rat aorta-ring assay

The dose response curve of eriodictyol on rat aorta ring model

Image 1 show 6 serial dilution conc. (200, 100, 50, 25, 12.5 and 6.25 µg/ml) used for the determination of dose response curve.



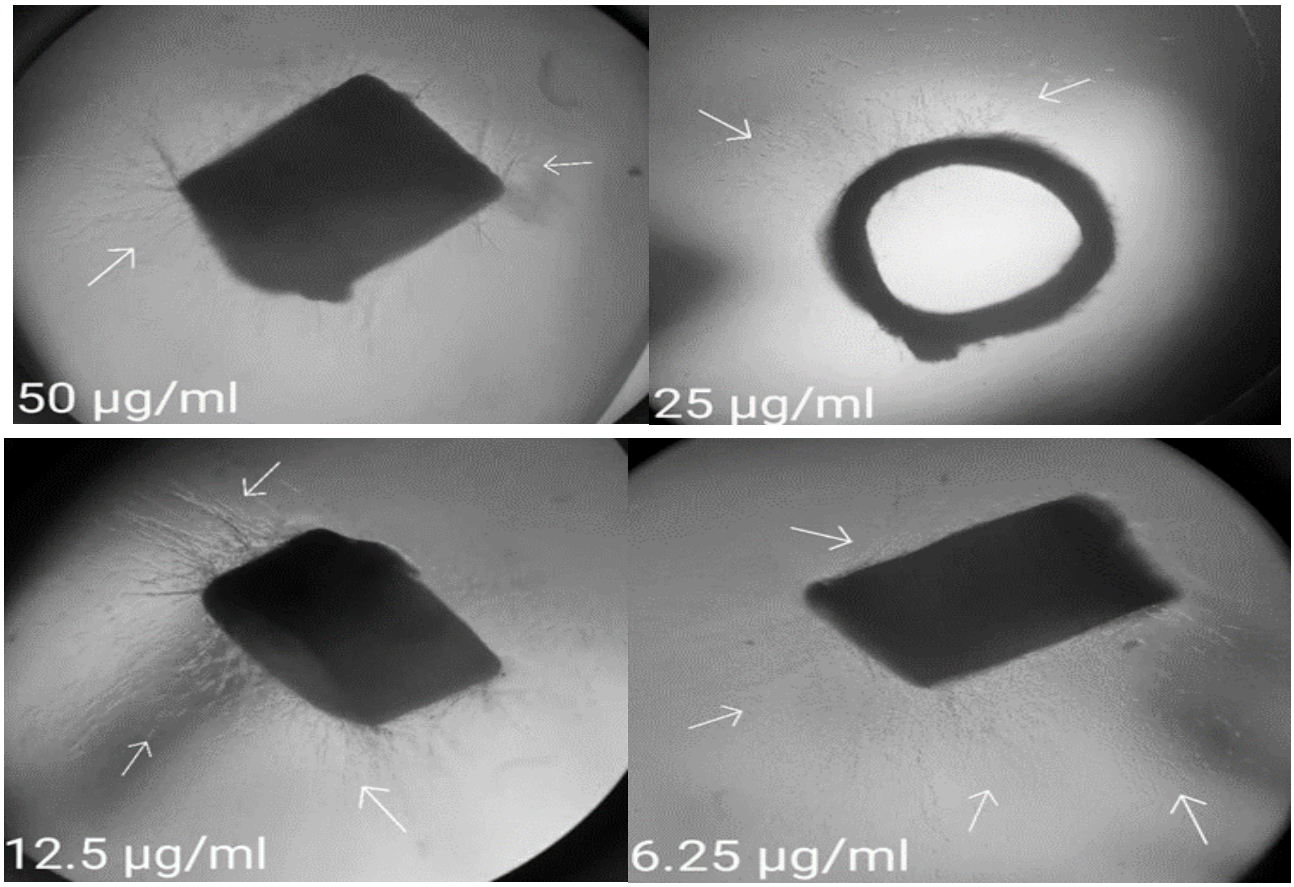


Image 1

Table2 the data was presented AS percent of inhibition ±SD which was showing a highly significance dose dependent inhibition of the blood vessels growth at P value < 0.05. The IC50 is determined by the use of logarithmic-equation in which, Y = percent of inhibition, X = concentrations which was obtained equaling 5.34 µg/milliliter. See in Figure1.

Table 2. the six serial Concentrations and The Respective Inhibition Percent with Eriodictyol

Conc. (µg/ml)	percent of inhibition±SD
200	96.70 ± 3.5
100	97.31 ± 1.4
50	82.70 ± 4.9
25	76.50 ± 10.6
12.5	68.60 ± 7
6.25	57.40 ± 7

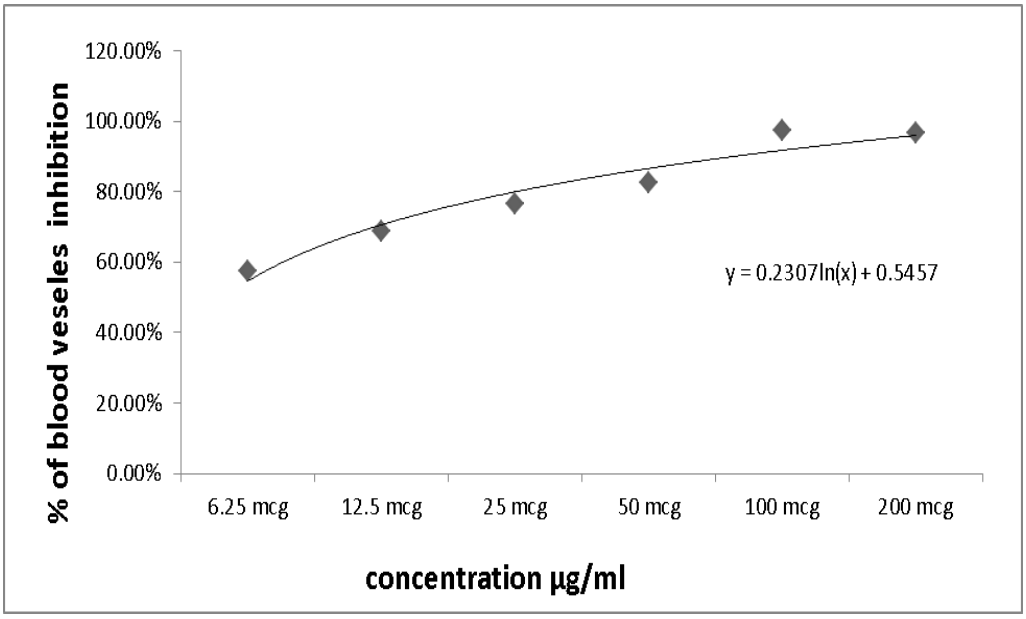


Figure 1. The Serial Dilutions Dose Response Curve of Eriodictyol in the assay of Rat Aorta Rings

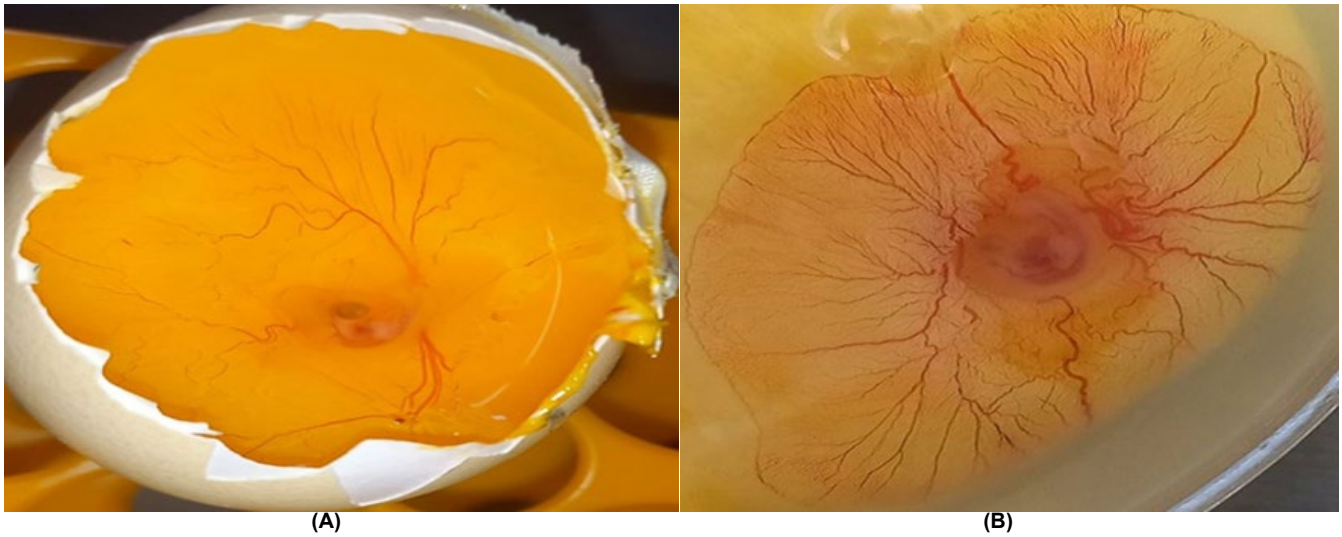
Invivo chick chorio-allantoic membrane CAM assay for Eriodictyol

Inhibition zone was measured at day seven in accordance to scoring system and it was significant through scoring (+++). The blood vessels of the CAM were undergoing regression and disorganization and the appearance of a pale-yellow color.

It also showed a significant inhibition at P value < 0.05. The inhibition is mainly known through the appearance of vascularzone throughout the disc which contain 0.5mg/millilitre of Eriodictyol like that shown in Table3 and Image 2.

Table 3. The Zone of Inhibition of Blood Vessels Growth of Eriodictyol plus the Scorings by Using the Chick Chorio-allantoic Membrane assay

Number of eggs	Inhibition-zone (MM)	Scorings
one	12	+++
Two	13	+++
Three	10	+++
Four	8	++
Five	10	+++
six	7	++
Mean ± SD	10 ±5.3	+++



(A) (B)

Image 2. invivo Chick Chorio-allantoicMembrane Assay. (A)Treated Group with Eriodictyol; (B)Treated Group with DMSO 0.1% as Negative Control

Assessment of proliferation inhibition of human umbilical vein endothelial cells (HUVEC) cell line

Cell viability was evaluated by MTT assay on human umbilical vein endothelial cells obtained from ATCC, passage 7, authenticated and tested. Data was presented as mean ± SD and IC50 values were calculated through the use of algorithmic equation as seen in figure 2. The data obtained from the MTT assay was presented as mean ± SD, where SD represents the standard deviation. This format provides information about the central tendency (mean) and the variability (standard deviation) of the measured values.

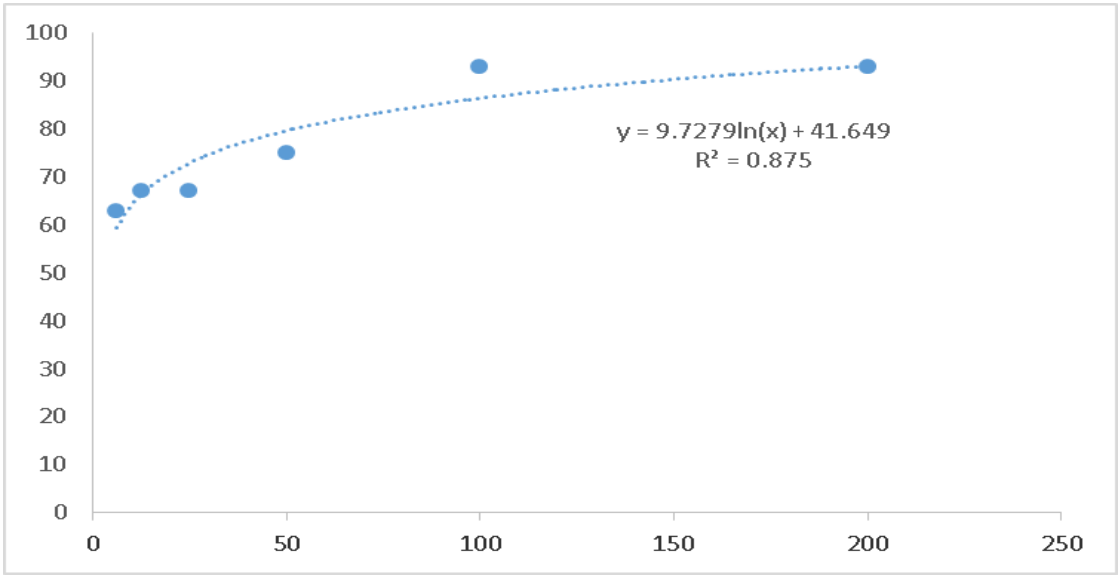


Figure 2. cell viability Curve for Serial Dilutions of Eriodictyol in human umbilical vein endothelial cells (HUVEC) cell line

Effect of Eriodictyol on cell viability

The following Table (4) and the previous Figure (2) showed that there was a significant dose dependent decrease in cell viability on HUVEC cells at ($P < 0.05$) after 48 h of treatment.

Table (4): Serial concentrations and their percentage of cell viability of Eriodictyol treated HUVEC cell lines.

Conc.(µg/ml)	HUVEC (Viability ± SD)
200	93 ±0.07
100	93±0.16
50	75± 0.08
25	67±0.22
12.5	67±0.008
6.25	63±0.009

Discussion

25% of deaths in the USA is majorly because of cancer, and the rate is increasing attributed to the growth of population, prolonged life expectancy, and many other risk factors such as smoking, lack of activity, obesity [15]. Cancer has the ability to spread to adjacent or distant organs, which makes it life-threatening. Tumor cells can penetrate blood or lymphatic vessels, circulate through intravascular stream, and then proliferate at another site, metastasis [16]. The common characteristic in a lot of tumors is the low O2 level, which is called hypoxia, different tumor types have different hypoxia severity. In severely proliferating tumor tissues, O2demand will be surpassed by O2supply, also distance in-between cells and existing vasculature rise, hindering O2diffusion which creates higher hypoxia [17, 18].

Blood vessels make A NETWORK consists of capillaries plus tubes that supply whole body with O2 and needed nutrient. In order to that, the technique they form plus function is highly essential in embryogensis and physiology in general. Blood vessels mainly consist of endothelium cell that make an impenetrable barrier in among tissues and blood, and interact with extra cellular matrix. While the blood vessels are formed denovo in embryogenesis, through the process vasculogenesis which involves bone marrow derived endothelial progenitor cell (EPCs)) [19] following that the process of angiogenesis, a mechanism through which new capillaries are made from preexisting vasculature. [20] Lastly, those vessels mature by physical-interaction to smooth muscle cell plus pericyte.an unusual Angio genesis process indicates a sign of diseased condition like progression of tumor, in which hyper proliferating cancer cells surpass the blood then become hypoxic tissue. Hypoxia enhance the imbalance between pro angiogenic plus anti angiogenic factors production, leading to induced and quick formation of blood vessels [21]. Hypoxia and potent transcription factor (HIF - 1α, HIF - 2α) showed to be contributing in all the levels of blood vessel formation. The first one to present the antiangiogenic therapy for the treatment of cancer in the year 1971 was Folkman [22]. The successful use of monoclonal antibodies against vascular endothelial growth factor VEGF, (bevacizumab) which is approved for treating metastatic colorectal cancer [23] following that a multiple solid tumors, stimulated development of other anti-angiogenic therapy [24].

This study that confirmed previous studies of the role of flavonoids in reducing cancer risks. In the recent years, many researchers have used the rat aortic ring as an exvivo assay to investigate the antiangiogenic activity in full or partial organ culturing. To prove the activity of the anti-angiogenesis assays and to better understand the mechanisms, it was required to analyses the sequential concentration of Eriodictyol against rat aorta in an exvivo experiments. The aim of the study was to investigate if the quantity of Eriodictyol have an anti-angiogenic effect. In this present study, Eriodictyol with a concentration of 200µg///ml was noticed to have a bigger antiangiogenic effect on rat aortic-rings comparing to other concentrations with IC50 value of 5.34µg/ml that indicates a nontoxic compound.

The flavonoid Eriodicttyol belonging to flavanone subclass [25] majorly found in citrus fruits, vegetables, and most of medicinal plants [26]. Flavonoids are polyphenol that can be found mainly in human digest regularly also majorly found within herbs found in nature [27]. Phyto-medicine has a crucial role of the traditional Chinese medicine which mainly work by scavenging free radical and own an anti-inflammatory affect. It's supposed that phytochemicals possess major role for human healthcare systems and account for more than forty percent of the prescribed medicine that're mainly home grown [28]. Therapeutically, Phyto-medicine have a lot of advantages over synthetic drugs with regard to low toxicity and little or no side effects [29].

A lot of flavonods found in herbs are mixed with carbohydrrates like β-glycosidis. The flavonoid Eriodictyol have a role that gradually expended in the GIT such as glucuronidatiion after the action of guts micro-biota and metabolized by methoxylationn in liver and becoming a restructured homo eriodictyol. The formed metabolits are eventually used in kidney, carrying glucuronicacids having corrosive or sulfate groups and inducing bilary and urinary discharges. Eriodictyol like mentioned also in blood-plasma and urine samples in 4 hrs. plus 24 hrs. subsequently [30].

Other than that, ER has a lot of pharmacological effects and activity, also recently it was reported that their role in healing a different type of disease attracted more attention for this drug. The result for the study showed that Eriodictyol possess a significant dose dependent inhibitions of micro-vessels sprouting when comparing them to untreated ring tissues. As mentioned, 200 micrograms/milliliter of Eriodictyol was noticed that have a max antiangiogenic effect on rat-aortic-rings comparing them to the other concentrations[31]. The main advantages of the CAM method assay are that it is a sensitive, practical, easiest, and not expensive invivo assay to determine the anti-angiogenic potential of each drug. The assay does not only providing information on a compound's activity but also indicates toxicity effects

invivo [32]. The recent research used the CAM method assay, which is one of many other standards invivo methods for the studying of angiogenesis production. As recently shown in Table 3, the inhibition zone areas are about 10 mm (score three-plus), while the score one-plus was much less effective, depending on the conditions. This observation was confirmed by the results that were obtained in Image 2, which showed the by blood vessel growth inhibitions in the control and the treated blood vessels of CAAM. The CAM inhibition of blood vessels was treated with a disc contains 10 mg of Eriodictyol that was applied to the area with numerous blood vessels, resulting in a strong antiangiogenic action, a reduction in the number of blood vessels, and a pale-yellow look of the vessels.

The MTT colorimetric method was used to assess cytotoxicity, cell proliferation and metabolic activity. The MTT assay works on the idea of cellular redox enzymes converting the soluble yellow tetrazolium salt into a water-insoluble purple formazan crystal. This assay is commonly used to measure metabolism, viability and adherent animal cell growth. In the present study the in vitro screening revealed that Eriodictyol owns an anti-proliferative activity against HUVEC cell line in a dose-dependent manner after 48 hours. The inhibitory concentration that inhibits 50% of cell viability was deduced and it was found to be (2.36 µg/ml). For pure compound or drug, IC50 value less than 4 µg/mL is considered potent. In conclusion, in accordance to the finding of the study, Eriodictyol has a promising effect against tumor cells as a target for angiogenesis-related or chemotherapy-related disorders. Eriodictyol, which contains flavonoids and polyphenols, significantly inhibits the formation of blood vessels in CAM and RAR assay. Furthermore, the chemical groups investigated were found to inhibit angiogenesis, or the growth of tumor cells.

References

1. A. M. Peluzzo and M. V. Autieri, "Challenging the paradigm: Anti-inflammatory interleukins and angiogenesis," *Cells,* vol. 11, no. 3, p. 587, 2022.
2. G. A. Jasim, A. A. Al-Zubaidy, S. M. Hussain, and H. B. Sahib, "The Antiangiogenic Activity of Commiphora molmol oleo-gum-resin Extracts," *Int. J. Pharm. Sci. Rev. Res,* vol. 33, no. 1, 2015.
3. H. B. Sahib *et al.*, "Anti-angiogenic and antioxidant properties of Orthosiphon stamineus Benth. methanolic leaves extract," *Int J Pharmacol,* vol. 5, no. 2, pp. 162-167, 2009.
4. S. HB, A. AA , and J. GA, "Iranian Journal of Pharmaceutical Sciences," vol. 52, no. 2, 2016.
5. M. E. Swift, H. K. Kleinman, and L. A. DiPietro, "Impaired wound repair and delayed angiogenesis in aged mice," *Laboratory investigation; a journal of technical methods and pathology,* vol. 79, no. 12, pp. 1479-1487, 1999.
6. S. Y. Yoo and S. M. Kwon, "Angiogenesis and its therapeutic opportunities," *Mediators of inflammation,* vol. 2013, 2013.
7. S. Marohn, *The Natural Medicine First Aid Remedies: Self-Care Treatments for 100+ Common Conditions*. Hampton Roads Publishing, 2001.
8. Y. Miyake, K. Yamamoto, N. Tsujihara, and T. Osawa, "Protective effects of lemon flavonoids on oxidative stress in diabetic rats," *Lipids,* vol. 33, no. 7, pp. 689-695, 1998.
9. Z. K. Ali and H. B. Sahib, "Antiangiogenic Activity of Sweet Almond (Prunus dulcis) Oil Alone and in Combination with Aspirin in both in vivo and in vitro Assays," *Asian Pacific Journal of Cancer Prevention: APJCP,* vol. 23, no. 4, p. 1405, 2022.
10. M. Al Nayf Mantas, J. Párraga Montilla, E. Lozano Aguilera, S. López-García, and J. Moral-García, "MUSCLE STRENGTH, GAIT SPEED, AND REACTION TIME IN ACTIVE ELDERLY PEOPLE," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.
11. J. Stiffey-Wilusz, J. A. Boice, J. Ronan, A. M. Fletcher, and M. S. Anderson, "An ex vivo angiogenesis assay utilizing commercial porcine carotidartery: Modification of the rat aortic ring assay," *Angiogenesis,* vol. 4, pp. 3-9, 2001.
12. R. F. Nicosia, Y. J. Lin, D. Hazelton, and X. Qian, "Endogenous regulation of angiogenesis in the rat aorta model. Role of vascular endothelial growth factor," *The American journal of pathology,* vol. 151, no. 5, p. 1379, 1997.
13. A. A. Al-Zubaidy, H. B. Sahib, and S. H. Ganduh, "Anti-angiogenic activity of matricaria chamomilla flowers methanol extract-In vivo study," *Int J Pharm Sci Rev Res,* vol. 41, pp. 367-71, 2016.
14. J. Murray, "Angiogenesis Protocols. In Angiogenesis Protocols," *Humana Press,* 2001.
15. L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent, and A. Jemal, "Global cancer statistics, 2012," *CA: a cancer journal for clinicians,* vol. 65, no. 2, pp. 87-108, 2015.
16. J. Folkman, "Tumor angiogenesis theraperutic implications," *Engl J Med*.
17. R. H. Thomlinson and L. H. Gray, "The histological structure of some human lung cancers and the possible implications for radiotherapy," *British journal of cancer,* vol. 9, no. 4, p. 539, 1955.
18. G. L. Semenza, "Hypoxia, clonal selection, and the role of HIF-1 in tumor progression," *Critical reviews in biochemistry and molecular biology,* vol. 35, no. 2, pp. 71-103, 2000.
19. E. M. Conway, D. Collen, and P. Carmeliet, "Molecular mechanisms of blood vessel growth," *Cardiovascular research,* vol. 49, no. 3, pp. 507-521, 2001.
20. P. Carmeliet, "Angiogenesis in life, disease and medicine," *Nature,* vol. 438, no. 7070, pp. 932-936, 2005.
21. G. L. Semenza and G. L. Wang, "A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation," *Molecular and cellular biology,* 1992.
22. J. Folkman, "Tumor angiogenesis: therapeutic implications," *New england journal of medicine,* vol. 285, no. 21, pp. 1182-1186, 1971.
23. H. Hurwitz *et al.*, "Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer," *New England journal of medicine,* vol. 350, no. 23, pp. 2335-2342, 2004.
24. G. Mountzios, G. Pentheroudakis, and P. Carmeliet, "Bevacizumab and micrometastases: revisiting the preclinical and clinical rollercoaster," *Pharmacology & therapeutics,* vol. 141, no. 2, pp. 117-124, 2014.
25. L. Marín, I. Gutiérrez-del-Río, P. Yagüe, Á. Manteca, C. J. Villar, and F. Lombó, "De novo biosynthesis of apigenin, luteolin, and eriodictyol in the actinomycete Streptomyces albus and production improvement by feeding and spore conditioning," *Frontiers in microbiology,* vol. 8, p. 921, 2017.
26. P. He *et al.*, "Eriodictyol alleviates lipopolysaccharide-triggered oxidative stress and synaptic dysfunctions in BV-2 microglial cells and mouse brain," *Journal of Cellular Biochemistry,* vol. 120, no. 9, pp. 14756-14770, 2019.

27. K. B. Pandey and S. I. Rizvi, "Plant polyphenols as dietary antioxidants in human health and disease," *Oxidative medicine and cellular longevity*, vol. 2, pp. 270-278, 2009.
28. R. Ober, *The National Herb Garden Guidebook*. Potomac Unit, Herb Society of America, 1996.
29. K. Patel and D. K. Patel, "Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: A concise report," *Journal of traditional and complementary medicine*, vol. 7, no. 3, pp. 360-366, 2017.
30. A. Islam, M. S. Islam, M. K. Rahman, M. N. Uddin, and M. R. Akanda, "The pharmacological and biological roles of eriodictyol," *Archives of pharmacal research*, vol. 43, pp. 582-592, 2020.
31. J. Barbado Solorzano and A. Martínez-Moreno, "SPORTS CENTRE WORKERS'PERCEPTIONS OF LEADERSHIP, ENGAGEMENT AND STRESS," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte*, vol. 22, no. 85, 2022.
32. R. Chopra, S. Nayar, and I. Chopra, "Glossary of Indian medicinal plants (including the supplement), Council Sci," *Ind. Res., New Delhi, India*, 1986.