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AI-IKOSA® BASED SCREENING OF ANTI-ANGIOGENIC PROPERTY OF BIOACTIVE SESAME LIGNAN USING IN-OVO CAM ASSAY

CHENGAMA RAJU NS*[1], PRABHAKAR AK[2], LAMANI N[2] AND RAJ B[3]

1: Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana, India

2: Department of Pharmacology, T. John College of Pharmacy, Bangalore, Karnataka, India

3: Professor, East West college of Pharmacy, Bangalore, Karnataka, India

***Corresponding Author: Dr. Nisha Shri Chengama Raju: E Mail: nisha8091@grcp.ac.in**

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ABSTRACT

Angiogenesis is defined as the process of formation of new blood vessels. Inhibition of angiogenesis helps in the treatment of cancer, opthalmic condition, rheumatoid arthritis, and other diseases. In this study the anti-angiogenic potential of sesamol, a lignan from sesame seeds, was screened by in-ovo chorioallantoic membrane assay using chicken egg. The images were captured during 0hrs, 24hrs and 48hrs after treatment and was quantified using an AI tool IKOSA. The vascular parameters like vessel total area, vessel total length, vessel mean thickness and vessel number branching points was obtained from the image analysis. The findings of our study revealed that sunitinib did not decrease the vessel area and length in the region of interest. However sunitinib exhibited a significant decrease in the number of branching points when compared with control. The effect of sesamol in altering area, length and thickness was not significant whereas it significantly ($p<0.05$) decreased the number of branching points when compared with control group. In our study we conclude that sesamol has potential to inhibit angiogenesis but not similar to sunitinib. The conclusions need further support from similar studies which are conducted with longer observation period.

Keywords: Anti-Angiogenesis, Sesamol, *In-Ovo* CAM Assay, IKOSA, Sunitinib

1. INTRODUCTION:

Vasculogenesis and angiogenesis are the sequential fundamental processes involved in the development of the vascular network in living tissues **[1-3]**. The first sign of vasculature occurs in the early phases of embryonic development when mesoderm-derived endothelial progenitor cells (EPCs) proliferate and form a primitive network of vessels during vasculogenesis **[3]**. In angiogenesis, new capillaries are formed from the pre-existing blood vessels either by sprouting mechanism or by intussusception mechanism **[3-5]**. The tumour cells are fast multiplying the supply of blood is crucial for the survival of the tumour tissue. Hence, tumour progression is often accompanied by the ingrowth of blood vessels **[6]**. Endothelial cells are normally quiescent but can be induced to sprout and advance to angiogenesis by pro-angiogenic factors like vascular endothelial growth factor (VEGF) which are released in response to hypoxia, tissue ischemia, oxidative stress, inflammatory mediators **[1, 2, 6, 7]**. Matrix metalloproteinase (MMP) expressed by endothelial cells cleaves collagen thereby regulating and controlling angiogenesis **[5]**. One of the root causes for conditions like age-related macular degeneration, atherosclerotic plaque formation, diabetic retinopathy, diabetic neuropathy, tumorigenesis, metastasis, etc. is found to be an imbalance in the angiogenic switch and excessive angiogenesis **[2]**. Hence, controlling excessive or unwanted angiogenesis using anti-angiogenic drugs is one of the rational for treating the above conditions.

Anti-angiogenic drugs like bevacizumab, ranibizumab, pegaptanib, sunitinib and other medications work against angiogenic growth factors or inducers **[1]**, but they have their own set of side effects. Moreover, in cancer patients, they may result in thrombosis and endocrine disruption **[1, 7]**. Because of minimal adverse effects and less toxic nature, the components from the natural source may help in alleviating excessive angiogenesis with less incidences of complications. Also, these natural compounds could serve as lead molecules for obtaining new anti-angiogenic drugs. Hence, the aim of this study was to screen the anti-angiogenic property of sesamol, a phytochemical from *Sesamum indicum* which is one of the regular dietary components in the Asian cuisine **[8]**.

Lignans (methylenedioxyphenyl) of sesame are progressively being investigated for their health benefits **[9]**. Sesamol, episesamin, samin and many lignans are identified in traces in the raw seeds **[8, 10]**.

These minor lignans are said to be formed during the seed processing procedures. Sesamol (3,4-methylenedioxyphenyl), is a sesamolin-derived by-product which is present in traces in raw seeds **[8]**. Sesamol content is high in roasted seeds and in processed sesame oil. Sesame is reported to have antioxidant, anti-hyperlipidemic, anti-hypertensive, antimicrobial, antipyretic, anti-inflammatory, anti-atherosclerotic, and anti-mutagenic properties **[8, 11, 12]**. Sesamol suppresses carcinogenesis, reduces monoamine oxidase (MAO) activity in the CNS, alleviates neurodegenerative disorders and also exhibits potent inhibitory effects on lipid peroxidation **[8, 11, 12]**. Current research is focusing on establishing the medicinal values of these minor lignans, and we want to support the researchers by exploring the anti-angiogenic potential of Sesamol.

1.1. CAM as antiangiogenic screening model:

The chick chorioallantoic membrane (CAM) is an extraembryonic membrane that is commonly used to study both angiogenesis and anti-angiogenesis. The mesodermal layer of the allantois in embryo becomes fused with the adjacent mesodermal layer of the chorion to form the CAM **[13]**. On days 10–12 of incubation, the capillaries resemble those in the 8-day membrane and are now near the surface of the chorionic epithelium **[13]**. Recent developments in artificial intelligence (AI) have opened new possibilities for image analysis, especially in life sciences. The IKOSA platform **[14]** provides various analytical tools where one of which named as "CAM assay" facilitates AI-based analysis of vascular networks on CAM.

2. MATERIALS AND METHODS:

Phosphate buffer saline, sunitinib, sesamol (Sigma aldrich®), biological incubator, hot air oven (Biotech), laminar airflow (Labline), IKOSA (KML Vision GmbH, Graz, Austria), autoclave, fertilized chicken eggs, cutter (pen knife), whatman filter paper.

2.1. Preparation of Phosphate buffer saline (PBS) 7.4 pH: Buffer saline of 7.4 pH was prepared and the pH of the solution was adjusted to 7.4 by adding few drops of dilute sodium hydroxide or dilute hydrochloric acid as required **[15]**. The buffer was sterilized and after sterilization the pH of the solution was double checked and was stored at 4°C until further use.

2.2. Preparation of Study solutions: Sunitinib standard solution (0.01µM/mL) and sesamol solution of 100 µM/mL **[16]** concentration were prepared by serial dilution.

2.3. Preparation of CAM: Fertilized chicken eggs of local breeds were used for the assay. The fertilized eggs were procured from a local

hatchery and were carefully shifted to the lab facility with utmost care. The eggs were cleaned using wet sponge and tissue paper and the incubator was disinfected to avoid contamination. The incubator was pre-treated for 3 days to ensure that the temperature and humidity was maintained in the range of 37.0 - 37.5°C and 55-60% respectively **[2, 17]**.

Eggs were incubated and were turned manually in the horizontal direction 3-4 times in a day to avoid sticking of CAM to the shell which may tear it during window opening **[2, 18]**. Egg candling was performed on the 3rd day for visualization of the developing embryo and blood vessels (**Figure 1**). On day 3 of incubation, the CAM was exposed in the eggs by opening window of 1cm x 1cm. Before opening the window the air sac displacement was carried out by removing 2ml of albumin under aseptic conditions as shown in **Figure 1**. This procedure of air sac displacement allows the CAM to stay intact during window grafting. In order to dislodge the air sac it was identified, marked and using a pen knife a small hole was made in the area without disturbing the developing embryo. A sterile needle was inserted through the hole and slowly suction was applied to remove about 1-2 ml of egg albumin depending on the size of the egg. During the windowing process care was taken to prevent the contamination of the CAM with the shell dust. After windowing (**Figure 1**), the openings were sealed and the eggs were moved back into the incubator for further incubation **[2, 18, 19]**.

Figure 1: Samples of candling, albumin removal, windowing and IKOSA image analysis

On the fifth day of incubation, the embryos were checked for viability. The eggs with viable embryo were split into three groups containing 6 in each group. The treatment groups were Group A: Negative control (PBS with pH 7.4); Group B: Positive control (sunitinib, 0.01µM); Group C: Sesamol solution (100 µM).

2.4. CAM assay: The sample discs were prepared using whatmann filter paper. The discs loaded with the standard or study sample were applied onto the CAM membrane. The

CAM was observed and images were captured immediately after placing the drug loaded discs into the appropriately labeled embryos. The windows were sealed with paraffin tape and the eggs were returned to the incubator. After 24 and 48 hours the eggs were observed for viability and the images of CAM layers were captured to analyze the changes of blood vessels **[20]**. CAM Image magnification at 9x were analyzed in IKOSA (**Figure 1**), an AI tool for quantitative image analysis **[4]**.

3. RESULTS & DISCUSSION:

Angiogenesis is the process of growth of new blood vessels from the existing vessels which occurs as a normal physiology as well as due to pathological conditions. Blood capillaries, which are developed through the process of angiogenesis, are never more than a few hundred micrometers away from any metabolically active tissue in the body **[21]**. Angiogenesis is very essential for the supply of nutrients, gaseous matter and waste exchange from and to the cells. A balance in the angiogenesis process is required for proper functioning of tissues and organs. In few diseases conditions like ischemic heart disease, peripheral arterial disease and wound healing processes promoting angiogenesis is considered as one of the therapeutic rational whereas conditions like rheumatoid arthritis, tumour growths and metastasis demand for inhibition or suppression of angiogenesis to avoid progress of the condition **[21, 22]**.

Angiogenesis occurs either by sprouting mechanism or intussusceptive mechanism. In sprouting angiogenesis, small sprouts arise from the pre-existing blood vessels under the stimulus of VEGF **[21]**. The vascular sprouts may extend further towards the tissue which is completely devoid of vascular supply. Intussusceptive angiogenesis undergoes splitting action of the blood vessels resulting in transvascular communication in the tissues already having vascular supply. Both these types of angiogenesis occur in almost all the tissues and organs in response to stimulatory conditions like hypoxia or any condition with promotes secretion of proangiogenic factors **[21]**. Anti-angiogenesis is the inhibition of blood vessels to repress the further development and growth of blood vessels. Angiogenesis is very essential for the development and growth of solid tumours since the rapid growth of tumour mass requires nutrients for their survival. Suppressing the angiogenesis process in the solid tumours is a successful rational which makes the tumour mass deficit of their essential nutrients.

Sesame seeds are one of the unavoidable ingredients in many of the Asian food preparations. Many alternative treatment

strategies like naturopathy, homeopathy, aromatherapy, Traditional Chinese Medicine (TCM), etc. **[23]**. Compendium of Materia Medica conveys that consumption of black sesame seeds for more than 100 days cures almost all chronic illnesses **[24]**. Many studies have reported evidences regarding the benefits of phytochemicals isolated from sesame seeds **[23]**. Sesamol is one of the bioactive lignan that is present in roasted seeds and oil extracted from raw seeds. Sesamol is reported to exhibit antioxidant and antibacterial properties **[25]**. A study by Aparna *et.al.* concluded that sesamol inhibits the process of angiogenesis mediated by lipopolysaccharides by downregulating the VEGF-A and VEGF receptors **[24]**. A study published in 2018 **[26]** reported that topical application of sesamol may prevent corneal neovascularization either as monotherapy or as combination with bevacizumab. Sesamol is a non-toxic compound that acts by blocking the transcription of inflammatory markers such as cytokines, redox status, protein kinases, and inflammatory enzymes. Furthermore, sesamol triggers apoptosis in cancerous cells by activating caspase cascades and triggering mitochondrial and receptor-mediated pathways can serve better version of anti-angiogenic than the synthetic standard drugs which focus on blocking the pro-angiogenic receptors. Background study of the literature states that sesamol contain anti-angiogenic property and very few studies have studied under CAM model. Hence, the objective of this study was to screen the anti-angiogenic potential of sesamol by *in-ovo* CAM assay.

CAM model has long been a favoured system for the study of angiogenesis. Immunocompetence in birds develops only after hatching **[13]**. Sprouting angiogenesis occurs in chick embryo during the development age of 1st to 8th day whereas intussusceptive angiogenesis occurs from day 11 **[20]**. Immature blood vessels that lack a complete basal lamina and smooth muscle cells will be scattered in the mesoderm and will grow very rapidly until day 8. At day 14, the capillary plexus will be located at the surface of the ectoderm adjacent to the shell membrane. Rapid capillary proliferation continues until day 11 thereafter, the endothelial cell mitotic index declines rapidly, and the vascular system attains its final arrangement on day 18, just before hatching **[13]**. To study anti-angiogenesis effect, growing blood vessels serve as an important platform for which developing chick embryos are used in assay. *In-ovo* studies were reported with sustained and better survivability than the *ex-ovo* study. Anti-angiogenic activity can be expressed against sprouting angiogenesis or

proliferation angiogenesis. In our study, we have mainly focused on studying the effect of sesamol in inhibiting the sprouting angiogenesis which occurs during the initial stages of embryo development.

Sunitinib is an oral tyrosine kinase inhibitor with antiangiogenic and antitumor properties. As sesamol will be screened for its anti-angiogenic potential, sunitinib was chosen as a positive control for comparison. The IC_{50} of sunitinib for angiogenesis was found to be 0.01µM **[2, 27, 28]** which was used in the study.

Fertile chicken eggs were incubated and CAM assay was carried out as mentioned in the methodology. The images were captured using a high definition camera. The images captured were restricted to a specific region of interest (ROI) of 1228800 squared pixel around the disc region. The impact of the sample on the development of blood vessels was analysed using a novel AI-powered quantification tool IKOSA® **[29, 30]**. CAM-analysis of IKOSA platform has been a reliable tool in research quantification of angiogenesis parameters like vessel total area (VTA), vessel total length (VTL), vessel mean thickness (VMT), vessel number branching points (VNBP) **[30-32]**. The image data of few samples analysed in each study group is presented in **Figure 2**.

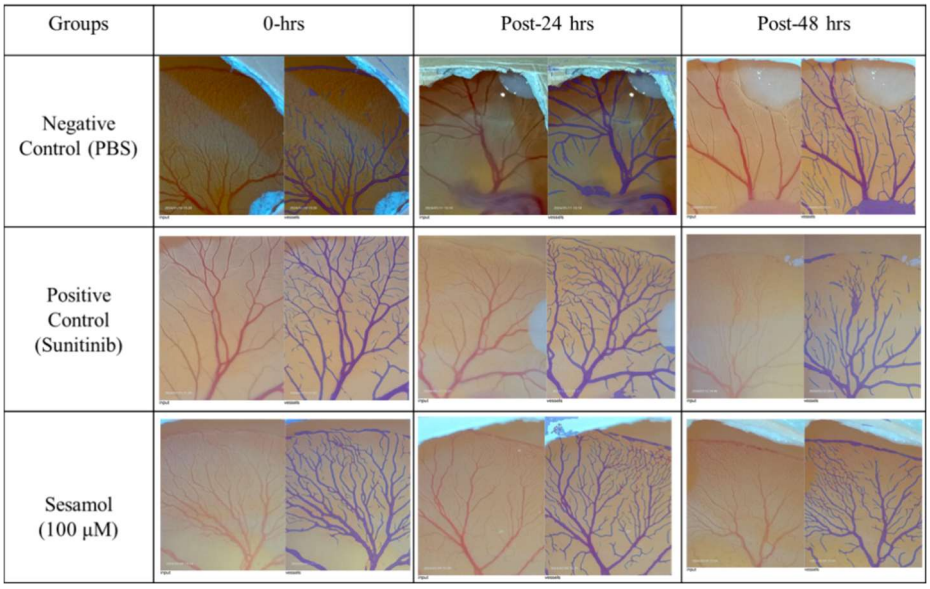

Figure 2: Images of CAM their IKOSA analysis

Images captured at different time interval of 0-, 24- and 48- hours were imported into the IKOSA® and their respective data is given in **Figure 2** and **Table 1**. All the results obtained were normally distribution according to Shapiro-Wilk test **[33]**. Hence, parametric

one-way ANOVA and Tukey test was adopted for comparison of variables between and within the groups **[34]**.

In control group vascular growth parameters like blood vessel area, length, thickness and number of branching points has increased (**Figure 3**) indicating that the administered PBS did not affect the angiogenesis process. Administration of sunitinib did not reduce VTA and VTL, however decline in VMT and VNBP was observed (**Figure 3**). Statistically significant decrease in the number of branching points after 24 and 48 hours of treatment was observed (p=0.02, p=0.0003) when compared with control. Sunitinib has selective action in inhibiting VEGF receptor which has important role in sprouting angiogenesis that occurs predominantly during the first 8 days of embryo development **[20]**. In our study it was observed that sesamol did not reduce VTA and VTL. A slight decline was observed in the VMT which has to be confirmed with the support of multiple study data. Sesamol reduced the number of branching points which was a significant (p=0.02) reduction when compared with control at 48 hours duration.

Table 1: IKOSA analysis parameters

Parameter	Groups	0 hrs	24 hrs	48 hrs
VTA (Px^2)	Control (Mean ± SD)	205489.33 ± 32303	276826.17 ± 73277	319719 ± 93019
	Standard (Mean ± SD)	222638.5 ± 99786.1	266196.83 ± 99672	305569.75 ± 93689.7
	Sesamol (Mean ± SD)	186296.33 ± 19950.3	235653.33 ± 25925.2	253565.5 ± 48516.6
VTL (Px)	Control (Mean ± SD)	13520.85 ± 4454.13	18771.49 ± 3527.77	23297.37 ± 4060.65
	Standard (Mean ± SD)	15214.75 ± 7812.03	20793.13 ± 7284.1	24193.85 ± 6235.36
	Sesamol (Mean ± SD)	15857.42 ± 1808.91	20400.35 ± 3758.2	19929.45 ± 3888.87
VMT (Px)	Control (Mean ± SD)	13.10 ± 1.91	13.82 ± 2.37	17.32 ± 5.22
	Standard (Mean ± SD)	15.35 ± 3.16	13.31 ± 3.35	12.71 ± 2.39
	Sesamol (Mean ± SD)	13.62 ± 1.47	11.76 ± 1.53	12.97 ± 2.71
VNBP	Control (Mean ± SD)	119 ± 35.38	163.33 ± 24.76	191 ± 32.48
	Standard (Mean ± SD)	124.67 ± 40.90	105 ± 37.84*	64 ± 23.17*
	Sesamol (Mean ± SD)	136 ± 35.29	131 ± 35.05	122.5 ± 41.91*

*** Statistically significant (p<0.05)**

The percentage change of the 3 study groups (**Table 2**) indicates that in control group, PBS has not affected the neoangiogenesis process. However, the changes in the groups treated with sunitinib and sesamol showed decline in the vascular ramifications. This specifies that the standard and the sample substances do not support formation new vascular sprouts.

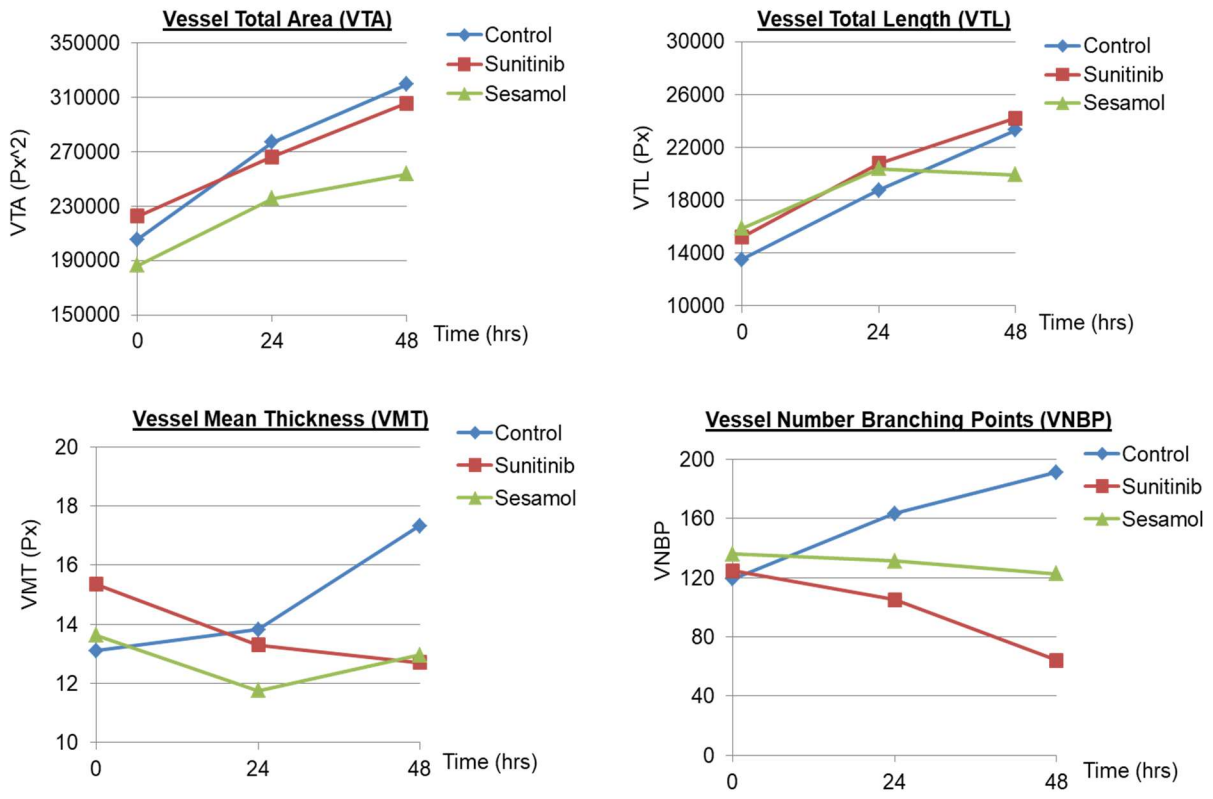

Figure 3: Graphical comparison of blood vessel parameters

Table 2: Percentage changesVascular ramification and Vascular complexity in CAM

Parameter	Groups	0 hrs	24 hrs	48 hrs
Vascular Ramification VNBP/ROI (%)	Control (Mean ± SD)	0.009 ± 0.003	0.013 ± 0.002	0.016 ± 0.002
	Standard (Mean ± SD)	0.010 ± 0.003	0.009 ± 0.003	0.005 ± 0.001
	Sesamol (Mean ± SD)	0.011 ± 0.003	0.011 ± 0.003	0.010 ± 0.003
Vascular Complexity VNBP/VTA (%)	Control (Mean ± SD)	0.058 ± 0.017	0.064 ± 0.023	0.064 ± 0.020
	Standard (Mean ± SD)	0.068 ± 0.038	0.045 ± 0.020	0.023 ± 0.012
	Sesamol (Mean ± SD)	0.073 ± 0.016	0.055 ± 0.009	0.051 ± 0.026

The quantitative measure of the complexity of the vascular network branching structure can be assessed by dividing VNBP by VTA **[30]**. If the vascular network is more complicated and extensively branched then the ratio will be higher. From our study results it was observed that the sunitinib and sesamol reduced the ratio as the time progresses to 48 hours post treatment.

4. CONCLUSION:

Anti-angiogenic property of sesamol was screened by *in-ovo* CAM assay. From the obtained results it is observed that PBS did not interfere in the growth process of blood

vessels. Sunitinib and sesamol has shown a notable angiogenesis inhibition property with respect to the number of branching points in the sprouting angiogenesis. However, the observation was made only for 48 hours and hence further observations till extended hours and many studies with multiple parameters are required to approve the anti-angiogenic potential of sesamol.

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REFERENCES:

[1] Rajasekar J, Perumal MK, Vallikannan B. A Critical review on anti-angiogenic property of phytochemicals. J Nutr Biochem. 2019;71:1-15. https://doi.org/10.1016/j.jnutbio.2019.04.006

[2] Kei WW, Raju NSC. Anti-Angiogenic screening of *Moringa oleifera* leaves extract using Chorioallantoic Membrane Assay. Iraqi J Pharm Sci. 2022;31(1):225-232. https://doi.org/10.31351/vol31iss1pp225-232

[3] D'Alessio A, Moccia F, Li JH, Micera A, Kyriakides TR. Angiogenesis and Vasculogenesis in Health and Disease. Biomed Res Int. 2015;2015:126582. https://doi.org/10.1155/2015/126582

[4] Faihs L, Firouz B, Slezak P, Slezak C, Weibensteiner M, Ebner T, *et al.,* A Novel Artificial Intelligence-Based Approach for Quantitative Assessment of Angiogenesis in the Ex Ovo CAM Model. Cancers. 2022;14:4273. https://doi.o1g/10.3390/cancers14174273

[5] Kole D, McClung JA, Aronow WS. Chapter 6. Vasculogenesis and Angiogenesis In Translational Research in Coronary Artery Disease. 2016. Elsevier. 49-65. https://doi.org/10.1016/b978-0-12-802385-3.00006-1

[6] Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci. 2020 May;77(9):1745-1770. https://pubmed.ncbi.nlm.nih.gov/31690961/

[7] Kennedy DC, Coen B, Wheatley AM, McCullagh KJA. Microvascular Experimentation in the Chick Chorioallantoic Membrane as a Model for Screening Angiogenic Agents including from Gene-Modified Cells. Int J Mol Sci. 2021 Dec 31;23(1):452.

https://pubmed.ncbi.nlm.nih.gov/31690961/

[8] Andargie M, Vinas M, Rathgeb A, Möller E, Karlovsky P. Lignans of Sesame (*Sesamum indicum* L.): A Comprehensive Review. Molecules. 2021 Feb 7;26(4):883. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7914952/

[9] Moazzami AA, Haese SL, Kamal-Eldin A. Lignan contents in sesame seeds and products. Eur J Lipid Sci Technol. 2007;109:1022-1027. https://doi.org/10.1002/ejlt.200700057

[10] Jayaraj P, Narasimhulu CA, Rajagopalan S, Parthasarathy S, Desikan R. Food Funct. 2020. https://doi.org/10.1039/C9FO01873E

[11] Mushtaq A, Hanif MA, Ayub MA, Bhatti IA, Jilani Mi. Chapter 44. Sesame In Medicinal Plants of South Asia. 2020. Elsevier. 601-615. https://doi.org/10.1016/B978-0-08-102659-5.00044-6

[12] Shah A, Lobo R, Krishnadas N, Surubhotia R. Sesamol and Health - A Comprehensive Review. IJPER. 2019;53(2):S28-S42. https://archives.ijper.org/sites/default/files/IndJPhaEdRes_53_2s-28.pdf

[13] Ribatti D, Nico B, Vacca A, Roncali L, Burri PH, Djonov V. Chorioallantoic membrane capillary bed: A useful target for studying angiogenesis and anti-angiogenesis in vivo. Anat Rec. 2001;264:317-324. https://doi.org/10.1002/ar.10021

[14] IKOSA Application "CAM Assay"- Version 1.0.0 IKOSA ® Application Documentation Application Name CAM Assay. [(accessed on 30 June 2022)]. Available online: www.Ikosa.Ai

[15] Indian pharmacopoeia 1996, 4th edition, Government of India. Ministry of Health and Family Welfare, Volume 2, Appendix 13.2, A-147

[16] Siao AC, Hou CW, Kao YH, Jeng KC. Effect of sesamin on apoptosis and cell cycle arrest in human breast cancer mcf-7 cells. Asian Pac J Cancer Prev. 2015;16(9):3779-83. https://doi.org/10.7314/apjcp.2015.16.9.3779

[17] Nowak-Sliwinska P, Segura T, Iruela-Arispe ML. The chicken chorioallantoic membrane model in biology, medicine and bioengineering. Angiogenesis. 2014 Oct;17(4):779–804. https://doi.org/10.1007/s10456-014-9440-7

[18] West DC, Thompson WD, Sells PG, Burbridge MF. Angiogenesis Assays Using Chick Chorioallantoic Membrane. Methods Mol Med. 2001;46:107–29. https://doi.org/10.1385/1-59259-143-4:107

[19] Ribatti D. Chicken Chorioallantoic Membrane Angiogenesis Model. Methods Mol Biol. 2012;843:47–57. https://doi.org/10.1007/978-1-61779-523-7_5

[20] Raju NSC, Ying TS. Anti-Angiogenesis screening of Moringa oleifera Pod Extracts by In-Ovo Chorioallantoic Membrane Assay. Hacet Univ J Fac Pharm. 2023; 43(4): 301-309. https://dergipark.org.tr/en/download/article-file/2724077

[21] Adair TH, Montani JP. Angiogenesis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. Chapter 1, Overview of Angiogenesis. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53238/

[22] Zhang R, Yao Y, Gao H, Hu X. Mechanism of angiogenesis in tumour. Front Oncol. 2024 Mar; 14:1-15. https://doi.org/10.3389/fonc.2024.1359069

[23] Wei P, Zhao F, Wang Z, Wang Q, Chai X, Hou G, *et al.,* Sesame (*Sesamum Indicum* L.): A Comprehensive Review of Nutritional Value, Phytochemical Composition, Health Benefits, Development Of Food, And Industrial Applications. Nutrients. 2022; 14(19): 4079. https://doi.org/10.3390%2fnu14194079

[24] Kumar CM, Singh SA. Bioactive Lignans from Sesame (*Sesamum Indicum L.*): Evaluation of Their Antioxidant and Antibacterial Effects for Food Applications. J Food Sci Technol. 2015;52(5):2934-2941. https://doi.org/10.1007%2fs13197-014-1334-6

[25] Huseyin K, Gokhan P, Aygun Y, Can HM, Barbaros S. Is Sesamol effective in Corneal Neovascularization? Eye & Contact Lens: Science & Clinical Practice. 2018;44:S414-S419. https://journals.lww.com/claojournal/toc/2018/11002

[26] Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(3):213-9. https://doi.org/10.2147/vhrm.2006.2.3.213

[27] Demetri GD, Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, *et al*., Efficacy and safety of sunitinib in patients with advanced gastrointestinal

stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006;368(9544):1329–1338. https://doi.org/10.1016/s0140-6736(06)69446-4

[28] Annese T, Tamma R, Ribatti D. IKOSA® CAM Assay Application to Quantify Blood Vessels on Chick Chorioallantoic Membrane (CAM). Methods mol biol. 2023;2572:129-139. https://doi.org/10.1007/978-1-0716-2703-7_10

[29] https://www.kmlvision.com/ikosa-prisma-in-action-assessing-angiogenesis-hallmarks-within-the-cam-assay-model/

[30] Faihs L, Firouz B, Slezak P, Slezak C, Weibensteiner M, Ebner T, *et al.,* A Novel Artificial Intelligence-Based Approach for Quantitative Assessment Of Angiogenesis In The Ex Ovo CAM Model. Cancers (Basel). 2022 Sep;14(17):4273. https://doi.org/10.3390%2fcancers14174273

[31] Kuri PM, Pion E, Mahl L, Kainz P, Schwarz S, Brochhausen C, *et al.,* Deep Learning-Based Image Analysis for the Quantification of Tumor-Induced Angiogenesis in the 3D In Vivo Tumor Model-Establishment and Addition to Laser Speckle Contrast Imaging (LSCI). Cells 2022;11(15):2321. https://doi.org/10.3390/cells11152321

[32] Salvante ERG, Popoiu AV, Saxena AK, Popoiu TA, Boia ES, Cimpean AM, *et al.,* Glycosaminoglycans Modulate the Angiogenic Ability of Type I Collagen-Based Scaffolds by Acting on Vascular Network Remodeling and Maturation. Bioengineering. 2024;11(5):423. https://doi.org/10.3390/bioengineering11050423

[33] Lee S, Lee DK. What is the proper way to apply the multiple comparison test? Korean J Anesthesiol. 2018 Oct;71(5):353-360. https://doi.org/10.4097%2Fkja.d.18.00242

[34] Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. Application of student's *t*-test, analysis of variance, and covariance. Ann Card Anaesth. 2019;22(4):407–411. https://doi.org/10.4103/aca.ACA_94_19