Anti-angiogenic Activity of *Cuminum cyminum* seeds extract: *in vivo* study

Ahmed M Mahmood[1], Hayder B Sahib[2]

1 College of medicine Al Nahrain University

2 College of pharmacy Al Nahrain University

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ABSTRACT

The objective of this study is to identify the possible anti – angiogenic activity of Cuminum cyminum seeds extract in vivo assays. Cuminum cyminum seeds powder was extracted by methanol using the cold method "maceration" as extraction process. The in vivo study was done by Chick Chlrioallontoic Membrane assay (CAM assay).Then phytochemical analysis done by Gas Chromatography mass spectroscopy (GC). The obtained data revealed that methanolic extract of Cuminum cyminum seeds significantly inhibit blood vessels growth when it compared to negative control and the inhibition zone about (++). And according to GC mass the extract showed chemicals related poly phenol group like tannins and flavonoids that may be responsible on anti angiogenic activity of Cuminum cyminum seeds.

ألفعالية المضادة لتكوين الاوعية الدموية لمستخلصات بذور الكمون : داخل الجسم الحي

احمد محمد محمود1 , حيدر بهاء صاحب2

1 . كلية الطب جامعة النهرين

2 . كلية الصيدله جامعة النهرين

الكلمات المفتاحيه : تولد الاوعيه الدمويه, مستخلص, تجربة البيضه المخصبه

الخلاصة:

الهدف من هذه الدراسه هو معرفه احتمالية امتلاك مستخلصات بذور الكمون القابليه على منع تولد الاوعيه الدمويه داخل الجسم الحي. تم استخلاص مسحوق بذور الكمون بواسطة محلول الميثانول وتم اسنخدام طريقة التنقيع. تم اختبار المستخلص بواسطة تجربة البيضه المخصبه. بعد ذلك تم الاطلاع على مكونات المستخلص الكيميائيه النباتيه بواسطة Gas Chromatography Mass Spectrometry . اظهرت نتائج الدراسه ان

مستخلص الميثانول لبذور الكمون يثبط تولد الاوعيه الدمويه داخل الجسم الحي بصوره واضحه مقارنة مع المعيار حيث كانت درجة تثبيط تولد الاوعيه هي (++). كذلك اظهرت نتائج التحليل بواسطة ال GC ان المستخلص يحتوي على مركبات البولي فينول مثل التانين والفلافونايدات التي قد يعزى اليها فعالية مستخلص بذور الكمون كمثبط لتولد الاوعيه الدمويه.

Introduction

The formation of new blood vessels from an existing one is the definition of Angiogenesis process; Angiogenesis is very important physiological process in wound healing, placenta formation, embryonic growth, and tumour development [1].

There are 2 type of angiogenesis, Sprouting and intussusceptive. First type of angiogenesis is occur when the tissues are hypoxic and poorly perfused, then the oxygen sensing mechanisms recognize a level of hypoxia "where the oxygen tension is low" that requires the formation of new blood vessels to meet the metabolic requirements of endothelial cells [2].Second type is also named splitting angiogenesis because the vessel wall extends into the lumen causing a single vessel to divide in two. This kind of angiogenesis is believed to be fast and efficient compared with sprouting angiogenesis because it needs only reorganization of standing endothelial cells and does not depend on immediate endothelial migration or proliferation. Intussusceptive angiogenesis happens throughout life but plays the noticeable role in vascular development in embryos where growth is fast and resources are inadequate [3].Excessive angiogenesis occurs in diseases such as cancer, diabetic blindness, age related macular degeneration, rheumatoid arthritis, psoriasis. While Insufficient angiogenesis occurs in diseases such as coronary artery disease, stroke, and chronic wounds [4].The word cumin was derived from the Latin cuminum, which itself was derived from Greek (kyminon) [5]. Nowadays it cultivated extensively in Iraq, Turkey, India, China, Libya, and Palestine [6]. In traditional medicine, cumin was used to treat hoarseness, jaundice, dyspepsia and diarrhoea. Its seeds were used for stomachic, diuretic, carminative, stimulant, astringent and abortifacient properties [7]. The biological and pharmacological effect of *Cuminum cyminum* herb thought to be due to the variety of its chemical constituents like alkaloid , anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid [5].The objective of this study is to identify the possible anti – angiogenic activity of *Cuminum cyminum* seeds methanolic extract *in vivo* assays.

Material and method

A seed part of *Cuminum cyminum* was obtained by botanical expert from local market in Baghdad. The seeds were washed with tap water

then left to air dry. The dried seeds were ground to very fine powder. After that the powder extracted methanol, using Maceration (cold) method [8]. The mixture filtered by whatmann no.1 filter paper to get the extract. The extract was concentrated by rotary evaporator with vacuum, the final extract, stored in dry and well-sealed container for further works [9].

Chick Chorioallantoic Membrane Assay (CAM Assay)

Fertilized chicken eggs were obtained from a local hatchery in Baghdad were incubated for 72 hr at 37°C with humidity of 60 - 80%. The eggs then placed in horizontal position and rotated many times. After 72hr. 1-2ml albumen were sucked out through a pinpoint hole pierced down by the side and closed " in order to allow better side of view " where the CAM will separate from the sack that is attached to the egg shell, then incubated again for another 24hr. Then a round piece of shell (3-4 cm) was removed from the top of blunt end and the egg's sac punctured, then a round disc of filter paper which impregnated previously with the test sample placed on the CAM and the eggs were sealed with a sterile adhesive tape and incubated for further 72 hr.

The test sample was prepared as 50mg/ml and 20 µl (the final dose was 1mg/disc) sited on the disc of filter paper and left to dry prior to transfer to the CAM. On day 7 the zone of inhibition photographed and calculated; 6 CAM were used for each control and test sample [10].

Quantification and Immaging of CAM

The responses were graded are : + (3 - 6 mm); ++ (6 - 9mm); +++ (> 10mm). The quantification of zone of inhibition was done by using image analyzer [11].

Gas Chromatography – Mass Spectroscopy (GC – MS)

The (GC-MS) analysis was performed using a GC-MS (Model ; QP 2010S, Japan) equipped with aVF-5ms fused silica capillary column of 0.25mm, 30m. The temperature of the column oven was 70∘C to 240∘C. Ionization of sample components was carried out in electron impact (EI) mode (70 eV). And the temperature of the ion source was set on 200∘C and the interface to 240∘C. Helium (99.9995% purity) was the transporter gas at a flow rate of 1.21 mL/min. 1 µl of the extract of *Cuminum cyminum* was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis in injection technique. The solvent delay was 0 to 2 min, and the GC/MS running time was 30 min. The percentage amount of each component was calculated by comparing its average peak area to the total areas [12].

RESULTS

In vivo chick chorioallantoic membrane (CAM) assay of methanol extract of *Cuminum cyminum seeds.*

The zone of inhibition for methanol extracts was measured at day 7 of the experiment. Blood vessels in the CAM begin to regress by the result of the extract; the vessels were disorganized with pale yellowish form. The inhibition was recognized by the appearance of the avascular zone nearby the disc that contained the test extract, and the extent of inhibition zone was measured according to the scoring system mentioned previously.

Methanolic extract produced a significant inhibition zone of blood vessels in the CAM by scoring of (++) and as shown in the table (1).

Table (1): The scoring for the inhibition zone of blood vessels growth in *in vivo* (CAM) assay for Methanolic extract of *Cuminum Cyminum* seeds.

Scoring for the inhibition zone	NO.
+++	1
++	2
+++	3
+	4
+++	5
++	6

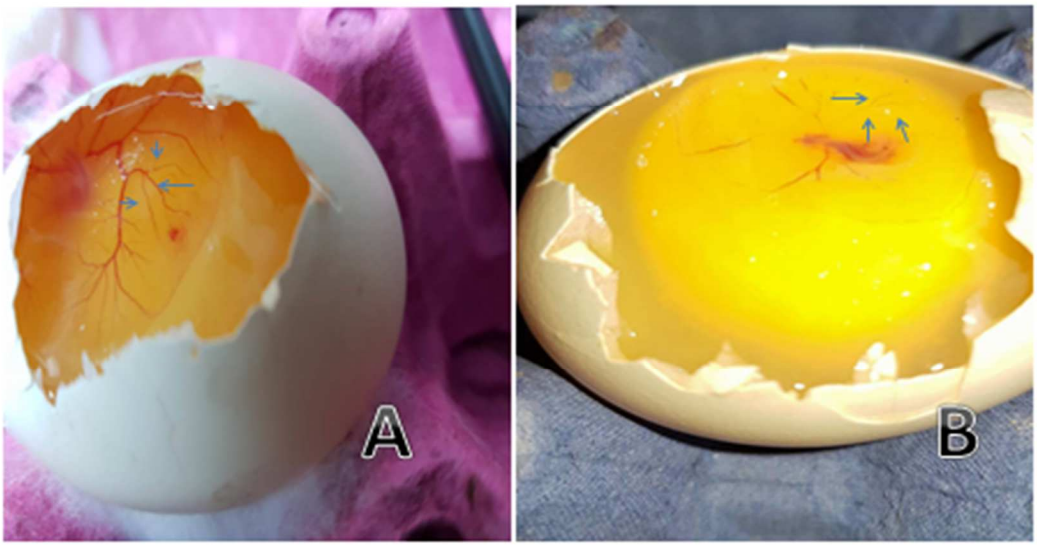

Figure (1) *In vivo* chick chorioallantoic membrane (CAM) assay of methanolic extract of *Cuminum Cyminum* seeds

Gas Chromatography Mass Spectrometry (GC-MS) Investigation of *Cuminum cyminum* Methanolic Extract

Upon GC–MS analysis Figure (2), chemical composition of the main constituents from methaolic extract Identification of the constituents was performed by comparing the recorded mass spectra with the standard mass spectra from the NIST Library.

The main constituents were considered “identified”, when their mass spectral fit values were at the default value of 90% or above and we are going to mention the constituent that inhibit or decrease blood vessels growth because these findings could provide an understanding for molecular mechanism underlying the antiangiogenic potential of *Cuminum cyminum* seeds extract.

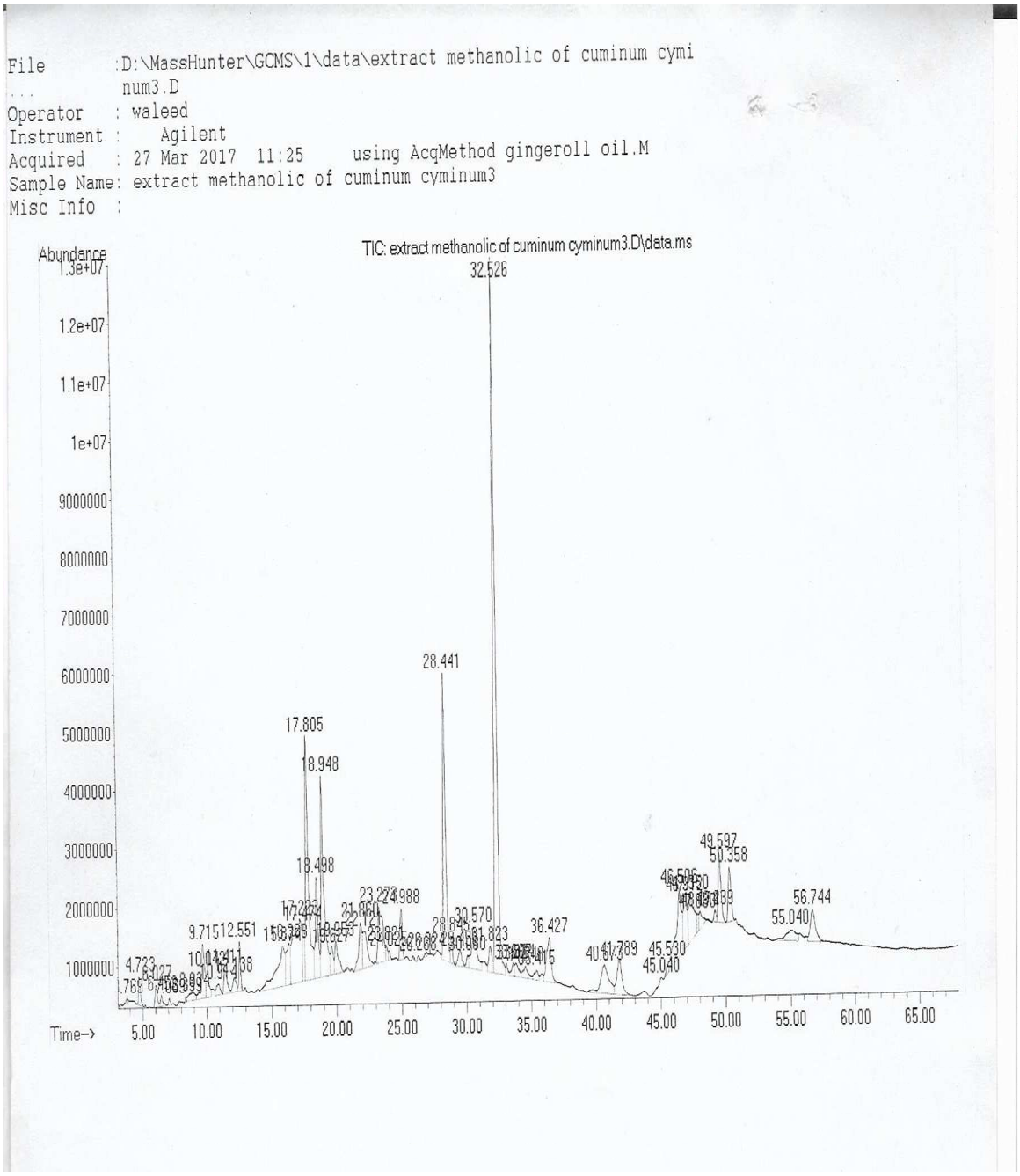

Figure (2) GCMS of *Cuminum cyminum* seeds methanolic extract.

DISCUSSION

CAM Assay one of the most used methods to investigate the anti-angiogenesis processes *in vivo*. In this study the data showed a significant perturbation in blood vessels growth in comparison to the negative control. CAM treated with the methanolic extract of *Cuminum cyminum* seeds inhibit new blood vessels formation and structure of existing vasculature was disrupted, the blood vessels number declined significantly with appearance light yellow in treated CAMs. This data may be attributed to the existence of many chemicals having anti-angiogenesis activity, from this point the searchers tested the extract with GC-MS. The data showed that monoterpenes (cuminol), Propanal, 2-methyl-3-phenyl and Cumin aldehyde are significantely existed. Blood vessels growth inhibition may be related to these constituents, previous study showed that *Cuminum cyminum* seeds contain terpenes and this agreed with this study [13]. Terpen has known of its effect as antioxidant, anticancer, tumor inhibitors through anti angiogenic effect [14].

Cumin aldehyde act via Annexin 2 which is extracellular proteolysis, implicated in the generation of several angiogenic regulatory molecules and play a role in the regulation of cellular growth and in signal transduction pathways. Cuminaldehyde found to be inhibits annexin 2 leading to suppress the VEGF-induced activity of hypoxic conditions [15].Also, the anticancer activity of cuminaldehyde in vitro involved the suppression of cell proliferative markers, topoisomerase I as well as II, together with increase of pro-apoptotic molecules, associated with upregulated lysosomal vacuolation. On the other hand, in vivo, cuminaldehyde diminished the tumor burden that would have a significant clinical impact. Furthermore, similar effects were observed in other tested cell lines [16].

Conclusion

Methanol extract of *Cuminum cyminum* seeds inhibit blood vessels growth in CAMs. The mechanism may be related to the existence of many constituents like terpenes and cuminol. These chemical groups proven to inhibit proliferation of tumour cells, angiogenesis and endothelial cells.

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