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Potency dependent angiogenic effect of *Bellis perennis* on in ovo chick chorioallantoic membrane assay

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Abstract

Background: Hormesis is the biological response of an organism for a particular dosage of a substance. The lower dose stimulates and higher dose inhibits. J Compton Burnett, an eminent homeopath, prescribed *Bellis perennis* for breast and uterine tumours, which suggests it could be due to the antiangiogenic property or angiogenic inhibiting property and clinically also it is used for healing of fractured bone and wound healing which may be due to angiogenic promoting property. This experiment deals with the dose dependent effect of *Bellis perennis* on In Ovo Chick Chorioallantoic Membrane Assay. **Objective:** To evaluate the Hormesis effect of *Bellis perennis* 12X, 6CH, 30CH by assessing the angiogenic effect in chorioallantoic membrane assay (CAM). **Methods:** CAM assay was done following the standard protocol and the antiangiogenic and angiogenic effect of *Bellis perennis* in 12X, 6CH, and 30CH was assessed by counting the number of vessels and densitometry study using Image J software at 24 h, 48 h, and 72 hours(h). Histological study was carried out under light microscopy for tissue thickening. **Results:** *Bellis perennis* had antiangiogenic property in 12X and angiogenic property in 6CH and 30CH. **Conclusion:** *Bellis perennis* shows potency dependent angiogenic activity.

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ORIGINAL ARTICLE

Potency dependent angiogenic effect of *Bellis perennis* on *in ovo* chick chorioallantoic membrane assay

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ABSTRACT

Background: Hormesis is the biological response of an organism for a particular dosage of a substance. The lower dose stimulates and higher dose inhibits. J Compton Burnett, an eminent homeopath, prescribed *Bellis perennis* for breast and uterine tumours, which suggests it could be due to the antiangiogenic property or angiogenic inhibiting property and clinically also it is used for healing of fractured bone and wound healing which may be due to angiogenic promoting property. This experiment deals with the dose dependent effect of *Bellis perennis* on In Ovo Chick Chorioallantoic Membrane Assay. **Objective:** To evaluate the Hormesis effect of *Bellis perennis* 12X, 6CH, 30CH by assessing the angiogenic effect in chorioallantoic membrane assay (CAM). **Methods:** CAM assay was done following the standard protocol and the antiangiogenic and angiogenic effect of *Bellis perennis* in 12X, 6CH, and 30CH was assessed by counting the number of vessels and densitometry study using Image J software at 24 h, 48 h, and 72 hours(h). Histological study was carried out under light microscopy for tissue thickening. **Results:** *Bellis perennis* had antiangiogenic property in 12X and angiogenic property in 6CH and 30CH. **Conclusion:** *Bellis perennis* shows potency dependent angiogenic activity.

Keywords: Angiogenesis, Antiangiogenic, *Bellis perennis*, Chorioallantoic membrane assay, Homoeopathy, Hormesis

Introduction

Angiogenesis is the process by which blood vessels proliferate from preexisting vasculature. Beginning in utero and extending into old age, it happens throughout life in both health and illness.¹

The term “hormesis” is used to describe a biphasic dose-response to an environmental substance, which is characterized by a low dose stimulating or positive effect and a high dose inhibiting or harmful effect.²

Serially diluted succussed solutions of an appropriate medication or toxic molecule can exhibit physical, chemical, and biological characteristics well beyond Avogadro’s limit. In homoeopathy the medicinal substance is diluted and succussed beyond Avogadro’s limit so they can produce hormesis when introduced to living system.^{2,3}

The therapeutic effects of serially diluted succussed solutions are not limited to compounds that are often utilized as source-drugs in homoeopathy. Even well above Avogadro’s limit, homoeopathically diluted chemicals like aspirin, thyroxine, and histamine have been discovered to have opposing effects on biological models compared to their medicinal doses.⁴ Various substances and medications can elicit hormetic responses in living cells when they are diluted even beyond Avogadro’s limit, according to several research groups.⁴

A hormetic dose-response can be produced by nanoparticles. Such nanoparticles are found in succussed ultrahigh dilutions of drugs, toxic substances utilized in homeopathic remedies.⁴

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Researchers in biology have rediscovered the Arndt-Shultz law, which asserts that biological systems react to substances in varying amounts significantly differently, which is called by the new name hormesis.⁵

Bellis perennis is a homoeopathic medicine; acts upon the muscle fibres of the blood vasculature. First drug used in treatment injuries or wound to the deeper tissues, after major surgical work⁶ and for deep trauma or septic wounds.⁷

J Compton Burnett cured tumours with well-trying anti-traumatic *Bellis perennis* homoeopathic medicine. Potency used was mother tincture and 3X to treat tumours.⁸

J Compton Burnett has successfully treated numerous cases of tumours that started from a blow. J.H. Clark says, J Compton Burnett is the chief authority on *Bellis perennis* in homoeopathic uses.⁹

J Compton Burnett treated many conditions with *Bellis perennis* medicine, like traumatic uterus, enlargement of the uterus after abortion, sub involuted uterus, sterility, misshapen uterus, enlarged spleen, inconveniences of pregnancy, hemorrhoidal uterus,¹⁰ rectal abscess, and fistula.¹¹

Bellis perennis having Antioxidant, Anticancer, Wound healing, Anti Haemorrhagic, Haemolytic, Neuroprotective, Anti-ulcerogenic activity.¹² From these it is understood *Bellis perennis* has Anti-angiogenic effect on both Tumours & Psoriasis and same *Bellis perennis* has Angiogenesis promoting effect on Deep Traumatic Injuries.

The chorioallantoic membrane (CAM) of the chick embryo is an extraembryonic membrane that functions as a surface for gas exchange and is supported by an immense capillary network. Because of its easy accessibility and major vascularization, CAM has been employed to research the morpho functional facets of angiogenesis.¹³

The goal of present research is to determine the angiogenesis effects of *Bellis perennis* in various potencies on the Chorioallantoic membrane.

Materials and methods

Study settings

Materials

Homoeopathy medicine *Bellis perennis* 12X, 6CH, 30CH is prepared manually according to Homoeopathic Pharmacopeia of India¹⁴ using *Bellis perennis* mother tincture (Q) as a source. (*Bellis perennis* Q was obtained from GMP Certified Homoeopathic pharmacy Schwabe, India Pharmaceutical company Batch No: 0151000). Fertilized domestic chicken eggs were purchased from poultry on (11/02/2023).

Intervention

According to the standard protocol¹³ of CAM assay fertilised domestic chicken eggs weighing 50 to 55g were incubated for a period of eight days in Egg incubator (TM&W) (from 13/02/2023 to 20/02/2023) at 37°C in “humid atmosphere” (>60% humid) and were slowly moved at least three times a day.¹⁵ The incubated eggs were divided into control group and medication group by marking them. Fertilized eggs were randomly distributed into experimental groups (control, 12X, 6CH, and 30CH) prior to incubation. Randomization was performed using a label random number sequence to ensure unbiased group allocation. Eggs showing any pre-incubation defects or weight deviations greater than $\pm 10\%$ were excluded before randomization. On the 9th day (21/02/2023) embryo was located by ‘candling’ and its position was marked on the shell with pencil. The egg shell was sterilised by wiping with 70 percent ethyl alcohol.

A hole was made in the egg’s convergent end to remove 0.5 to 1ml of albumin using needle to minimize adhesion of the shell membrane and a window 1cm² was cut in the shell. Forceps were used to separate the shell surrounding the embryonic air sac, and the shell membrane at the air sac’s base was peeled away. A window of one centimetre was created in the egg shell to introduce the medicine.

10 microliters (μl) of the remedy *Bellis perennis* 12X, 6CH, 30CH was implanted in the chorioallantoic membrane by instilling at the ratio of 1:11 (1 μl of *Bellis perennis* + 10 μl of distilled water) (1:11 dilution was chosen because it provides a mild, biologically tolerable concentration) in 3 different groups B, C, and D respectively on Whatman filter paper disc. Duplicates of each group were made. Each group has 3 eggs and a total of 24 eggs were used.

Control group (group A) treated with using 1 μl of dispensing alcohol + 10 μl of distilled water (in the ratio of 1:11) on Whatman filter paper disc implanted in the chorioallantoic membrane. The window and hole were sealed with micropore plaster. Then they were further incubated for 24, 48 and 72 hours (h).

CAM were exposed at 24 h, 48 h and 72 hours and photographed [Fig. 1]. The number of vessels were counted for three different time periods eggs (24, 48 and 72 hours) and densitometry study by using Image J Software.

Light microscope analysis

Bellis perennis-treated area of the CAM was examined under light microscope (Olympus CX21i) after careful dissection. The dissected membrane was placed on glass slides, and photographs of the capillary growth were captured using a light microscope at 4x and 10x magnifications [Figs. 2 to 5].

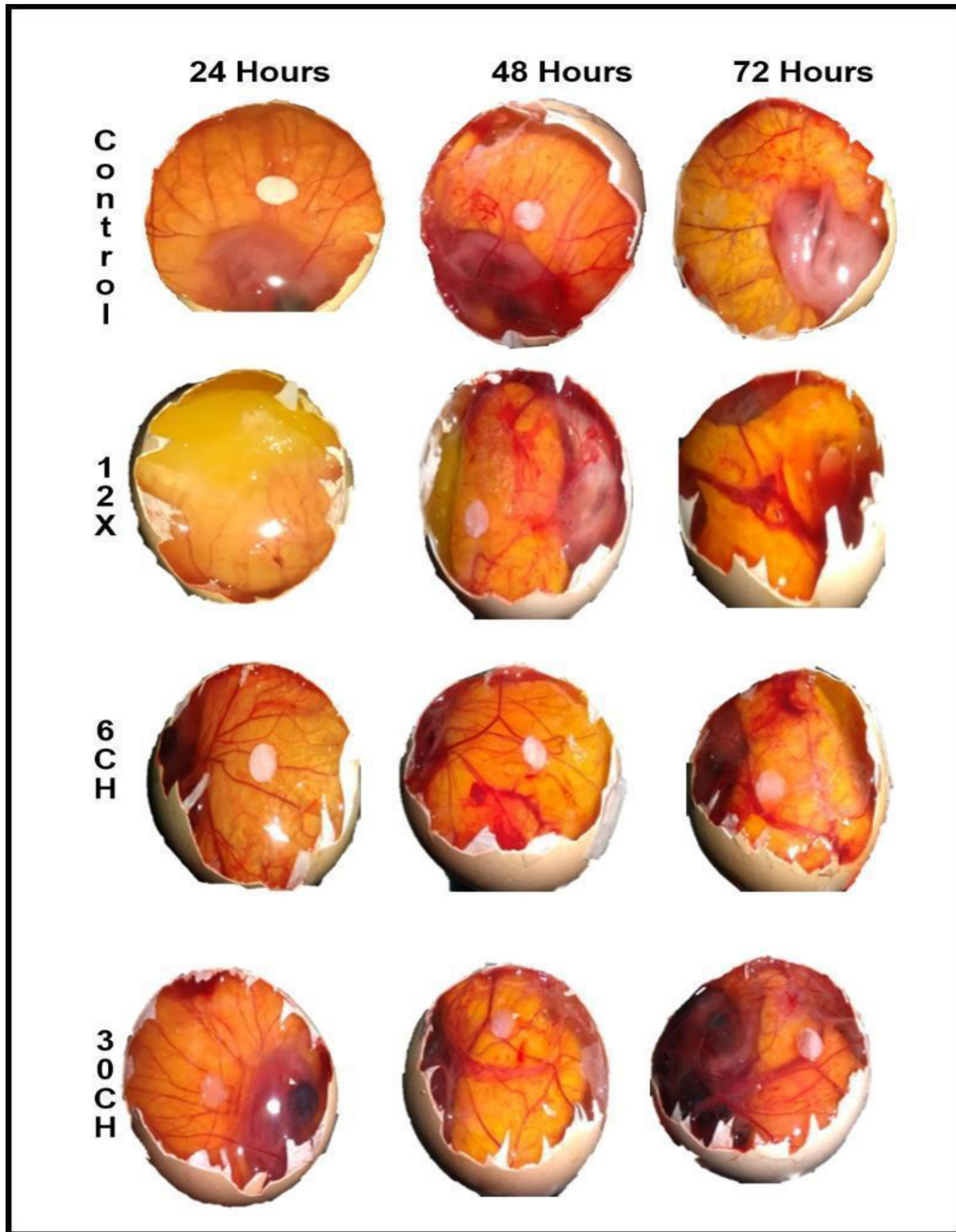


Fig. 1. Images of CAM treated with 12X, 6CH, 30CH Concentration of *Bellis perennis* and control for 24 h, 48 h, 72 h.

Histology

After 72 hours of incubation, Bouin's fixative solution was flooded over the *Bellis perennis*-treated area of the CAM and control.

The membrane that surrounded the treated area was carefully removed, dried using a graduated sequence of alcohol from (50%, 70%, 90%, and 100%), and then imbedded in paraffin wax.

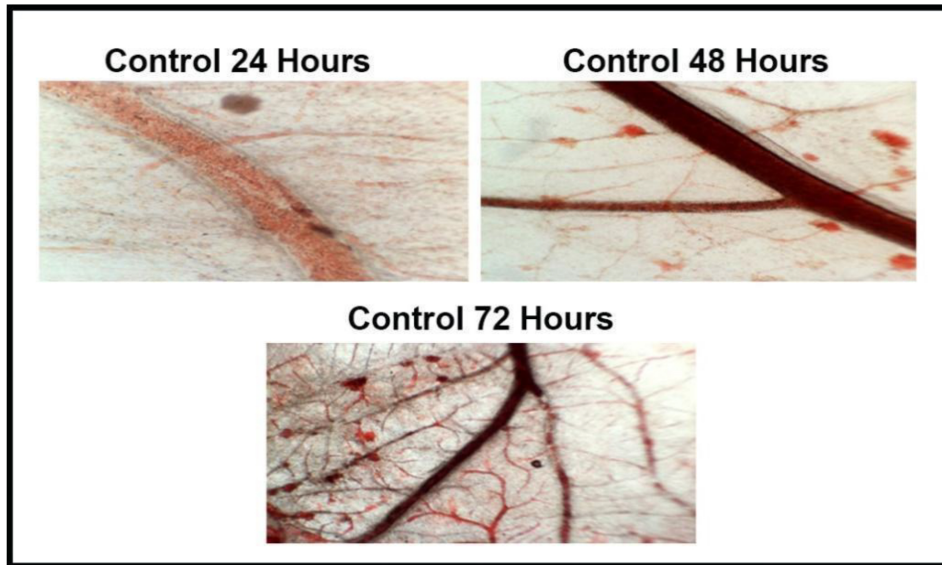


Fig. 2. Light microscopic images of CAM Control for 24 h, 48 h, 72 h.

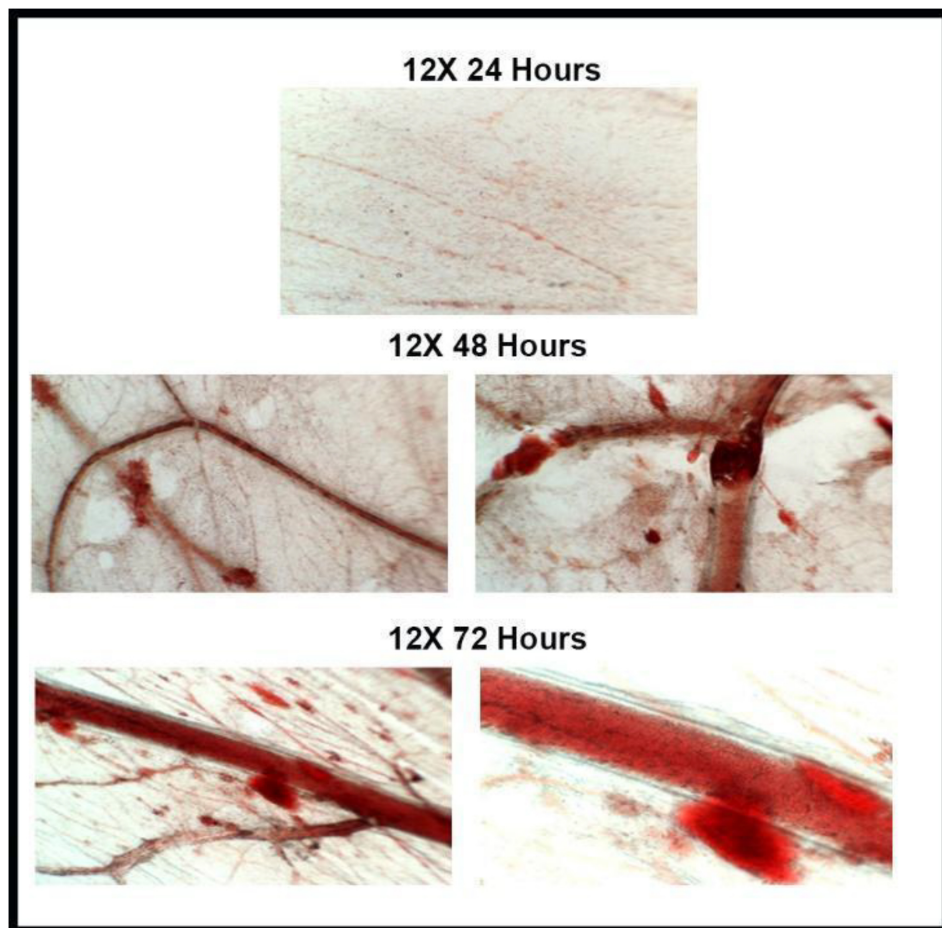


Fig. 3. Light microscopic images of CAM treated with *Bellis perennis* 12X for 24 h, 48 h, 72 h.

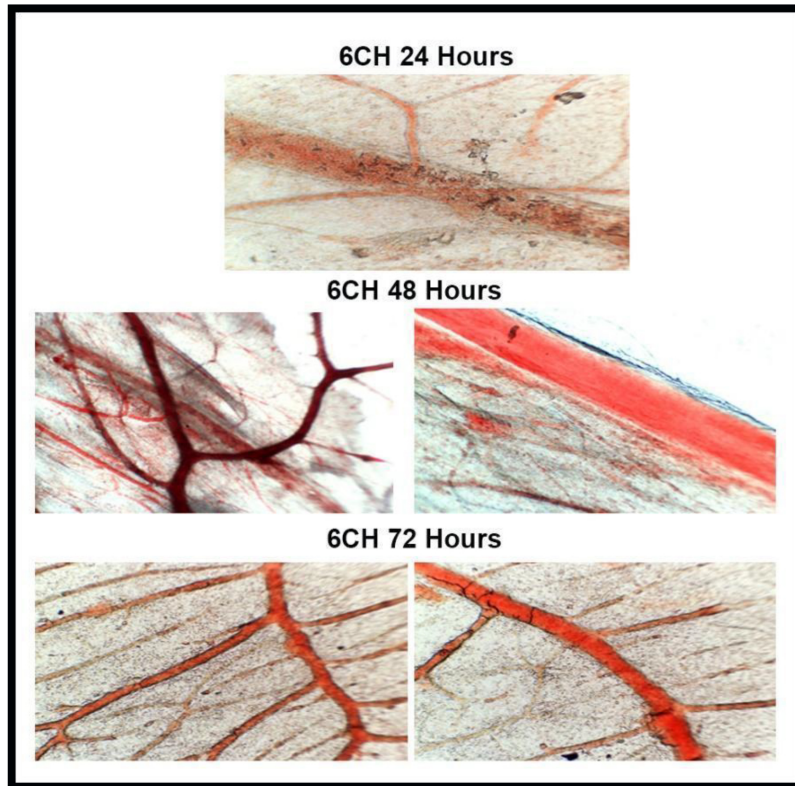


Fig. 4. Light microscopic images of CAM treated with *Bellis perennis* 6CH for 24 h, 48 h, 72 h.

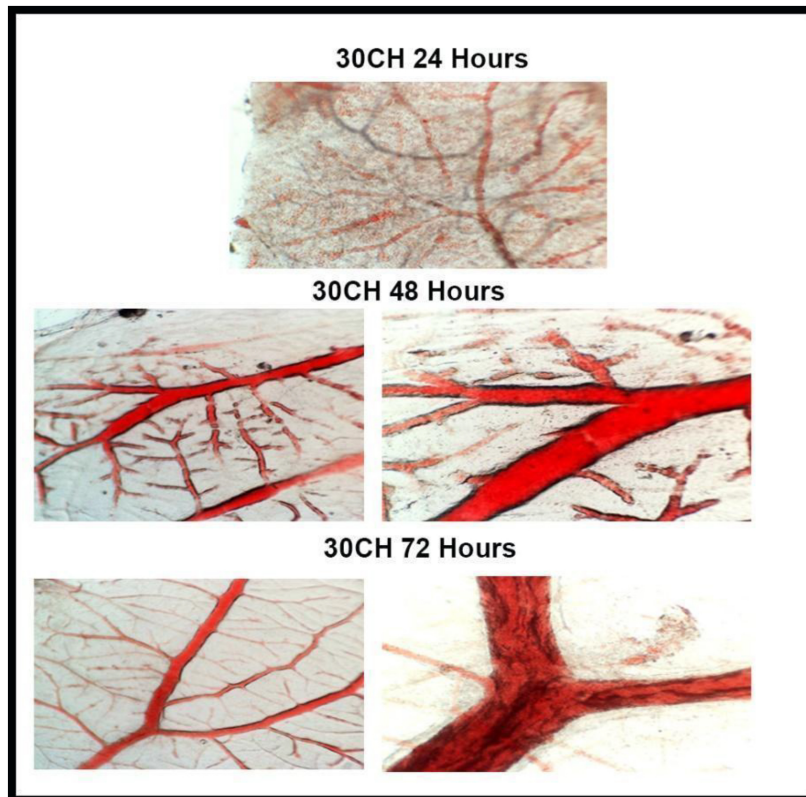


Fig. 5. Light microscopic images of CAM treated with *Bellis perennis* 30CH for 24 h, 48 h, 72 h.

Rotating microtomes (Histo-line laboratories Histo Cut – 17) were used to slice vertical cross-sections of tissue that were 7 micrometres thick. Sections were cleaned with xylene solution and treated with alcohol in descending order (100%, 90%, 70%, & 50%) and stained with haematoxylin and eosin.

The histological slides were mounted with DPX, evaluated qualitatively using light-microscope at a 40x magnification, and photographs were taken using a camcorder connected to light-microscope at a 10x magnification.^{16,17}

Results

The mean number of blood vessels (24 h, 48 h, 72 h) compared with control eggs [Fig. 6].

After 24 hours (22/02/2023) of inoculation of medicine and the vessels were counted and the following result was observed. Control had 22 blood vessels, 12X potency 13 blood vessels, 6CH potency 25 blood vessels, and in 30CH potency 28 blood vessels. The maximum number of vessels was observed in 30CH potency.

After 48 hours (23/02/2023) of inoculation, Control was observed to have 23 blood vessels, in 12X potency 12 blood vessels, in 6CH potency 27 blood vessels, and in 30CH potency 31 blood vessels. Again, 30CH potency with maximum number of blood vessels had been observed.

Vessel counts after 72 hours (24/02/2023) had 24 blood vessels in control, 12X potency had 10 blood vessels, 6CH potency had 28 blood vessels, and 30CH potency had 33 blood vessels.

After 24, 48 and 72 hours of incubation, 12X of *Bellis perennis* had a diminished number of blood vessels (13, 12, 10 blood vessels). It shows inhibition of capillaries around the disc and was visually compared to that of control CAM (control has 22, 23, 24 blood vessels).

Bellis perennis of 6CH showed numerous scattered allantoic vessels in and around filter paper disc at 24, 48, 72 hours (25, 27, 28 blood vessels) growth when compared to control (22, 23, 24 blood vessels).

Formations of new capillaries were more prominent with 30CH of *Bellis perennis* after 24, 48 and 72 hours and has (28, 31, 33 blood vessels) that was evident with development of reddishness around the disc indicating increased vessel growth at the treated area and with more vessels growth when compared to that of 6CH has (25, 27, 28 blood vessels).

From the above observation it can be inferred, that both 6CH and 30CH potencies of *Bellis perennis* has the ability to induce angiogenesis through new capillary formation at 72 hours of incubation and 12X potencies of *Bellis perennis* has the ability to inhibit angiogenesis at 72 hours of incubation.

In order to confirm this and also to arrive at the optimum working concentration for *Bellis perennis* we measured the growth of the vessels in terms of its

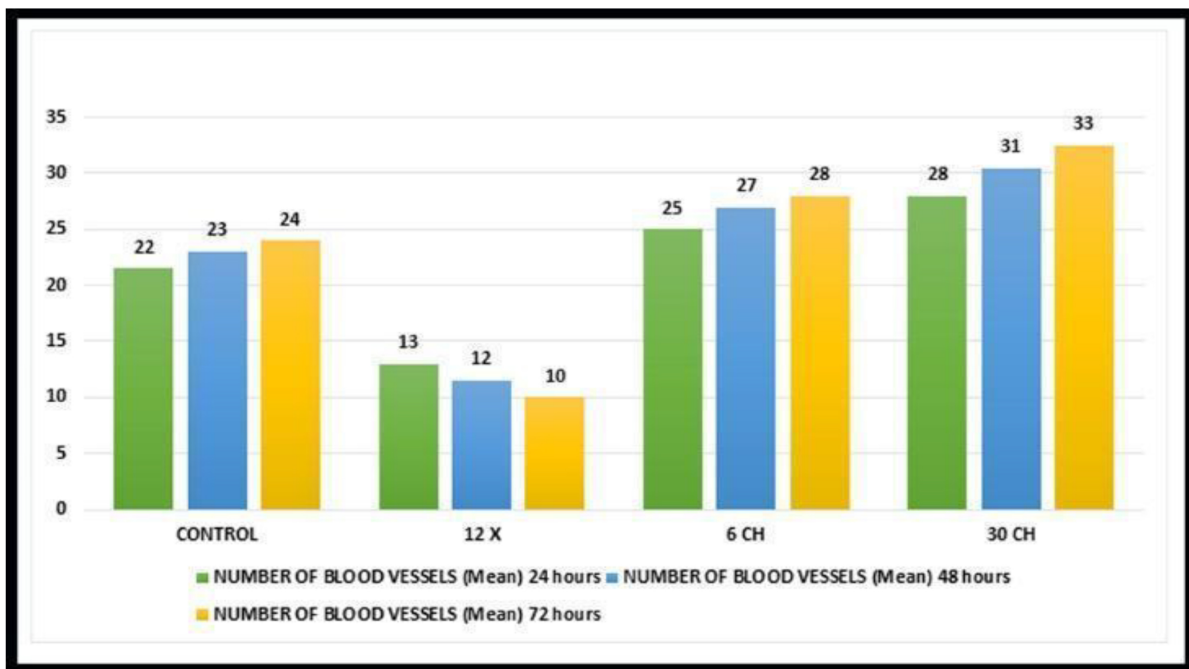


Fig. 6. Mean of number of blood vessels in CAM 24 h, 48 h, 72 h.

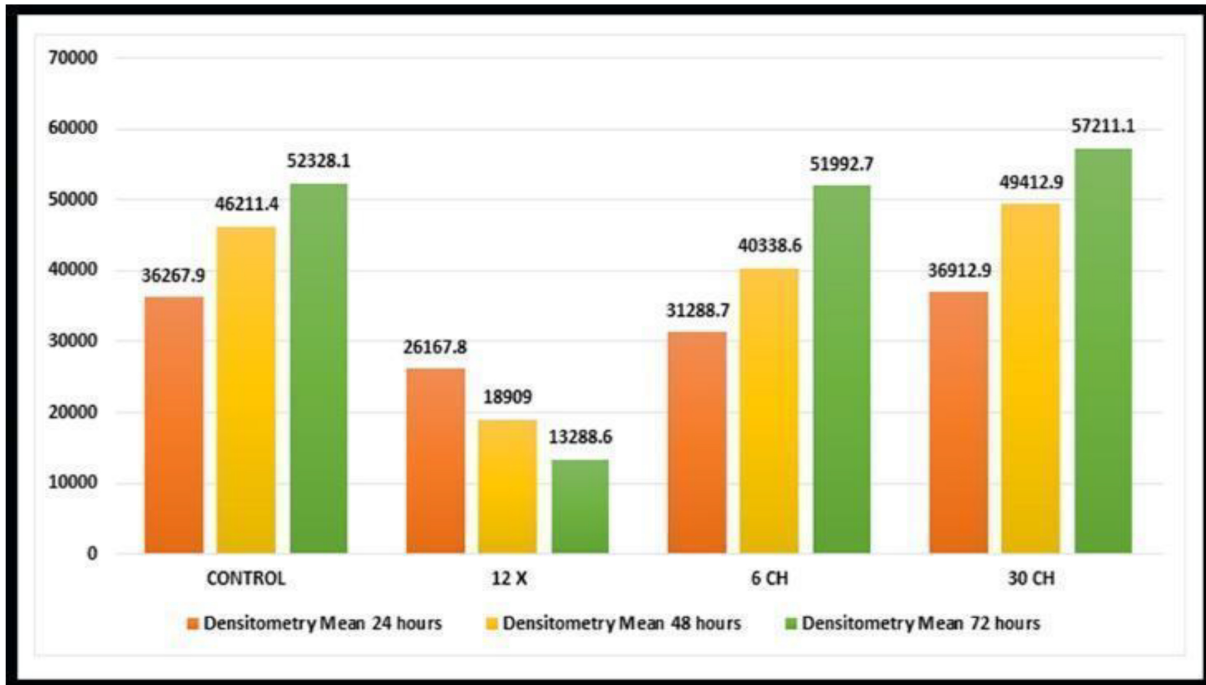


Fig. 7. Densitometry mean of CAM 24 h, 48 h, 72 h.

length and size, from the images taken at 24, 48, and 72 hours of incubation with Image J software of the three different concentrations, used 30CH of *Bellis perennis* demonstrates a significant increase in vessel growth and 12X of *Bellis perennis* shows inhibition in vessel growth [Fig. 7].

Morphology of blood vessels on CAM vascular bed

We analysed the morphology of blood vessels in order to confirm the sprouting angiogenic ability of *Bellis perennis* at the treated area. Images of CAMs were recorded immediately after 24, 48, and 72 hours of incubation using a microscope. It was found that *Bellis perennis* is able to induce growth of new blood vessels from existing ones in 6CH and 30CH potency [Figs. 4 and 5].

CAM incubated with 30CH of *Bellis perennis* shows a greater number of sprouting vessels along with branches and sub branches at the treated area. An enlarged view of the same treated area shows the presence of more branches from the main vessel and sub-branches from the newly formed vessels, a feature directly suggesting the fact that *Bellis perennis* of this particular concentration and time of incubation exerts its maximum angiogenic activity [Fig. 5].

CAM treated with 6CH also shows the presence of sprouting of vessels from the main one with appearances of branches and sub-branches, but its

potential to induce new vessel formation at the treated area was comparatively lesser than that of 30CH of *Bellis perennis*.

Bellis perennis of 12X doesn't show any such sprout formation from the existing vessels.

Control CAM shows the presence of sprouting structures from the main vessel, but it was not remarkably strong when compared to 6CH and 30CH concentrations.

Histological observation of the CAM vasculature

To confirm the angiogenic potential of *Bellis perennis*, we also analysed the changes in the cellular morphology of the CAM vasculature from cross sections of the treated area stained with haematoxylin and eosin. Changes in the morphology of the CAM vasculature has been observed for all three potencies of *Bellis perennis*.

CAM incubated with *Bellis perennis* 12X showed thin Chorionic and Allantoic epithelial layers with lesser number of sub epithelial capillary network. *Bellis perennis* 6CH shows presence of a lesser number of small vessels and slight thickness at the primary stratum. *Bellis perennis* 30CH shows the presence of numerous large vessels around the large one with increased thickness at the primary stratum. Control CAM shows thin chorionic and allantoic epithelial layers with sub epithelial capillary network.

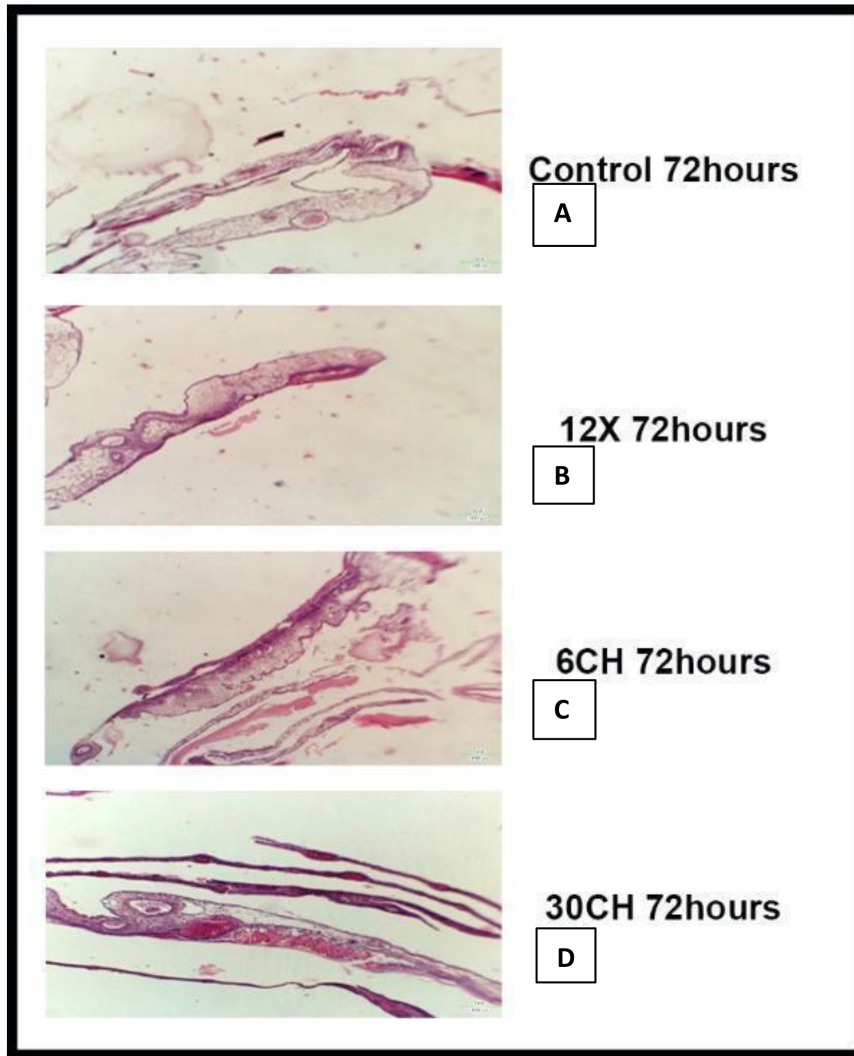


Fig. 8. Haematoxylin and eosin-stained histological images of CAM treated with *Bellis perennis* 12X, 6CH, 30CH and control for 72 h. [A]control shows thin chorionic and allantoic epithelial layers with sub epithelial capillary network, [B]*Bellis perennis* 12X shows thin chorionic and allantoic epithelial layers with lesser number of sub epithelial capillary network, [C]*Bellis perennis* 6CH shows presence of lesser number of small vessels and slight thickness at the primary stratum, *Bellis perennis* 30CH shows the presence of numerous large vessels at the surrounding of large one with increased thickness at the primary stratum.

Bellis perennis of 30CH concentration was capable of eliciting a promising cellular response that could lead to more angiogenic growth [Fig. 8].

Data analysis and statistical analysis

Each experiment was carried out twice independently. Standard deviation was calculated. The two-way ANOVA analysis of variance test was used to analyse the data. The two-way ANOVA revealed statistically significant effects for both the main factors as well as their interaction ($p < 0.0001$ for all comparisons). The column factor accounted for the largest proportion of total variation (69.81%), followed by the interaction effect (18.44%) and the row factor

(11.42%). The F-ratios indicated strong differences among the treatment groups, with $F(3, 12) = 834.9$ for the column factor, $F(2, 12) = 204.9$ for the row factor, and $F(6, 12) = 110.3$ for the interaction. These results demonstrate that both individual factors and their combined influence significantly affected the measured angiogenic response in the CAM assay Data were expressed as p-values of $p < 0.0001$. The result was statistically significant.

Discussion

Bellis perennis in a large dose (lower potency)¹⁸ has the properties to inhibit angiogenesis due to

the particle presence in the medicine, and the same medicine in a small dose (higher potency) has action on wound healing (higher potency).¹⁸

A dosage-response phenomena called hormesis is characterized by small dose stimulating and large dose inhibiting. The phenomenon is applied in *Bellis perennis* large dose inhibiting the angiogenesis and small dose promoting the angiogenesis.²⁰

This opposite effect is happening due to the dynamization of medicine. The large dose has the higher materialistic particles, and the small dose has the dynamization, which has no materialistic particles. While reducing the material particles in the large dose by the process of potentiation. While reaching higher and higher potencies, the material constituents will reduce, and the action of the remedy will promote angiogenesis.

Homoeopathic medicines have nanoparticles. Even though beyond the Avogadro number, these particles have a hormesis effect.^{21,22}

According to Arndt-Schulz law, for every substance, small doses stimulate, moderate doses inhibit, large doses kill. Large dose inhibiting and small dose promoting the angiogenesis. The smaller the dose of the medicine, the greater its effectiveness.²²

While the mathematical calculations for 12X and 6CH are identical (Each step involves diluting the substance to ten times its original volume. As a result, the X (Decimal) scale dilution is half that of the CH (Centesimal) scale dilution,^{23,24} the methods used to prepare the decimal and centesimal scales vary. Both the medicine 12X and the medicine 6CH have different angiogenesis activities. This variation can be due to preparation of medicine on different scales.

Angiogenesis is the process of which new blood vessels are developed from existing ones, it is vital to many physiological and pathological events.¹ The angiogenic capacity of the *Bellis perennis* using an In Ovo CAM test was seen as a transitional stage between a single cell culture and a complex mammalian model, the assay's key advantage is that it may be used as a rapid way to measure angiogenesis response. The use of chorioallantoic membrane for scoring tissue responses to angiogenesis activity of biological materials precisely and in a manner similar to responses seen in mammalian model responses, as well as for assisting in the maintenance of test materials at the administration place, is another significant advantage.^{25,26}

According to the findings of this study on Chorioallantoic membrane assay, *Bellis perennis* has both angiogenesis-promoting and inhibiting properties. *Bellis perennis* 12X has angiogenesis-inhibiting properties, and *Bellis perennis* 6CH and 30CH can induce neovascularization.

Therefore, in the current study, we conducted experiments on various groups of eggs for 24, 48, and 72 hours to examine the angiogenic activity of *Bellis perennis* because at this period, newly developed vessels get stabilized. Interestingly we discovered, both *Bellis perennis* 6CH and 30CH showed significant angiogenic effects at 72 hours of incubation, while *Bellis perennis* 12X showed significant anti-angiogenic effects, indicating that the angiogenic activity of *Bellis perennis* depends on length of incubation period.

Bellis perennis 30CH appears to have a greater ability than 6CH concentration to induce the sprouting of new blood vessels from the existing ones, according to an examination of the vessel growth pattern under a microscope. *Bellis perennis* can thereby induce neo-vascularization in a potency dependent way, The 30CH potency may therefore be more suited, resulting in an earlier and more reliable angiogenesis.

Some foreign substance placed on the CAM can cause an inflammatory reaction, which can then lead to a secondary Vaso proliferative reaction and a false positive result. To solve this problem, we thoroughly examined the histology after adding *Bellis perennis*. The changes in histology and cellular morphological changes in treated area showed that *Bellis perennis* can start the growth of new blood vessels without causing any localised inflammation. A *Bellis perennis* of 30CH exhibits significantly high angiogenesis responses by hastening the development of new blood vessels by sprouts from the main one. Additionally, the thickness of the CAM at the treated area can be used to analyse the angiogenesis or anti-angiogenic effects of a compound since the development and formation of new blood vasculature will increase tissue thickness at the stroma. As a result of the formation of capillary-like tubes and other structure, our study revealed that 30CH of *Bellis perennis* is able to utilize maximum angiogenesis response at the stroma region very close to chorionic epithelium rather than mesoderm, resulting in increased tissue thickness.

However, the authors acknowledge that the study has several limitations. The sample size in this study was small and may limit the statistical power of our findings. Due to practical constraints, increasing the number of samples was not feasible in the current study. The authors plan to include a larger sample size and perform power analysis in future work to strengthen the statistical robustness of the results. Another limitation was that the histology study was done qualitatively only and vessel density and thickness score could not be measured. Further, only vehicle control (alcohol+water) was used in this study. No positive angiogenic (e.g., VEGF, bFGF, heparin) or anti-angiogenic (e.g., thalidomide, endostatin) control were used to demonstrate assay

sensitivity. This limits the validation of assay sensitivity as well as the interpretive strength of the findings. The authors understand that without normal control and positive control, it is not possible to assess the actual sensitivity and specificity of the assay and risk of false positives.

Conclusion

Bellis perennis 6CH and 30CH showed significant Angiogenic effects at 72 hours of incubation, while *Bellis perennis* 12X showed significant Anti-angiogenic effects, indicating that the angiogenic potential of *Bellis perennis* depends on length of incubation period.

Increased tissue thickening and cellular morphological changes of the chorioallantoic membrane suggest that the treatment promotes *Bellis perennis* potency-dependent angiogenic ability.

Overall, our findings suggest that *Bellis perennis* exhibits dose-dependent modulation of angiogenesis in the CAM assay. As this study represents an early screening model, the results indicate potential for angiogenic or anti-angiogenic activity but cannot be directly extrapolated to therapeutic applications without further in vivo and clinical validation.

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Conflicts of interest

None.

Author contribution

Jayanthdevan V - Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Guarantor.

Winston Vargheese V - Concepts, Design, Definition of intellectual content, Data analysis, Statistical analysis, Experimental studies, Manuscript editing, Manuscript review, Guarantor.

Anish fathima K - Data acquisition, Data analysis, Statistical analysis.

Lohita S - Data acquisition, Data analysis, Statistical analysis.

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Effet angiogénique dose-dépendant de *Bellis perennis* sur le test de la membrane chorioallantoïque de l'embryon de poulet in ovo

Contexte: L'hormèse est la réponse biologique d'un organisme à une dose particulière d'une substance. Une faible dose stimule, tandis qu'une dose élevée inhibe. J. Compton Burnett, un homéopathe renommé, prescrivait *Bellis perennis* pour les tumeurs du sein et de l'utérus, ce qui suggère que cela pourrait être dû à ses propriétés anti-angiogéniques ou inhibitrices de l'angiogenèse. Cliniquement, cette plante est également utilisée pour la consolidation des fractures osseuses et la cicatrisation des plaies, ce qui pourrait être dû à ses propriétés angiogéniques. Cette étude porte sur l'effet dose-dépendant de *Bellis perennis* sur le test de la membrane chorioallantoïque de l'embryon de poulet in ovo. **Objectif:** Évaluer l'effet d'hormèse de *Bellis perennis* aux doses 12X, 6CH et 30CH en évaluant son effet angiogénique dans le test de la membrane chorioallantoïque (CAM). **Méthodes:** Le test CAM a été réalisé selon le protocole standard. L'effet anti-angiogénique et angiogénique de *Bellis perennis* aux concentrations 12X, 6CH et 30CH a été évalué par comptage du nombre de vaisseaux et par densitométrie à l'aide du logiciel ImageJ après 24 h, 48 h et 72 h. Une étude histologique a été menée en microscopie optique pour évaluer l'épaississement tissulaire. **Résultats:** *Bellis perennis* a présenté des propriétés anti-angiogéniques à la concentration 12X et des propriétés angiogéniques aux concentrations 6CH et 30CH. **Conclusion:** *Bellis perennis* présente une activité angiogénique dépendante de sa concentration.

Potenzabhängige angiogene Wirkung von *Bellis perennis* im In-Ovo-Chorioallantoismembran-Assay (CAM-Assay)

Hintergrund: Hormesis ist die biologische Reaktion eines Organismus auf eine bestimmte Dosis einer Substanz. Niedrige Dosen wirken stimulierend, hohe Dosen hemmend. J. Compton Burnett, ein bedeutender Homöopath, verschrieb *Bellis perennis* bei Brust- und Gebärmuttertumoren, was auf antiangiogene oder angiogenesehemmende Eigenschaften hindeutet. Klinisch wird *Bellis perennis* auch zur Heilung von Knochenbrüchen und Wunden eingesetzt, was auf angiogenetische Eigenschaften zurückzuführen sein könnte. Dieses Experiment untersucht die dosisabhängige Wirkung von *Bellis perennis* im In-Ovo-Chorioallantoismembran-Assay (CAM-Assay). **Ziel:** Bewertung der Hormesis-Wirkung von *Bellis perennis* 12X, 6CH und 30CH durch Bestimmung der angiogenen Wirkung im CAM-Assay. **Methoden:** Der CAM-Assay wurde gemäß Standardprotokoll durchgeführt. Die antiangiogene und angiogene Wirkung von *Bellis perennis* in 12X, 6CH und 30CH wurde durch Zählung der Gefäße und densitometrische Untersuchung mit der Software ImageJ nach 24, 48 und 72 Stunden (h) bestimmt. Die Gewebeverdickung wurde histologisch lichtmikroskopisch untersucht. **Ergebnisse:** *Bellis perennis* zeigte in 12X antiangiogene und in 6CH und 30CH angiogene Eigenschaften. **Schlussfolgerung:** *Bellis perennis* weist eine potenzabhängige angiogene Aktivität auf.

ओवो चिक कोरियोएलेंटोइक मेम्ब्रेन एसे पर बेलिस परेनिस का पोटेंसी डिपेंडेंट एंजियोजेनिक प्रभाव

पृष्ठभूमि: हॉर्मिसिस किसी चीज़ की एक खास डोज़ के लिए किसी जीव का बायोलॉजिकल जवाब है। कम डोज़ स्टिम्युलेट करती है और ज़्यादा डोज़ इनहिबिट करती है। जे कॉम्पटन बर्नेट, एक जाने-माने होम्योपैथ, ने ब्रेस्ट और यूटेराइन ट्यूमर के लिए बेलिस परेनिस का अच्छा असर पाया था, जिससे पता चलता है कि

यह एंटीएंजियोजेनिक प्रॉपर्टी या एंजियोजेनिक इनहिबिटिंग प्रॉपर्टी के कारण हो सकता है | इसका इस्तेमाल टूटी हुई हड्डी को ठीक करने और घाव भरने के लिए भी किया जाता है, जो एंजियोजेनिक प्रभाव के कारण हो सकता है। यह अध्ययन इन ओवो चिक कोरियोएलेंटोइक मेम्ब्रेन एसे पर *बेलिस परेनिस* के डोज पर निर्भर असर से जुड़ा है। **उद्देश्य:** कोरियोएलेंटोइक मेम्ब्रेन एसे (CAM) में एंजियोजेनिक असर का आंकलन करके *बेलिस परेनिस* 12X, 6CH, 30CH के हॉर्मोसिस असर का मूल्यांकन करना। **विधि:** स्टैंडर्ड प्रोटोकॉल के हिसाब से CAM एसे किया गया और 12X, 6CH, और 30CH में *बेलिस परेनिस* के एंटीएंजियोजेनिक और एंजियोजेनिक असर का आंकलन नसों की संख्या गिनकर और 24 घंटे, 48 घंटे, और 72 घंटे पर इमेज सॉफ्टवेयर का इस्तेमाल करके डेंसिटोमेट्री करके किया गया। टिशू के लिए लाइट माइक्रोस्कोपी में हिस्टोलॉजिकल स्टडी की गई। **परिणाम:** 12X में *बेलिस परेनिस* में एंटीएंजियोजेनिक प्रभाव और 6CH और 30CH में एंजियोजेनिक प्रभाव देखा गया। **निष्कर्ष:** *बेलिस परेनिस* पोटेंसी में एंजियोजेनिक एवं एंटीएंजियोजेनिक प्रभाव दिखाता है।

Efecto angiogénico dependiente de la potencia de *Bellis perennis* en el ensayo de membrana corioalantoidea in ovo en pollos

Antecedentes: La hormesis es la respuesta biológica de un organismo a una dosis específica de una sustancia. La dosis más baja estimula y la dosis más alta inhibe. J. Compton Burnett, eminente homeópata, prescribió *Bellis perennis* para tumores de mama y útero, lo que sugiere que podría deberse a su propiedad antiangiogénica o inhibidora de la angiogénesis. Clínicamente, también se utiliza para la curación de fracturas óseas y heridas, posiblemente debido a su propiedad promotora de la angiogénesis. Este experimento aborda el efecto dependiente de la dosis de *Bellis perennis* en el ensayo de membrana corioalantoidea in ovo en pollos. **Objetivo:** Evaluar el efecto de hormesis de *Bellis perennis* 12X, 6CH, 30CH mediante la evaluación del efecto angiogénico en el ensayo de membrana corioalantoidea (CAM). **Métodos:** El ensayo CAM se realizó siguiendo el protocolo estándar y se evaluó el efecto antiangiogénico y angiogénico de *Bellis perennis* en 12X, 6CH y 30CH mediante el recuento vascular y un estudio densitométrico con el software Image J a las 24 h, 48 h y 72 h. El estudio histológico se realizó con microscopio óptico para determinar el engrosamiento tisular. **Resultados:** *Bellis perennis* presentó propiedades antiangiogénicas en 12X y propiedades angiogénicas en 6CH y 30CH. **Conclusión:** *Bellis perennis* muestra actividad angiogénica dependiente de la potencia.

雏菊 (*Bellis perennis*) 对鸡胚绒毛尿囊膜血管生成作用的效价依赖性效应

背景: 兴奋效应是指生物体对特定剂量物质的生物学反应。低剂量刺激，高剂量抑制。著名顺势疗法医生J Compton Burnett曾用雏菊治疗乳腺和子宫肿瘤，这表明其疗效可能与其抗血管生成特性有关。临床上，雏菊也被用于促进骨折和伤口愈合，这可能与促进血管生成特性有关。本实验旨在研究雏菊对鸡胚绒毛尿囊膜血管生成作用的剂量依赖性效应。 **目的:** 通过评估绒毛尿囊膜 (

CAM) 血管生成作用, 评价雏菊12X、6CH和30CH的兴奋效应。方法: 按照标准方案进行鸡胚绒毛尿囊膜 (CAM) 实验, 并使用Image J软件在24小时、48小时和72小时 (h) 通过血管计数和密度分析评估雏菊 (*Bellis perennis*) 在12倍浓度 (12X)、6小时浓度 (6CH) 和30小时浓度 (30CH) 下的抗血管生成和促血管生成作用。在光学显微镜下进行组织学研究, 观察组织增厚情况。结果: 雏菊在12倍浓度下具有抗血管生成作用, 而在6小时浓度和30小时浓度下具有促血管生成作用。结论: 雏菊的促血管生成活性与浓度呈正相关。