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Annals of Biological Research, 2014, 5 (4):32-37
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ISSN 0976-1233
CODEN (USA): ABRNBW

Angiogenic effect of *Curcuma longa* Linn. (turmeric) tea powder on the chorioallantoic membrane of 10 - day old *Annas luzonica* (Duck) eggs

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ABSTRACT

Angiogenesis is a normal process in the body characterized by the formation of new blood vessels from existing vasculature. Abnormal angiogenesis is a denominator of many diseases. The study evaluated the effect of different concentrations of Curcuma longa Linn. tea powder on the angiogenesis of 10-day old duck embryos. 500 mg of the C. longa tea powder was used in preparing the stock solution for 100ppm (T_1), 200ppm (T_2), and 300ppm (T_3) experimental treatments. These are administered on the chorioallantoic membrane (CAM) of 10-day old duck embryos. After 48 hours, the secondary collaterals on the CAM are counted and compared with the control group and with each of the experimental groups. Results reveal that C. longa Linn. tea powder suppressed angiogenesis. The study found out that 200 ppm (T_2) and 300 ppm (T_3), significantly yielded anti-angiogenic effect. The control group (T_0) and 100ppm (T_1) ; and 100ppm (T_1) and 200ppm (T_2) have no significant difference which can be attributed to low amount and partial solubility of the phytochemicals in water. The synergistic effect of the phytochemicals present in C.longa powder, such as curcumin, quecetin, vitamin C, and vitamin E can affect various steps in angiogenesis. These were defined to be responsible for C. longa tea powder's anti-angiogenic activity.

Keywords: *Curcuma longa*, Turmeric, Tea powder, Anti-Angiogenesis

INTRODUCTION

A greater majority of Filipinos composed of 68.4% from rural areas and 51.5% from urban areas use Complementary and Alternative Medicine to alleviate their medical conditions [1]. Due to the popularity of plants as Complementary and Alternative Medicine modality, various herbal preparations such as infusion, commonly known as tea, became readily available in the market. A wide range of plants are already prepared and consumed as tea and one of the most known plants is luyang dilaw or turmeric. Dr. Andrew Weil noted that people in Okinawa, Japan who has the world's longest average life span of 81.2 years, drink fresh, canned or powdered turmeric tea daily [2].
Curcuma longa Linn., commonly known as turmeric, is a rhizomatous plant. It is mild digestive, stimulant, and a carminative. Being one of the cheapest spices, dried spice of turmeric is widely utilized in Southeast Asia. The most common preparation is hot water extracts of the dried rhizome grounded into a fine powder. In herbal medicine, pure or mixed tea preparations of the turmeric powder is known to be beneficial in treating intestinal problems, measles, sore eyes, and boils [3].

The rhizome of the plant is regarded as a rasayana herb in Ayurvedic medicine for its health benefits brought about by its active ingredient, curcumin [3]. Research studies on curcumin showed to have a wide range of therapeutic effects. Animal studies involving rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated the *C.longa*'s ability to inhibit tumor promotion, angiogenesis, and tumor growth [4].

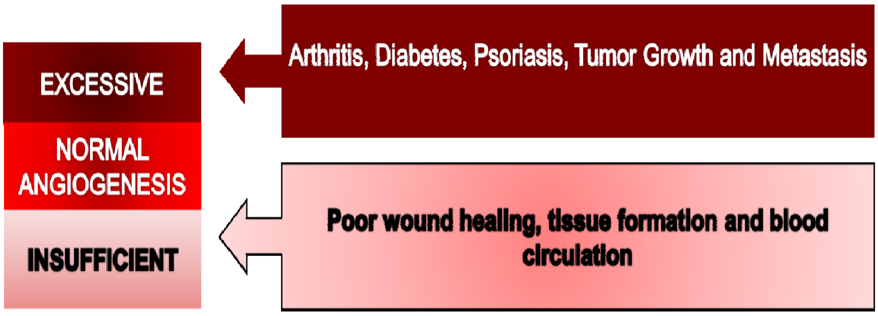


Figure 1. Diseases brought about by abnormal angiogenesis

Angiogenesis is the formation of new blood vessels from existing vasculature. This process is extensive during the embryo stage of human development and minimally continues on through old age [5]. Abnormal angiogenesis is a denominator of myriad diseases [6]. The effects of which are shown in Figure 1.

The present study established the angiogenic effect of *Curcuma longa* Linn. tea powder on the CAM of 10-day old *Annas luzonica* eggs.

MATERIALS AND METHODS

Collection of the C. longa tea powder and eggs

The *C.longa* tea powder was bought at a retailer of Delfa's Food Products in De La Salle University-Dasmariñas and was kept in a cool and dry place. Meanwhile, the sixty (60) 8-day old duck eggs were bought in a farm in 338 Malagasang 2C Open Canal Road, Imus, Cavite. The eggs were placed in an egg tray to prevent breakage.

Preparation of stock solution

500 mg of *C.longa* tea powder in 1 liter Erlenmeyer flask and was weighed using analytical balance. Afterwhich, the flask was filled with water up to 100 ml mark with continuous stirring.

Preparation of different concentrations of *C.longa* tea powder

Three different concentrations of the turmeric tea powder were prepared: T_1 – 100 ppm, T_2 – 200 ppm, and T_3 – 300 ppm. The desired concentrations were prepared by diluting the stock solution with distilled water using the formula:

$$C_1 V_1 = C_2 V_2$$

Where C_1V_1 were the initial concentration and volume of the turmeric tea stock solution and C_2V_2 were the desired concentration and the final volume of turmeric tea mixture. The dilution factor used to obtain desired concentrations. T_1 was prepared by a mixture of 4mL turmeric tea powder stock solution per 16mL of water. The same formula was used to prepare the 2^{nd} and 3^{rd} concentrations wherein 8 mL per 12 mL was used for the T_2 and 12mL per 8 mL was used for T_3.

Administration of the extract on the duck embryo

The 8 day old eggs were incubated at $37^{o}C$ and 70% humidity for two days until it reached its 10^{th} day. At the end of the 10^{th} day incubation period, the eggs were removed from the incubator. Each egg was swabbed with 70% alcohol. After which, the air sac where the concentrations were administered were located. Without damaging the membrane, four pieces of a 1 ml 26G sterile syringe were used to inject the test eggs with their corresponding concentrations, namely: 100 ppm, 200 ppm, 300 ppm turmeric powder and distilled water. The opening of the punctured egg was resealed using a micropore tape, masking tape, and melted wax of a candle. Then, the eggs were placed back in the incubator for 48 hours [7].

Observation of CAM

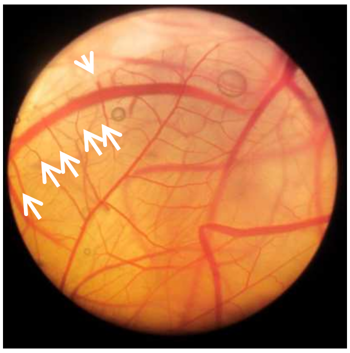
After 48 hours of administration, each of the test eggs was removed from the incubator and the vascularization of the CAM was examined and determined. The CAM was harvested by removing the shell of the egg and extracting it from the embryo. The embryos were observed on a petri dish where the membrane was observed using a dissecting microscope. The number of primary collaterals that was seen as the first branching blood vessels from the main blood vessels and their intersection were counted and compared to the eggs with different concentrations [8].

Data Gathering and Statistical Analysis

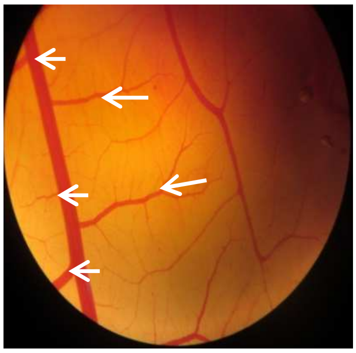
The gathering of data was made two days after the administration of the turmeric tea powder solutions and the control samples to the developing duck embryo. After two days, each of the test embryos was sacrificed and their CAM was prepared for observation of angiogenic activity. The number of collaterals in the CAM of the duck eggs were counted and compared. The observation of the number collaterals in the CAM of the duck eggs was done through a dissecting microscope. Photo documentation was also conducted.

One-way ANOVA was employed in determining the significant differences between the treatments. For further analysis, Tukey method was used to compare the significant differences between the experimental treatments and the angiogenic effects on the CAM of the test duck embryos of the treatments in comparison with the control group. All statistical analyses were done at 5% probability level.

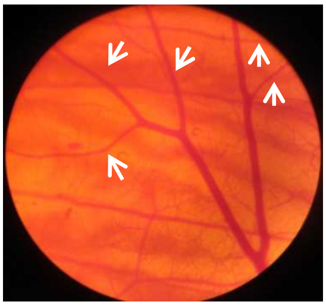
RESULTS



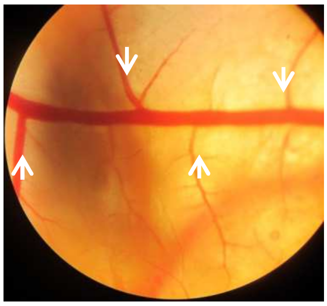
(A) T_0 Control
86.6 average collaterals



(B) T_1 -100ppm
70.8 average collaterals



(C) T_2 -200ppm
49.6 average collaterals



(D) T_3 -300ppm
17.4 average collaterals

Figure 2. Chorioallantoic membrane of Annas luzonica eggs subjected to control and experimental treatments. Arrows point to secondary collaterals.

As shown in Figure 2, it is found out that the control group (T_0) had the highest average collateral count at 86.6 collaterals per test egg, followed by the experimental treatment with 100ppm (T_1) having an average number of 70.8 collaterals. Afterwhich was 200 ppm (T_2) with the average number of collaterals of 49.6. The lowest count of collaterals is observed on the experimental treatment with 300ppm (T_3) with an average number of 17.4 collaterals.

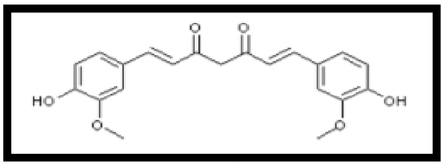
Table 4.1 Average Number of Collaterals Formed by the Control Group and Different Concentrations of *Curcuma longa* Linn. Tea Powder

Treatments	Average number of collaterals	
	Control Group (Distilled water)	Applied with Turmeric tea powder
T_1:100 ppm	T0: 86.6^{A}	T_1: 70.8^{AX}
T_2:200 ppm	T0: 86.6^{A}	T_2: 49.6^{BX}
T_3:300 ppm	T0: 86.6^{A}	T_3: 17.4^{CY}

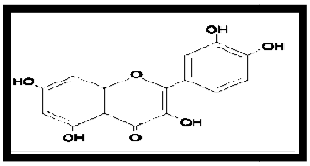
Letters A, B and C compare the different treatments (rows) and letters X and Y compare the experimental treatments. Means followed by a common letter are not significantly different (p<0.05), Tukey Test Method.

The results revealed that there was a decreasing trend in the average number of the collaterals formed in the test eggs as the concentration of *C. longa* tea powder increases. Further, the current study revealed that 200 ppm (T_2) and 300 ppm (T_3) of *C.longa* tea powder yielded significant difference in reference to the control group. On the other hand, it is observed that there was no significant difference between the control group (T_0) and 100 ppm (T_1).

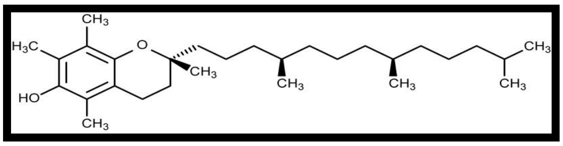
DISCUSSION



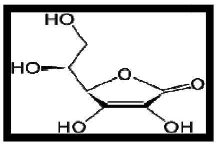
Curcumin



Quercetin



Vitamin E alpha-tocopherol



Vitamin C

Figure 3. Phytochemicals present in *Curcuma longa* Linn.

To suppress angiogenesis, anti-angiogenic factors must be introduced to the system. One of which is curcumin, the main phytochemical of *C.longa* that downregulates proangiogenic factors such as VEGF (Vascular Endothelial Growth Factor), thus, interferes with endothelial activation, proliferation and migration [9]. The angiosuppressive property of the highest dose, 300 ppm (T_3) of *C.longa* tea powder could be brought about by curcumin, present at $3.912x10^{-5}$ mg in the aforementioned concentration.

Flavonoids, particularly quercetin, also play a role in *C.longa*'s angiosuppressive ability by interfering with the VEGF pathway and expression of matrix metalloproteases. Considering this, quercetin slows down endothelial activation, proliferation and migration plus extracellular matrix ECM remodelling. Quercetin, a phytochemical of *C.longa* exhibited no effect at its lowest dose, 25 nmol or 2.5 µl per egg using CAM assay. By applying mathematical methods, approximately, 0.00000187µl quercetin was found to be in each of the 100 ppm test eggs. It was observed that the approximation was below the 2.5µl, so the concentration elicited no angiogenic effect [9]. Noting this, it was verified that the decrease in phytochemicals present in the *C.longa* tea powder mixture can be one of the reasons of the non-significant difference between the control group (T_0), 100 ppm(T_1) and 200 ppm(T_2). Another, phytochemicals of the plant material such as quercetin is only partially soluble in water, it is a possibility that low amounts of phytochemicals are dissociated in the polar solvent [11]. The water-soluble vitamins with anti-angiogenic properties such as vitamins C and E also contributed to angiogenesis inhibition [12, 13].

Combinations of dietary inhibitory compounds may be more effective in eliciting an anti-angiogenic response if each compound targets different steps of angiogenesis. Based on the gathered literature, combination of curcumin and quercetin can interfere with the following processes in angiogenesis: endothelial cells activation, proliferation and migration [9, 14] and basement membrane and extracellular matrix (ECM) degradation [10]. This was supported by a study that found out that combination of curcumin, quercetin and reservatrol resulted to least number and finest new blood vessel growth compared to control set-ups [15]. Also, it was mentioned in a research on the turmeric, a cohort study (N=5) wherein a decrease in size and number of adenomas in patients with familial adenomatous polyposis was observed after a mean of 6 months of treatment with curcumin and quercetin [16]. To sum up, the inhibition of angiogenesis of *C. longa* tea powder seen using the CAM assay can be attributed to the synergistic effect of different phytochemicals present in *C. longa.*

CONCLUSION

Curcuma longa Linn. tea powder is an inhibitor of angiogenesis. This is shown by the decrease on the vascularization of the CAM of a 10-day old duck embryo as the concentration of *C. longa* tea powder increased. 200 ppm (T_2) and 300 ppm (T_3) significantly yielded an anti-angiogenic effect. Further, these concentrations showed no fatality on the test eggs, only reduced number of blood vessels. Thus, it can be said that these concentrations of *C.longa* tea powder are anti-angiogenic at the same time these are safe and efficient. Meanwhile, the control group (T_0) and 100ppm (T_1) ; and 100ppm (T_1) and 200ppm (T_2) had no significant difference which can be attributed to low amount and partial solubility of the phytochemicals in water. The synergistic effect of the phytochemicals present in *C.longa* powder such as curcumin, quecetin, vitamin C and vitamin E can affect various steps in angiogenesis. These were defined to be responsible for *C. longa* tea powder's anti-angiogenic activity.

Recommendations

To further enhance the results of the study, the researchers hereby recommend the following:

1. An experimental phytochemical characterization and analysis of the *C.longa* tea powder from Silang farm harvest, which was utilized in this study be done.
2. A comparative study on the greatest anti-angiogenic effect of *C. longa* using solvents that can dissolve its phytoconstituents in different plant preparations that can be directly consumed.
3. A toxicity test may be conducted to establish the lethal dose of *C. longa* tea powder on its inhibitory effect on angiogenesis.

Acknowledgements

The researchers would like to express their profound gratitude and appreciation to their panel members Ms. Ruth B. America, Ms. Myra N. Lagat and Dr. Jonathan S. Rubio for giving them the opportunity to present their paper; to Dr. Janet P. Macawile,Mr. Danilo F. Gatdula, Mr. Juan B. Almoguera, Ms. Cynthia M. Naca and Mr. Manuel R. Llanillo Jr. for their unending patience and assistance; Mr. Michael R. Sandig, the owner of Silang Farm Harvest for their *C.longa* tea powder; their family members Mr. Manuel N. Maniago, Mrs. Bella Procesa N. Maniago, Mr. Lito Mari Mrs. Myrna S. Mari and Mr. Carl Gregory Mari for their encouragement; and above all, to the Loving Father, for being their immense source of strength, dedication, wisdom and knowledge to fulfil their study, and for continuously showering spiritual grace daily.

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