

## The Effect of Turmeric Decoctum to the Angiogenic Molecules Expression on Chicken Embryo

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## ABSTRACT

Turmeric (*Curcuma longa*) is widely used as herbal medicine, not exception by pregnant women. Turmeric consumption by expectant mothers requires standard dose, because of its antiangiogenic effect could be harmful on placentation process and embryonic development. This experiment was undertaken to determine the effect of different concentrations of turmeric decoctum to the expression of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) and Angiopoietin 1 (Ang-1) on the 48-hours-old chicken embryo. In this study, turmeric were extracted using decoction method to mimic the common method as adopted by people. The turmeric decoctum were freeze dried into a powder form, and was used in preparing the stock solution for 200 ppm (P1), 300 ppm (P2), and 400 ppm (P3) as experimental treatments. The control group (P0) received 2% DMSO without turmeric decoctum. These were administered on the yolk sack of 16 hours incubation of fertile chicken egg by number of 200 µL. After 48 hours incubation, the expression of VEGFR-2 and Ang-1 on the chicken embryo was counted by ImageJ software. The results revealed that there is no significant effect of turmeric decoctum to the expression of VEGFR-2 and Ang-1. This suggested that turmeric decoctum was safe up to 400 ppm on chicken embryo.

**Keywords:** *Turmeric, angiogenic molecules, VEGFR-2, Ang-1*

## INTRODUCTION

Turmeric (*Curcuma longa*) is often used as herbal medicine (hereafter referred to as “Jamu”) for it is believed to have many benefits, e.g. anti-inflammatory, antioxidant, antimicrobial and anticancer [1]. Several descriptive studies revealed that the consumption of Jamu by expectant mothers is still common [2, 3]. There are several types of Jamu which contain turmeric that commonly consumed by pregnant woman to strengthen their pregnancy. Those variants can be found in the form of pre-packed consumer products although they can also be self-prepared (i.e. home-brewed) [4].

Some research suggests that turmeric, which curcumin is the major compound, has the effect of inhibiting angiogenesis [5]. Angiogenesis is the process of new blood vessel formation from the pre-existing vessel [6]. Angiogenesis plays an important role in multitude

physiological processes, including the placentation process and embryonic development [7]. There are several molecules that regulate the angiogenesis process, two of which are VEGFR-2 and Angiopoietin-1 (Ang-1) [7, 8, 9].

VEGFR-2 is an early marker of endothelial cells and hematopoietic precursor cells during embryonic blood vessel development, as a response to the increase of VEGF quantity—especially VEGF-A, generated by mesenchymal cells around it. The binding between VEGF-A with VEGFR-2 initiates the formation of endothelial cells which in turn will form the primary blood vessels [10]. VEGFR-2 show tyrosine kinase activity 10 fold higher than VEGFR-1 [11]. Another important molecule, Ang-1, play an important role in the development and maturation of embryo’s blood vessels [12]. Abnormalities during angiogenesis process can lead to embryonic development disorder and even mor-

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tality [7].

Chicken embryo has been cited as a fit model for studying angiogenesis [13]. The purpose of this study was to determine the effect of different concentrations of turmeric decoctum to VEGFR-2 and Ang-1 expression on chicken embryos.

## MATERIALS AND METHODS

### *Extraction method*

Fresh turmeric rhizome were purchased from Materia Medica Batu, Health Departement of East Java. 500 g turmeric were cut down into small pieces, added to 2500 mL distilled water then boiled for 15 minutes to obtain aqueous extract of the turmeric. The solution were allowed to cool, filtered using muslin cloth and store at -20°C. The solution was freeze dried into powder samples and stored at 40°C. Thin Layer Chromatography (TLC), a qualitative phytochemical test, was used to detect the presence of phenolic and terpenoid compound by using standard procedures.

### *Preparing experimental treatment*

As much as 500 mg of the turmeric decoctum powder were dissolved in DMSO 2% as the stock solution. This solution were used for preparing the concentration of 200 ppm (P1), 300 ppm (P2), and 400 ppm (P3). The control group (P0) received DMSO 2% without turmeric decoctum.

### *Administration of turmeric decoctum to the chicken embryo*

The protocol applied for this experiment was approved by the ethics committee of Faculty of Medicine, Universitas Brawijaya, Malang. Fertile chicken eggs were purchased from avian breeder group “Lestari Sejahtera”- Modopuro - Mojosari - Mojokerto – East Java. The eggs were stored at 15°C for less than 1 week and incubated at 37.5°-39°C up to 16 hours (reach to the stage 3+ according to the criteria Hamburger and Hamilton [14]) in horizontal position with respect to the long axis [15].

Treatments were done in ovo by injection using disposable syringe 1 mL (23 gauge) at the beginning of the primitive streak (stage 3+ according to the criteria Hamburger and Hamilton [14]). 48 fertile chicken egg were divided into 4 group (n=12) as control group (P0) and treatment group (P1, P2, and P3). Fertile chicken eggs were prepared for injection by cleaning the blunt end of egg with alcohol swab. A small hole was made in the blunt end of egg by blood lancet, then 200 µL of

turmeric decoctum was injected into the center of egg yolks. After injection, holes were closed with vinyl tape, and the eggs were turned 180° and re-incubated until stage 12+ according to the criteria Hamburger and Hamilton (48 hours incubation) [14, 15].

### *Collecting the sample*

Each egg was cracked carefully and the content was pour into a petri dish. The frame from thick filter paper (external dimension 1.4 × 1.4 cm with internal window 1 cm<sup>2</sup>) was laid down on the surface of the yolk with the placing of embryo was central within the window. The outside of the frame was cut around when the frame getting wet. The frame was lifted up gently with forcep then washed in a dish with normal saline. The frame contain the embryo was transferred into paraformaldehyde 4% and stored at 40°C [16].

### *Whole mount immunohistochemistry and data collection*

Whole mount immunohistochemistry was performed to determine the expression of VEGFR-2 and Ang-1 by using specific antibody anti VEGFR-2 (A-3): sc-6251 and Ang-1 (C-19): sc-6320 (Santa Cruz Biotechnology®). DAB was used in colour development process. Embryo were observed using stereo microscope with 20 times magnification. Two dimension (2D) picture was taken by using camera DSLR Panasonic DMC-GH2 fixed to the microscope. The expression of VEGFR-2 and Ang-1 were shown as mean of 2D picture colour density intraembryonic region that is counted by software ImageJ [17]. Higher density of the colour means higher expression of VEGFR-2 or Ang-1 on chicken embryo. The data were analyzed statistically by One way ANOVA at p = 0.05 using *SPSS Statistic 21*.

## RESULTS AND DISCUSSION

### *Phytochemical detection*

TLC, as a qualitative test, detected the presence of curcuminoid molecule on the turmeric decoctum. However, terpenoid molecule could not be detected.

### *Turmeric decoctum effect to expression of VEGFR-2*

Expression of intraembryonic VEGFR-2 were shown as brown colour in Figure 1. Turmeric decoctum effect to expression of VEGFR-2 were just as shown in Figure 3A. It shown there were variation expression of VEGFR-2 among experimental group. They were expected as body physiological response to the

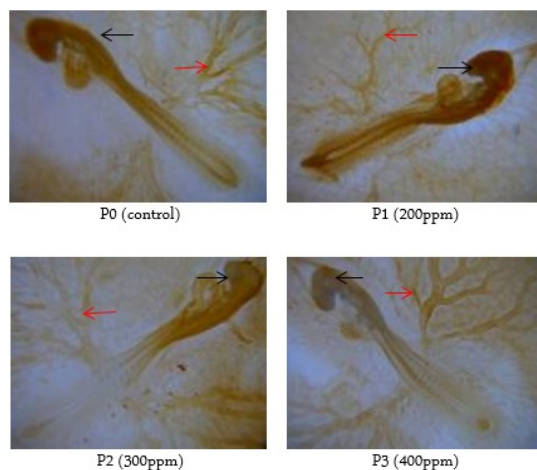


Figure 1. Expression of VEGFR-2 in each experimental group.- Expression of VEGFR-2 were shown as brown colour intraembryonic (black arrow). Higher density of the colour means embryo expressed more VEGFR-2. Red arrow shows extraembryonic vascular.

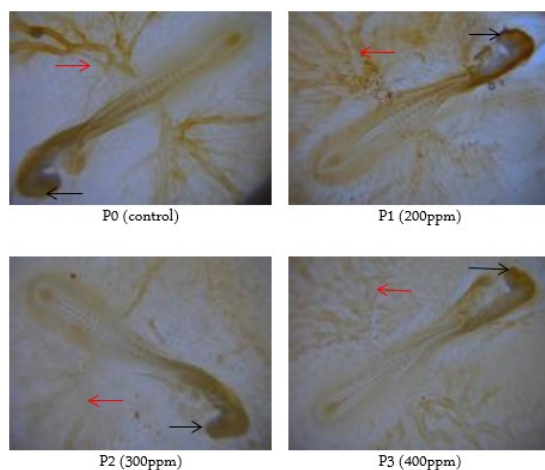


Figure 2. Expression of Ang-1 in each experimental group. Expression of Ang-1 were shown as brown colour intraembryonic (black arrow). Higher density of the colour means embryo expressed more Ang-1. Red arrow shows extraembryonic vascular.

stressor. That response involves multiple cellular signaling pathways and molecular mechanisms to produce cytoprotective proteins including growth factors [25]. Recent study suggested that body responded vary in the expression of VEGFR-2 to anticipate the negative effect that could be caused by administration of various dose of turmeric decoction.

According to statistical analysis by One way ANOVA, it shown that there was no significant differences in expression of VEGFR-2 among experimental group

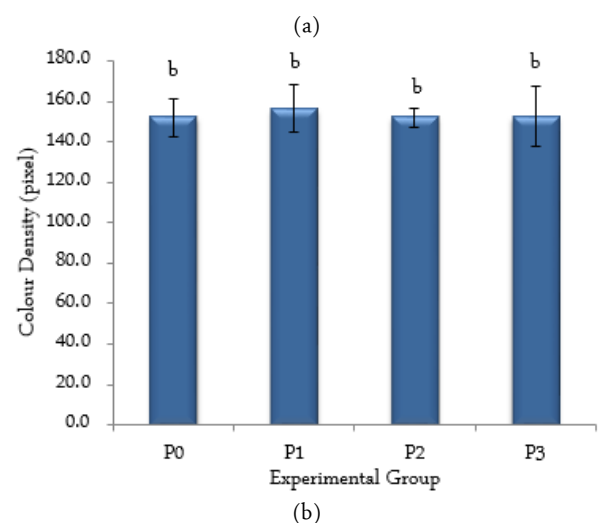
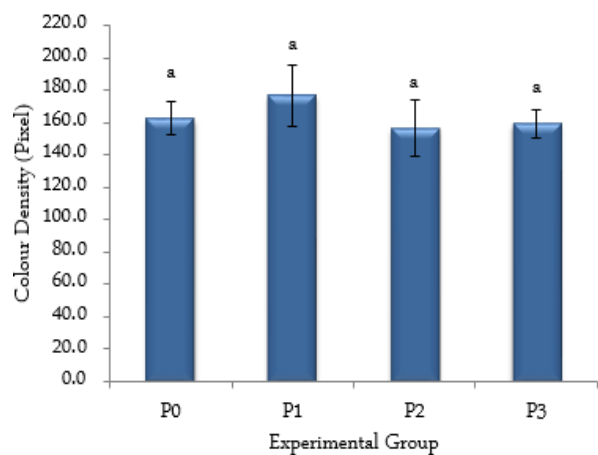


Figure 3. Average number of expression of VEGFR-2 (A) and Ang-1 (B) in each experimental group. P1 showed the highest average of expression of either VEGFR-2 or Ang-1. P0 = control; P1 = treated with 200 ppm of turmeric decoction; P2 = treated with 300 ppm of turmeric decoction; P3 = treated with 400 ppm of turmeric decoction.

( $p = 0.099$ ). It could be attributed to low amount and partial solubility of the turmeric phytochemicals in water. The property of curcumin (turmeric's most significant component) which is pleiotropic, by working in multiple mechanisms and regulating many different target molecules, could also affect the expression of VEGFR-2. Moreover, the dose used in this study was too low to register meaningful effects on the expression of VEGFR-2. Although many prior research suggest that turmeric, which curcumin is a major active substance, has the effect of inhibiting angiogenesis process [5].

Liu et al. had reported that turmeric extract has pharmacological activity 5 fold more potent as antian-

giogenic than pure curcumin compound [18]. Curcumin has been known to be potential in downregulation of proangiogenic factors, such as VEGF[5]. Inhibition on VEGF expression can also decrease the expression of VEGFR-2, due to the presence of receptor also determined by its ligands[8]. The study on tumor cell in mice shown that curcumin can decrease the percentage of tumor cells that express VEGFR-2 up to 28.6% [19].

Some studies proved that curcumin significantly decreases HIF-1 $\alpha$ , HIF-1 $\beta$  and creb-binding protein (CBP)/p300 through increasing proteosomal degradation [20, 21, 22, 23]. Moreover, this caused decrease the level and also activation of HIF-1 as well as disrupt transcriptional some growth factor molecule, such as VEGF and VEGFR-2 [8, 24].

#### ***Turmeric decoctum effect to expression of Ang-1***

Expression of Ang-1 were shown as brown colour intraembryonic area in Figure 2. Turmeric decoctum effect to expression of Ang-1 was just as shown in figure 3B. According to One way ANOVA, it shown that there was no significant differences in expression of Ang-1 among experimental group ( $p = 0.858$ ). Low amount and partial solubility of the turmeric phytochemicals in water, the pleiotropic effect of curcumin could also affect the expression of Ang-1. The same as effect on expression of VEGFR-2, the dose used in this study was too low to register meaningful effects on the expression of Ang-1.

This result disperated from prior research that suggested Ang-1 has the regulation process that similar with VEGFR-2 through mechanism by HIF. Some studies proved that curcumin significantly decreases HIF-1 $\alpha$ , HIF-1 $\beta$  and creb-binding protein (CBP)/p300 through increasing proteosomal degradation. Downregulation of HIF-1 $\alpha$ , HIF-1 $\beta$ , and CBP/p300 suppress the activity of HIF-1 which is a transcriptional factor for angiogenic molecules including Ang-1 [20, 21, 22, 23]. Gururaj, et al. suggest that curcumin has antiangiogenic effect by lowering gene expression of Ang-1 and Ang-2 molecules [26].

This result contrast with other research could be due to differences in methods of extraction. Although the dose used was based on previous research, in this study turmeric was extracted through decoction methods, based on general public in consuming turmeric. It may caused a number of different active substances that is extracted.

## **CONCLUSION**

This study suggested that administration of turmeric decoctum until 400 ppm did not significantly influence the expression of VEGFR-2 and Ang-1 on the chicken embryo.

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## **REFERENCES**

1. Akram M, Uddin S, Ahmed A et al. (2010) Curcuma longa and curcumin: A review article. *Romanian Journal of Biology - Plant Biology* 55 (2): 65-70.
2. Paryono AK (2014) Kebiasaan Konsumsi Jamu untuk Menjaga Kesehatan Tubuh pada Saat Hamil dan Setelah Melahirkan di Desa Kajoran Klaten Selatan. *Jurnal Terpadu Ilmu Kesehatan* 3 (1): 64-72
3. Purnamawati D, Ariawan I (2012) Konsumsi Jamu Ibu Hamil sebagai Faktor Risiko Asfiksia Bayi Baru Lahir. *Jurnal Kesehatan Masyarakat Nasional* 6 (6): 267-272
4. Handayani L (2003) Membedah rahasia ramuan Madura. Jakarta, Agromedia Pustaka
5. Bhandarkar SS, Arbiser JL (2007) Curcumin as an inhibitor of angiogenesis. In: *Mol. Targets Ther. Uses Curcumin Heal*. Boston, Springer.
6. Wang Y, Zhao S (2010) Vascular biology of the placenta. Morgan and Claypool Life Science, California.
7. Zygumunt M, Herr F, Münstedt K et al. (2003) Angiogenesis and Vasculogenesis in Pregnancy. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 110: S10–S18.
8. Olsson AK, Dimberg A, Kreuger J, Lena CW (2006) VEGF receptor signalling — in control of vascular function. *Nature Reviews-Molecular Cell Biology* 7 (5): 359-371.
9. Patel-Hett S, D'Amore PA (2011) Signal transduction in vasculogenesis and developmental angiogenesis. *The International Journal of Developmental Biology* 55 (4–5): 353–363.
10. Carlson BM (2014) Human embryology and developmental biology 5th edition. Sanders Elsevier, Philadelphia
11. Shibuya M (2011) Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti- and pro-angiogenic therapies. *Genes and Cancer* 2 (12): 1097–1105.
12. Syrjala S (2014) Critical role of angiopoietin pathway in ischemia reperfusion injury in cardiac transplantation. [dissertation]. Helsinki: University of Helsinki

13. Drake VJ, Koprowski SL, Lough JW, Smith SM (2006) Gastrulating chick embryo as a model for evaluating teratogenicity: A comparison of three approaches. *Birth Defects Research (PartA): Clinical and Molecular Teratology* 76: 66–71.
14. Hamburger V, Hamilton HL (1951) A series of normal stages of development of the chick embryo. *Journal of Morphology* 88: 4962.
15. Drake VJ, Koprowski SL, Lough JW, Smith SM (2006) Gastrulating chick embryo as a model for evaluating teratogenicity: A comparison of three approaches. *Birth Defects Research (PartA): Clinical and Molecular Teratology*. 76: 66–71.
16. Mason I, Sharpe PT (1999) *Molecular embryology methods and protocols* second edition, Sharpe, P and Mason, I editors. Heidelberg, Humana Press part of Springer Science and Business Media, LLC doi: 10.1007/978-1-60327-483-8.
17. Rahayu ID, Djati MS, Indra MR (2011) Hambatan EGCG terhadap ekspresi VEGF dan VE-Chaderin Embrio Ayam. *The Journal of Experimental Life Science* 1 (2): 56-110
18. Liu D, Schwimer J, Liu Z et al. (2008) Antiangiogenic Effect of Curcumin in Pure Versus in Extract Forms. *Pharmaceutical Biology* 46 (10–11): 677–682.
19. El-Azab M, Hishe H, Moustafa Y, El-Awady S (2011) Antiangiogenic effect of resveratrol or curcumin in ehrlich ascites carcinoma bearing mice. *European Journal of Pharmacology* 652: 7–14.
20. Bae MK, Kim SH, Jeong JW et al. (2006) Curcumin inhibits hypoxia-induced angiogenesis via down-regulation of HIF-1. *Oncology Reports* 15 (6): 1557-1562.
21. Choi H, Chun YS, Kim SW et al. (2006) Curcumin inhibits hypoxia-induced factor-1 by degrading aryl hydrocarbon receptor nuclear translocator: A mechanism of tumor growth Inhibition. *Molecular Pharmacology* 70 (5): 1664-1671.
22. Hasima, Aggarwal B (2014) Targeting proteasomal pathways by dietary curcumin for cancer prevention and treatment. *Current Medicinal Chemistry* 21 (14): 1583-1594.
23. Marcu MG, Jung YJ, Lee S et al. (2006) Curcumin is an inhibitor of P300 histone acetyltransferase. *Medicinal Chemistry* 2 (2): 74-169. doi: 10.2174/157340606776056133.
24. Krock BL, Skuli N, Simon MC (2011) Hypoxia-induced angiogenesis: good and evil. *Genes and Cancer* 2 (12): 1117–1133.
25. Mattson MP (2008) Hormesis defined. *HHH Public Access Author Manuscript* 7 (1): 1–7.
26. Gururaj AE, Belakavadi M, Venkates DA et al. (2002) Molecular mechanisms of anti-angiogenic effect of curcumin. *Biochemical and Biophysical Research Communications* 297 (4): 934–942. doi: 10.1016/S0006-291X(02)02306-9.