Kaempferia galanga L. Inhibiting Effect on Vascular Endothelial Growth Factor (VEGF) and Cyclooxygenase-2 (Cox-2) Expression on Endothelium of Chorioallantoic Membrane

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Kaempferia galanga L. Inhibiting Effect on Vascular Endothelial Growth Factor (VEGF) and Cyclooxygenase-2 (Cox-2) Expression on Endothelium of Chorioallantoic Membrane

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Abstract

This study was aimed to examine the extract of *Kaempferia galanga L.* to inhibit the expression of VEGF and Cox-2 on the chorioallantoic membrane of embryonated chicken eggs (ECE). This study used 24 ECE which were divided into six groups: (C+) inoculated with solvent, (C-, T1, T2, T3 and T4) inoculated with bFGF 60 ng then (T1, T2, T3 and T4) treated with the extract doses of 30, 60, 90 and celecoxib 60 mg, respectively. Expression of VEGF and Cox-2 was observed by the immunohistochemical method. It was concluded that the extract of *Kaempferia galanga L.* can inhibit the expression of VEGF and Cox-2 on the chorioallantoic membrane.

Key words: *Kaempferia galanga L*, VEGF, Cox-2, celecoxib

Development of cancer cells was induced by activating some growth factors such as Vascular Endothelial Growth Factor (VEGF), cyclooxygenase-2 (Cox-2) and tumor necrosis factor (TNF) (Prior *et al.*, 2004). *Kaempferia galanga L* contains Ethyl p-methoxycinnamate (EPMC), which has anti-inflammatory and anti-angiogenesis action through tyrosine kinase (Ekowati *et al.*, 2015). The study was conducted to assess these inhibitory effect of EPMC, on Cox-2, VEGF on Choriaollantoic membrane of embryonated eggs.

Materials and Methods

This study was approved by the ethical committee No: 923-KE, Faculty of Veterinary Medicine, Universitas Airlangga. Nine day old specific pathogen free (SPF) eggs from Pusat Veterinaria Farma, Surabaya. *Kaempferia galanga L.* isolated from Malang, East Java. Angiogenesis inductor used recombinant human Basic Fibroblast Growth Factor (bFGF) (Nako Japan Pure Chemical Industries Ltd.) diluted with Tris-HCl pH 7.5.

24 nine day old embroyonated eggs were divided into six groups of 4 each. The positive control C+ eggs were in inoculated with Tris-HCl; the negative control eggs are inoculated with DMSO solvent. The treatment groups T1, T2, T3 and T4 were inoculated with EPMC at doses of 30, 60, 90 and 120 mg in 2% DMSO and incubated at 38°C with 60% RH for 72 hr (Ribatti, 1997). The VEGF and Cox-2 expression were observed by immunohistochemical methods (Purnama *et al.*, 2019).

Results and Discussion

Observation of VEGF and Cox-2 expression was carried out on brownish-looking endothelial cells. The expression was caused by an antigen reaction with VEGF and Cox-2 antibodies as markers. The results (Table I) showed significant ($p<0.05$) on VEGF expression after being inoculated with (T2) *Kaempferia galanga L* dose of 60 mg. Cox-2 expression was significant ($p<0.05$) after being inoculated with (T1) *Kaempferia galanga L* dose of 30 mg.

The VEGF expression shows a brownish color in the cell cytoplasm that binds to the complex with VEGF receptor ligands (Chen *et al.*, 2016). The VEGF receptor bonds with specific ligands occur in the transmembrane domain and cytoplasm. The VEGF receptor is an inductor of angiogenesis and regulates the

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Table I. Mean and standard deviation of the VEGF and Cox-2 expression

Treatments	Mean±SD	
	VEGF	Cox-2
C-	48.60^{d}±9.34	69.60^{d}±9.73
C+	26.60^{c}±4.44	35.40^{c}±4.32
T1	23.40^{bc}±4.28	25.40^{b}±3.05
T2	17.40^{ab}±5.32	16.20^{a}±4.09
T3	13.80^{a}±3.29	15.80^{a}±5.13
T4	11.85^{a}±2.77	13.60^{a}±9.99

Different superscripts in the same column indicate significant differences among treatments ($p<0.05$)

integrity of the endothelium. Some of VEGF compounds inhibit endothelium formation and decrease angiogenesis (Di Marco *et al*., 2009).

One of the factors that trigger the growth of angiogenesis when tumors occur, namely VEGF which initiates the formation of blood vessels (Red-Horse and Ferrara, 2006). Ethyl p-methoxycinnamate acts to inhibit angiogenesis by decreasing VEGF expression. Quercetin and Ethyl p-methoxycinnamate contained in *Kaempferia galanga L* inhibits angiogenesis and tumor necrosis factors. Quercetin can suppress tumor growth in vitro and in vivo acting as a barrier to the activity of tyrosine kinase (Kubo *et al*., 2004).

Other active compounds in *Kaempferia galanga L* extracts such as alpha sitosterol, silymarin, 4-hydroxy-4-methyl-2-pentanone, quercetin and resveratrol are considered to have anticancer activities through a mechanism of signal transduction, inhibiting cell cycle, apoptosis, and inhibiting metastasis (Umar *et al*., 2012). Resveratrol and quercetin at a concentrations of 100 µg/ml were able to inhibit the proliferation of angiogenesis, migration of endothelial cells, and formation of intima vascular layers (Igura *et al*., 2001). Silymarin was developed as a cyclooxygenase-2 (Cox-2) enzyme inhibiting agent. This compound reduces the number of Human Vascular Endothelial Cells (HUVEC) at levels of 50 µg/ml (Tosetti *et al*., 2002).

The compounds contained in *Kaempferia galanga L* extract can inhibit Cox-2 activity induced by bFGF angiogenic agents. Flavonoid extract from *Kaempferia galanga L* decreased VEGF receptor (VEGFR2) through barrier activity from Matrix Metallo Proteinase (MMP), tyrosine kinase, and Cox-2 (Masferrer *et al*., 2000; Hamid *et al*., 2018).

Summary

Kaempferia galanga L EPMC extract at 60 mg can inhibit VEGF expression in chorioallantoic membrane blood vessel endothelial cells induced by bFGF. A minimum dose of 30 mg can inhibit Cox-2 expression. There was no significant difference in the administration of extracts of at the dosage of 90 mg with celecoxib 60 mg.

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