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Antiangiogenesis Activity of *Centella Asiatica* on the Macrophage Number and Matrix Metalloproteinase-9 in Blood Vessels of Chorioallantois Membrane Induced by Basic Fibroblast Growth Factor

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Abstract

This study aimed to examine the potential of aqueous extract of *Centella asiatica* on the macrophage number and matrix metalloproteinase-9 (MMP-9) in blood vessels of chorioallantois membrane induced by basic Fibroblast Growth Factor (bFGF). This study used 20 embryonated chicken eggs (ECE) which were divided into five groups i.e. positive control (C+) given 60 ng of bFGF; negative control (C-) given DMSO 2%; treatment groups (T1; T2; T3) given 60 ng of bFGF and *Centella asiatica* extract (75; 90; 110) µg. It was concluded that *Centella asiatica* at a dose of 110 µg decreased the macrophage number and the expression of MMP-9.

Key words: *Centella asiatica*, Chorioallantois membrane, Macrophage, MMP-9

Angiogenesis is a phenomenon associated with the growth of new blood vessels (Murata *et al.*, 2008). Angiogenesis of cancer pathophysiology takes a role of supplying oxygen and metabolic products of cancer cells (Seavey *et al.*, 2009). Cancer incidence were unable to be separated from the contribution of angiogenesis. The inhibitory, *Centella asiatica* was assumed to have the role on the mechanisms that initiate cancer through angiogenesis.

Materials and Methods

This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga. Induction of the new blood vessels in the Chorioallantois Membrane performed by induced basic Fibroblast Growth Factor (bFGF). *Centella asiatica* aqueous extract of 10 mg was dissolved in 2% DMSO in sterile

aquadest to obtained a solution with a concentration of 1 µg/µl. This study used 20 embryonated chicken eggs (ECE) which were divided into five groups i.e. positive control (C+) given 60 ng of bFGF; negative control (C-) given DMSO 2%; treatment groups (T1; T2; T3) given 60 ng of bFGF and *Centella asiatica* extract (75; 90; 110) µg, respectively. All samples were incubated at a temperature of 38°C and a relative humidity of 60% for 72 hours (Hamid *et al.*, 2018). Immunohistochemistry method was done to measure the macrophage number and expression of MMP-9. The data tested by one way Anova and followed Duncan test.

Results and Discussion

There was a significant difference in the provision of aqueous extract of *Centella asiatica* at various doses to decrease the number of macrophages (Table I). The mean number of macrophages were the lowest in the T3 group. These results were not significantly different ($p>0.05$) with the C- group. It means that the administration of *Centella asiatica* aqueous extracts at 110 µg capable of inhibiting the development of macrophages. While the T1 group at the dose of 75 µg was not significantly different ($p>0.05$) from C+ group. However, the results of this study indicated that there was decreased number of macrophages ($p<0.05$) in all three treatment groups treated with *Centella asiatica* aqueous extract, compared to C+ group.

The result of the MMP-9 expression in endothelial cells of new blood vessels (Fig.2) indicated that the highest average number expression was obtained in the C+ group. These results were significantly different ($p<0.05$) compared to all other treatment groups (Table

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Table I. Mean and standard deviation of the amount of macrophage and MMP-9

Treatments	Mean±SD	
	Macrophage	MMP-9
C+	90.50 ^a ±56.80	310.75 ^a ±23.76
C-	35.00 ^c ±7.52	97.00 ^c ±11.40
T1	82.25 ^a ±9.17	276.75 ^a ±30.01
T2	51.00 ^b ±8.60	151.00 ^b ±24.61
T3	34.50 ^c ±5.19	130.50 ^b ±19.39

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

D). While the mean results of the MMP-9 expression the least were shown in the C- group. On comparison no significant differences ($p > 0.05$) were noticed between by treatment groups with *Centella asiatica* extract at 90 µg and 110 µg.

Centella asiatica contains triterpenoid glycosides or asiaticoside which is used in leprosy wound healing. While compounds that function for anti-cancer properties are asiatic acids.

Centella asiatica is used as a tonic, antirheumatic, sedative, accelerates wound healing, and diuretics. The content of other compounds, namely: asiaticoside, thankuniside, medecassoside, brahmoside, brahminoside, madastic acid, vitamins B1, B2, and B6 (Sampson *et al.*, 2001).

bFGF or FGF-2 is part of the Fibroblast Growth Factor group that affects most cellular activity. Interacting with no more than 23 ligands, four receptors and with various co-receptors that complexly spread into a sign of effects on various receptors (Coults *et al.*, 2005). bFGF is generally identified as mitogen with prominent angiogenic, which is now better known as multifunctional growth factors (Przybylski, 2009). The ligand binding of the permissible and autophosphorylated receptors follows the binding and activation of cytoplasmic target proteins (Backhaus *et al.*, 2004).

Macrophages stimulate the body defense systems which functions as phagocytosis. Congenital body defense is a non-immunological

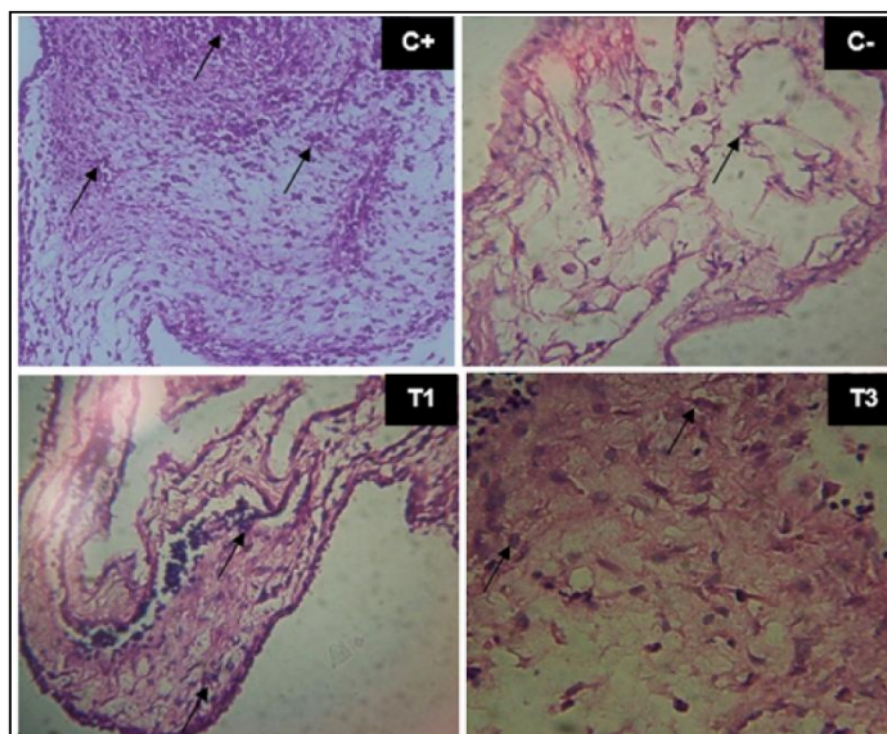


Fig.1. Histopathological features of chorioallantoic membrane of blood vessels after *Centella asiatica* and bFGF induction. The black arrows indicate macrophage (□).

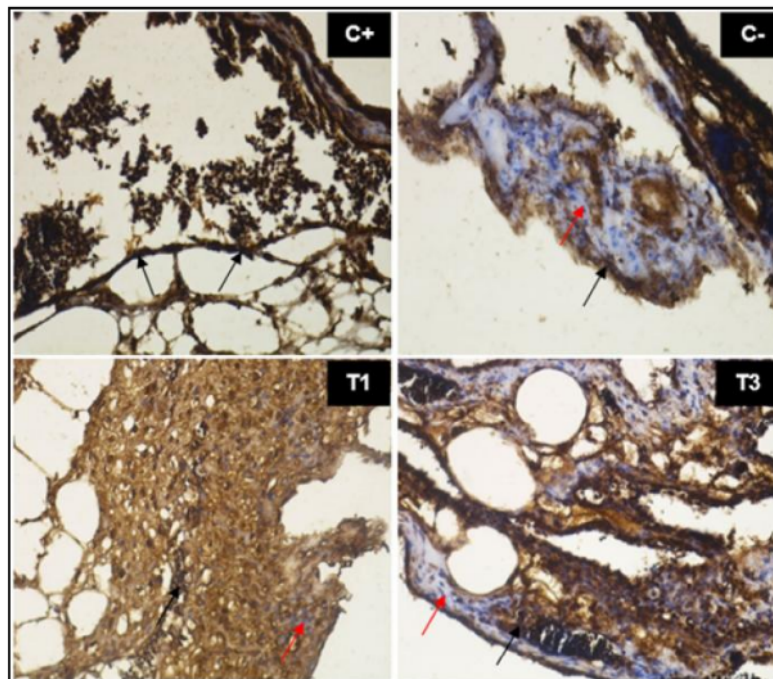


Fig.2. Expression of MMP-9 by Immunohistochemistry staining. The black arrows (□) indicate the MMP-9 expression and red arrows (□) indicate inactive MMP-9 in cells.

defense that has existed since the birth (Jetten *et al.*, 2014). Matrix metalloproteinase-9 and Matrix metalloproteinase-13 are families of MMP that play a role in angiogenesis. The role of MMP in angiogenesis was noticed when inflammatory cells or tumor cells secrete angiogenic factors such as VEGF and bFGF which then bind to their receptors on the endothelial cell surface (Iwasaki *et al.*, 2004). This will activate endothelial cells to secrete MMP and MMP that will degrade the extracellular matrix and assists their proliferation (Rosell *et al.*, 2008).

The observation of the overall number of macrophages in the treatment group *Centella asiatica* showed decreased significance (Fig.1). These results indicate that the higher the dose of *Centella asiatica* aqueous extract bigger the growth inhibition of new blood vessels. In addition, the growth of new blood vessels includes a complex process involving immunological processes, one of which is played by macrophages. Macrophages play a role in inflammatory processes and tumor angiogenesis (Jetten *et al.*, *loc. cit.*).

It is commonly known that MMP-9, often referred to as Chicken gelatinase B (chMMP-9), is a specific marker for heterophile, which is an analog of blood cells with neutrophils in mammals. It can be revealed by the development of angiogenesis induced by purified growth factors or by the Human HT-1080 cells fibrosarcoma which is linked to the presence of heterophile chMMP-9 (Rosell *et al.*, *loc. cit.*). The existence of heterophile followed by infiltration of monocytes or macrophages is sufficient to support the formation of new blood vessels. Disturbances or hindrance in cell inflammatory mechanism mediated by the presence of anti-inflammatory medications such as cortisone and ibuprofen showed significant differences in inhibiting angiogenesis. MMP-9 functionally contributes to angiogenesis triggered by inflammation and tumor growth (Zijlstra *et al.*, 2006).

Summary

The aqueous extract of *Centella asiatica* at 110 µg decreased the macrophages number and the MMP-9 expression. It is advisable to observe

some function of factors that play a role in neoangiogenesis such as MMP-13, FGF and several cytokines involved are recommended.

Acknowledgment

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