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Efficacy of epigallocatechin gallate gel on VEGF and MMP-9 expression on ulcerations

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ABSTRACT: Background: Green tea (Camelia sinensis) has bioactive components such as epigallocatechin gallate (EGCG), which has been found to help in wound healing. In the healing process of ulcerations, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) play a very important role, especially in angiogenesis and tissue remodeling. Objective: To determine the effect of EGCG gel administration on VEGF and MMP-9 levels on mucosal ulceration of Wistar rats. Methods: This study is a laboratory experimental study with a post-test-only control group design. 24 Wistar rats were selected randomly and divided into 3 groups, negative control, where the ulceration was given only a covering agent, positive control, where the ulceration was given triamcinolone acetonide, and treatment group, where the ulceration was given 10ppm EGCG gel, expression of VEGF and MMP-9 seen in days 3, 5, 7, and 9 days by immunohistochemical staining. Results: There is a difference, albeit not significant between the treatment group and the positive or negative control group. Conclusion: Administration of EGCG gel did increase the expression of VEGF and decrease the expression of MMP-9 in oral epithelial ulcerations in Wistar rats, albeit not significant compared to control groups, this may be influenced by the characteristics of EGCG, which is less stable and has low bioavailability.

1 INTRODUCTION

Aphthous stomatitis is the most prevalent oral lesion in the general population. These lesions often cause pain that interferes with daily activities, such as talking, eating, and drinking. The etiology is still unknown, but there are many related trigger factors, such as local trauma, genetic factors, nutritional deficiencies, and disorders of the endocrine and hormonal systems (Edgar et al. 2017). As many as 25% of the global population experience aphthous stomatitis, and according to the 2018 Basic Health Research (Riskesdas) data, 8% of the population experienced aphthous stomatitis, which is repeated at least 4 times and occupies the 3rd position of most frequent oral health problems in Indonesia (Kesehatan 2019).

Vascular endothelial growth factors (VEGF) are present in the wound healing process, namely at the proliferative stage, especially at the formation of granulation tissue, which requires an adequate blood supply. VEGF serves to signal the formation of new blood vessels, as a mechanism to restore the blood supply to tissues, and will accelerate the healing process (Duffy et al. 2013). VEGF is secreted by various cells, such as fibroblasts,

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macrophages, keratinocytes, and tumor cells. VEGF is one of the factors of angiogenesis and lymphangiogenesis. Angiogenesis is a physiological process of the formation of new blood vessels, while lymphangiogenesis is a physiological process of the formation of new lymph vessels, and angiogenesis and lymphangiogenesis play an important role in the wound healing process.

Matrix metalloproteinases (MMP) are present in the process of healing acute and chronic wounds at the proliferative stage, especially at the tissue remodeling stage. MMP and tissue inhibitor of metalloproteinase (TIMP) have an important role in the wound healing process, where in acute wounds, MMP will regulate the degradation of the extracellular matrix, so that extracellular matrix remodeling occurs (P. Chen & Parks 2009). One type of MMP, namely MMP-9 (gelatinase B), is expressed by keratinocytes at the periphery of the wound and has a role in cell migration, reepithelialization, and regulation of angiogenesis (Caley et al. 2015). In a study conducted with MMP-9 deficient mice, the wound-healing process was inhibited (Caley et al. 2015).

Green tea (*Camellia sinensis*) is one of the most popular teas and is often drunk throughout the world, and nourishes the body without side effects. Green tea extract has also been investigated, in certain doses, to increase the induction of keratinocyte migration in wound closure (Hagiu et al. 2020). One of the bioactive compounds in green tea that has been studied to have an effect on wound healing is epigallocatechin gallate (EGCG). EGCG is one of the catechins of green tea, is the most abundant catechin found in green tea, and is thought to have a significant role in biological activity (Bartosikova & Necas 2018). Topical administration of EGCG in incision wounds was observed to increase the rate of dermal granulation tissue formation in diabetic rats. In this study, it was concluded that EGCG has strong potential in wound healing, particularly at higher dosages (Kim et al. 2008). Topical administration of EGCG has also been carried out on the skin of white rats with second-degree burns, and it has been observed that it can accelerate the healing process of second-degree burns.

To date, no research has been conducted to determine the potential of EGCG administration in wound healing of the oral mucosa due to aphthous stomatitis, so further research is needed. VEGF and matrix metalloproteinase-9 (MMP-9) were observed because they are important parameters in the wound-healing process. This research could be used to explore the efficacy of EGCC usage in the healing process of aphthous stomatitis.

2 MATERIAL AND METHODS

2.1 *Ethical approval*

This research acquired an ethical approval letter for the use of animal subjects from the Ethics Research Committee Pusat Studi Biofarmaka Tropika Institut Pertanian Bogor, Bogor, Indonesia with number 001-2022 KEH TROP BRC.

2.2 *Epigallocatechin gallate gel preparation*

Epigallocatechin gallate powder was obtained from Sigma Aldrich and was mixed with orabase gel to create epigallocatechin gallate gel, with 10ppm concentration, as it is found in another study that 10ppm concentration was better for wound healing (Kim et al. 2008).

2.3 *Animal subjects preparation*

24 male Wistar rats (*R. norvegicus*) were acclimated for seven days with a rat diet consisting of 10% of their mass. Usage of a Ball Burnisher Tip triggered thermal injury to the labial mucosa of the upper lips of research subjects, which resulted in the development of ulceration. Animal models were administered with 0.05 to 0.1 ml/10 g of their mass with rodent anesthetic (ketamine and xylazine). After 24 hours, ulcer development was observable (Ernawati & Puspa 2018).

2.4 *The effect of epigallocatechin gallate gel*

24 male Wistar rats with ulcers on upper labial mucosa were divided into 3 groups, negative control groups, positive control groups, and treatment groups, each group was then divided into sub-groups (days 3, 5, 7, and 9), totaling 12 sub-groups consisting of 4 negative control groups of rats treated with Orabase gel; 4 positive control groups of rats treated with Triamcinolone acetonide 0.1% gel; and 4 treatment groups of rats treated with Epigallocatechin gallate gel, each sub-groups comprising 2 rats across all sub-groups, VEGF and MMP-9 expression were evaluated on days 3, 5, 7, and 9.

2.5 *The effect of epigallocatechin gallate gel on VEGF and MMP-9 expression in the ulcer healing process*

Tissue from the upper labial mucosa was processed for imaging using immunohistochemistry technique using monoclonal antibody with antigen reaction for both VEGF and MMP-9 in conjunction with diaminobenzidine substrate. VEGF-expressing endothelium cells appear brown at 40x10 magnification, while MMP-9-expressing fibroblast cells also appear brown at 40x10 magnification in 5 distinct areas reviewed by three experts, and cells were counted. The acquired data were further analyzed using Analysis of Variance (ANOVA), followed by Tukey's test in the event of any significant differences between the treatment groups.

3 RESULTS

Clinical observations in the treatment group showed faster healing compared to the negative control group, and areas of erythema were seen on day 5, the mucosa was whitish-red on day 7, and the mucosa had healed from the ulceration and on day 9 (Figure 1).

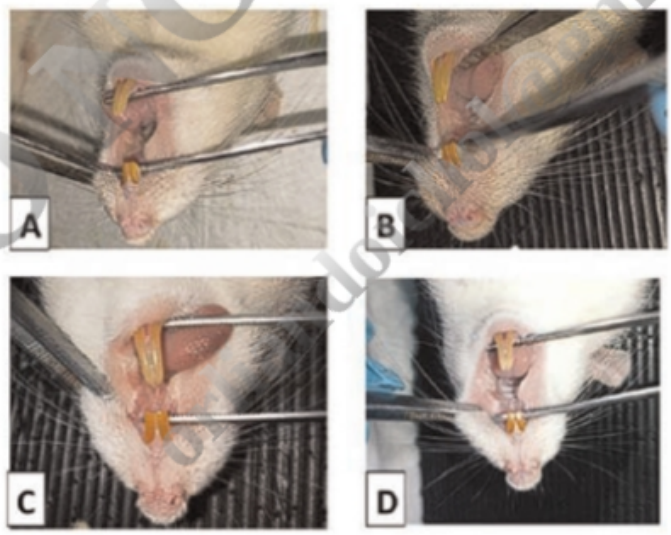

Figure 1. (A) H+3 after EGCG administration; (B) H+5 after EGCG administration; (C) H+7 after EGCG administration; (D) H+9 after administration of EGCG.

Microscopic observations showed that endothelial cells expressed VEGF in the cytoplasm, which was indicated by brown staining of the cytoplasm (Figure 2).

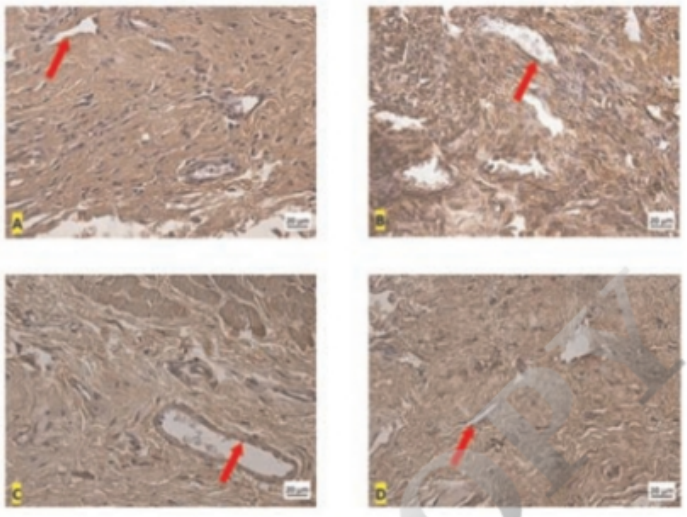

Figure 2. VEGF expression in endothelial cells (red arrows) indicated by brown staining of the cytoplasm on (A) day 3 (B) day 5; (C) day 7; and (D) day 9 (40X10 Magnification).

Microscopic observations showed that fibroblasts expressed MMP-9 in the cytoplasm, which was indicated by brown staining in the cytoplasm (Figure 3).

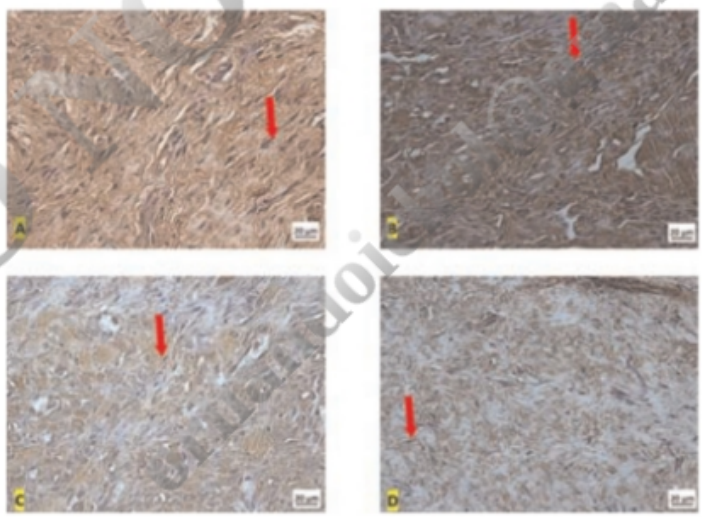

Figure 3. MMP-9 expression in fibroblast (red arrows) indicated by brown staining of the cytoplasm on (A) day 3 (B) day 5; (C) day 7; and (D) day 9 (40X10 Magnification).

The endothelial cell count was obtained from the observations of 3 interpreters, where the sample was blinded before being counted. The test was continued by performing one-way ANOVA, and the results of endothelial cell counting were obtained between the groups (Figure 4)

The fibroblast cell count was obtained from the observations of 3 interpreters, where the sample was blinded before being counted; after the results were obtained, the intraclass correlation coefficient test was carried out to assess the reliability between interpreters, and the results were 0.88 (excellent reliability), then continued with the normality test using Shapiro- Wilk, which shows the data have a normal distribution with $p>0.05$. The test was continued by performing one-way ANOVA, and the results of fibroblast counting were obtained between the groups (Figure 5).

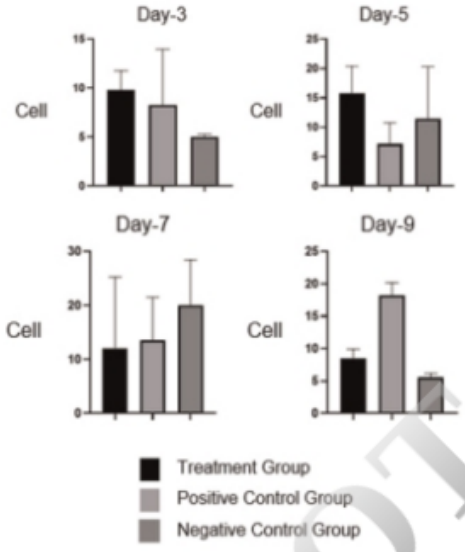

Figure 4. Endothelial cells expressing VEGF.

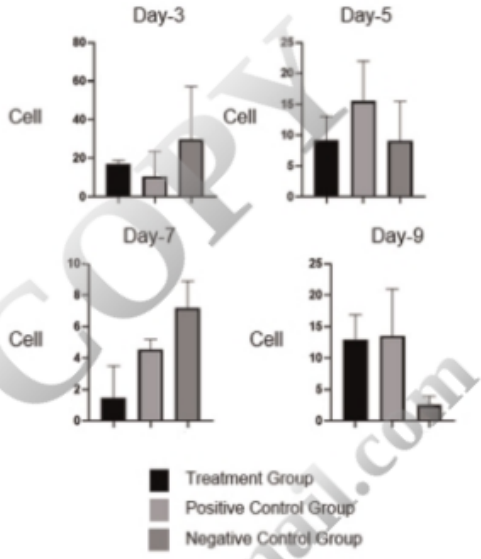

Figure 5. Fibroblast cells expressing MMP-9.

4 DISCUSSION

Wistar rats, weighing 200-250 g, were chosen as research subjects because their metabolism is similar to humans, and also easy to maintain (Ridwan 2013). Carboxymethylcellulose (CMC) is used as a vehicle for EGCG material due to its easy application to ulceration and because it does not affect the function or characteristics of EGCG, and the concentration of EGCG used is 10ppm because it has a high potential in wound healing activity, based on research conducted by Kim et al. (2008).

The treatment group showed faster healing compared to the negative control group, and areas of erythema were seen on day 5, the mucosa was red and whitish on day 7, and the mucosa had healed from ulceration on day 9. These results were also found in a study by Nugrahini (2018), where it was found that EGCG accelerates the reepithelialization of wounds. This increased reepithelialization could be because EGCG can improve wound healing by increasing transforming growth factor-beta 1 (TGF-β1), which causes increased migration of epithelial cells. Migration and proliferation of these epithelial cells will stop when the wound is completely closed. Epithelial cell proliferation and differentiation are influenced by several cytokines, including epidermal growth factor (EGF), keratinocyte growth factor, and TGF-β1, which is an important growth factor in reepithelialization. Beta 1 (TGF-β1) is the main factor for stimulating fibroblast proliferation, collagen production, wound contraction, and expression of connective tissue growth factor (CTGF) (Nugrahini 2018).

In the immunohistochemical picture, there is VEGF expression in endothelial cells. This result was also found in a study conducted by Inayati et al. (2020) that positive expression of VEGF was found in the alveolar bone tissue of Wistar rats given EGCG. This finding is in accordance with the results obtained that there is VEGF expression in EGCG-treated mucosal ulceration. However, the results of statistical calculations did not get similar results in this study, the results were not significantly different. Similar results were obtained in the study conducted by Gu *et al.*, who used EGCG orally on tumors in mice, where EGCG was found to have no effect on angiogenesis and VEGF expression in cardiac and skeletal muscle tissue but can inhibit VEGF expression in tumors.

In immunohistochemistry, there is MMP-9 expression in fibroblasts, similar results were found in the expression of MMP-9 in simulated wounds treated with EGCG (Klass et al. 2010). MMP-9 serves the breakdown of the extracellular matrix during the inflammatory phase and degrades tissue surrounding new blood vessels, and plays a role in the contraction and remodeling of tissues during the remodeling phase (Caley et al. 2015). In the study by Chen et al. (2016), it was found that the expression of MMP-9 was inhibited by EGCG *in vitro* in kidney cancer cells, and the study by Romi *et al.* found MMP-9 expression was inhibited by EGCG in several cancer cells such as fibrosarcoma, liver cancer, and glioblastoma. A study by Ciavarella et al. (2022) using EGCG as material for grafts for blood vessels performed on a scratch model found that MMP-9 was inhibited *in vitro*, this finding is inconsistent with studies conducted *in vivo*, which were not statistically significant. significant difference occurred.

This may be due to the low stability and bioavailability of EGCG. In a study conducted by Dube et al. (2010), it was found that EGCG is susceptible to oxidative decomposition due to several factors, such as temperature, slightly alkaline oral conditions, and catechins that are difficult to absorb due to EGCG's enterance to the cell through passive diffusion. By altering the structure of EGCG or utilizing nanomaterials as a carrier, the bioavailability of EGCG can be increased, which may boost its efficacy (Dai et al. 2020). This was also found by Yin et al. (2022) who found EGCG to have low stability *in vitro* and low bioavailability *in vivo*, so now there are many studies that encapsulate EGCG with various types of encapsulations, such as with nano-emulsion, protein-based particles, polymer-based particles, and also nanoparticles. Therefore, in the future, EGCG can be more effective *in vitro* and *in vivo* if used as medicine.

5 CONCLUSION

Based on the results obtained, it can be concluded that EGCG has an effect on the expression of VEGF and MMP-9 on oral mucosal ulceration in Wistar rats, although no statistically significant difference was found when compared with the positive and negative control groups.

COMPETING INTEREST

The authors declare that they have no competing interests.

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