Analysis of thickness of the epithelial in the cure of oral mucositis due to chemotherapy using 25% pegagan leaf (*centella asiatica*) extract gel spray

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ABSTRACT

Background: *Cancer treatment with radiotherapy or chemotherapy can cause oral mucositis. Pegagan leaf extract is an herbal alternative with minimal side effects and doesn't interfere with treatment procedures from chemotherapy cause contains triterpenoid acid, asiaticoside acid, madecoside acid, and saponins that stimulate collagen synthesis which affects epithelialization. This study aims to the difference in epithelial thickness between the administration of 25% pegagan leaf extract (Centella asiatica) spray gel and Gengigel® Hyaluronic acid spray to the healing process of oral mucositis in chemotherapy-induced rats.*

Method: *The design of this study was an experimental laboratory using a post-test only control group design, consisting of 2 groups, namely a positive control group using Gengigel® Hyaluronic acid spray, a group of 25% pegagan leaf extract (Centella asiatica) gel spray. The statistical analysis of the study used the Independent T-test.*

Result: *The results of the spray gel thickness of pegagan leaf extract (Centella asiatica) were 25% greater than Gengigel® Hyaluronic acid spray. The results of the Independent T-test were significant, with a value of p = 0.000 (P <0.05).*

Conclusion: *It can be concluded that there was differences in the increase of epithelial thickness in the application of 25% pegagan leaf extract (Centella asiatica) gel spray with Gengigel® Hyaluronic acid spray on the healing of oral mucositis due to chemotherapy.*

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INTRODUCTION

Cancer is a major health problem for people around the world. Current data from the 2018 Basic Health Research from the Health Research and Development Agency (Balitbangkes) of the Indonesian Ministry of Health stated that the prevalence of cancer in Indonesia reached 1.79% of sufferers or 1,017,290 residents[13]. Treatments for cancer that are often used are surgery, chemotherapy, and radiotherapy, treatment is often successful if the cancer is found early. Although it can kill cancer cells, chemotherapy drugs can also damage some normal cells and cause side effects. As many as 40% of head and neck cancer patients receiving chemotherapy treatment experience mucositis[11].

Oral mucositis can occur due to the toxic effects of chemotherapeutic agents on the oral mucosa and the severity increases on the 7th-10th day or the first week after treatment[7]. Oral mucositis can be characterized by the presence of erythema associated with clinical ulceration of the oral cavity. Ulceration that occurs causes pain and discomfort to the patient, this can affect the quality of the patient's diet and quality of life[10].

Judging from the high side effects of chemotherapy treatment, alternative treatments are needed which vary with minimal side effects and do not interfere with the treatment procedure of chemotherapy. One of the treatments is utilizing herbal ingredients that are potentially effective in wound healing.

Centella Asiatica leaf extract is often used as a traditional medicine because it has antioxidant, antibacterial, anti-fungal, anti-inflammatory, and anti-ulcer agents and can heal wounds[1,4]. Another study also explained that *Centella Asiatica* leaf extract applied to wounds in the form of a spray gel accelerated the epithelialization and keratinization effects of the infected area. The *asiticoside* content in *Centella Asiatica* leaves helps increase collagen production and angiogenesis in wound healing activity increases capillary permeability and has been shown to inhibit the inflammatory process as a trigger for hypertrophy[8]. *Centella Asiatica* extract contains triterpenoid acid, *asiaticoside* acid, madecosid acid, and saponins which stimulate collagen synthesis which affects epithelialization due to fibroblast keratinization factors which are young cells that make up connective tissue[5,15].

Based on this explanation, the authors wanted to carry out research to determine the effect of giving Centella Asiatica leaf extract spray gel 25% on increasing epithelial thickness in the healing process of oral mucositis induced by chemotherapy agents, namely 5-fluorouracil (5FU).

RESEARCH METHODS

The following research is an experimental laboratory study using a post-test-only control group design. A sample of 32 rats was obtained, and calculated using the Federer formula. Then, the samples were divided into 2 groups, namely 25% *Centella Asiatica* extract gel spray, and *Gengigel® Hyaluronic acid* spray.

The tools and materials used in the following research were divided into several parts: tools and materials for the manufacture of *Centella Asiatica* leaf gel spray, tools and materials for the 5-Fluorouracil treatment, tools, and sampling materials, and tools and materials for the manufacture and observation of preparations.

500 mg of *Centella Asiatica* herb was macerated using 2 liters of 70% ethanol while stirring occasionally for 3 days, followed by evaporation until a thick extract was obtained after being dissolved in a *water bath*. HMPC gelling agent was expressed into 30 ml of water at 80-90 C until it swelled and then stirred to form a gel.

Male Wistar rats were induced by 5-Fluorouracil on days 1-13 every 48 hours as much as 50 mg/kg each time intravenously until oral mucositis appeared which was characterized by erythema in the oral cavity. In the treatment group, rats were given food and drink in the morning and evening and were given a 25% *Centella Asiatica* extract gel spray sprayed on the area of oral mucositis twice every day of the study. In the positive control group, the rats were given food and drink in the morning and evening and Gengigel® *Hyaluronic acid* was sprayed on the oral mucositis area 2x a day until the 19th day.

Animals from each group were sacrificed using 5% ether inhalation on the 19th day. Furthermore, the inferior labial mucosa of rats with oral mucositis was removed with surgical scissors and a scalpel and placed in 10% neutral buffered formalin solution and labeled containing the type of organ, tissue, date of collection, animal species, and preservative. The sample was made of paraffin blocks and then cut into slices 5 µm thick on the microtome. Inferior labial mucosa samples were stained with *hematoxylin-eosin* (HE). All parts are mounted on glass slides.

Observation of histological preparations was carried out using a light microscope and support with image raster software with 400x magnification. The level of epithelial thickness will be checked by measuring the thickness of the epithelium from the basal layer (*stratum basale*) to the corneum layer (*stratum corneum*) in 3 selected fields of view and the results are averaged.

RESEARCH RESULT

This research has received approval from the Research Ethics Committee with Ethical Eligibility Number **253/B.1-KEPK/SA-FKG/XII/2020.**

The results of the study regarding the thickness of the epithelium in healing oral mucositis due to chemotherapy by administering 25% Centella Asiatica extract gel spray and Gengigel® Hyaluronic acid spray showed average values as in the following table:

Table 4.1 Average Results of Epithelial Thickness in each Treatment Group (µm)

No	Group	Average	± Standard Deviation
1	*Centella Asiatica* extract gel spray 25%	91.76377	4.06895
2	Gengigel® *Hyaluronic acid* spray	47.1375	4.98293

Based on **table 4.1** shows that the average thickness of the epithelium in the Centella Asiatica extract gel spray group was 25% thicker, namely 91.76 µm, compared to the average Gengigel® Hyaluronic acid spray control group, which was 47.13 µm.

	Group	*Shapiro-Wilk*	*Levene Test*	*Independent T-test*
Epithelial thickness	*Centella Asiatica* extract gel spray 25%	.252*	.800*	.000*
	Gengigel® *Hyaluronic acid* spray	.481*		.000*

Table 4.2 Normality Test Results and Homogeneity Test

Note: *Significant

Based on **table 4.2**, the *Shapiro-Wilk* was used in all groups to obtain a p-value > 0.05 and the results of the *Levene test* data for all groups > 0.05 showed that the data were normally distributed and homogeneous so that the *Independent T-test* requirements were fulfilled.

The *Independent T-test* for the two treatment groups has a significant value or p 0.000. Due to p <0.05, it was concluded that there was a significant

difference in epithelial thickness between the treatment group (spray *centella asiatica* extract gel 25%) and the control group (spray Gengigel® *Hyaluronic acid*).

DISCUSSION

Based on the study of epithelial thickness, it was found that the thickness of the epithelium in the gel spray group *Centella Asiatica* leaf extract was 25% thicker than the Gengigel® *Hyaluronic acid* spray group in healing oral mucositis in Wistar rats. These results are related to Choohuay's research which states that treatment using *Centella Asiatica* leaf extract spray accelerates wound healing. It can be seen that the average epithelial thickness in the treatment group (*Centella Asiatica* extract gel spray 25%) was higher, namely 91.76 (μm) compared to the average epithelial thickness in the control group (Gengigel® *Hyaluronic acid* spray), which was 47.13 (μm). This is in line with Tajul's research which stated that there was an increase in epithelial diameter by administering asiaticoside to diabetes-induced rats, the content stimulated several growth factors such as PDGF, VEGF, and KGF[16].

Tables 4.1 and 4.2 show that *asiaticoside* is effective in increasing the thickness of the epithelium. *Asiaticoside* helps the proliferation of fibroblasts and extracellular matrix, asiaticoside also facilitates the stage of wound healing by increasing the components of tensile strength, hydroxyproline, angiogenesis, collagen synthesis, and epithelialization[14]. *Asiaticoside* also stimulates macrophages to *activate growth factors such as keratinocyte growth factor* (KGF) which will increase epithelial proliferation[3]. In previous studies, *asiaticoside* which is a saponin supports the re-epithelialization process by modifying the expression of TGF-β receptors so that it can stimulate fibronectin synthesis by fibroblasts[16]. Fibronectin is a glycoprotein that plays an important role as a mediator of extracellular matrix components that will support keratinocyte adhesion and guide epithelial cells to cross the wound area. The maximal proliferation of keratinocytes occurs 48-72 hours after injury. According to Singer and Clark, two days after injury, keratinocytes began to actively proliferate which was stimulated by the release of *epidermal growth factor, transforming growth factor-α, and keratinocyte growth factor*. When a new basement membrane is formed, the keratinocyte phenotype will return to the stationary phenotype and begin to actively proliferate[14]. Keratinocytes migrate due to the "*free edge effect*", namely emptying the neighboring cells of the epithelial cells of the basal layer at the edges of the wound. Keratinocytes from the basal layer of the epithelium at the edge of the wound tissue dissolve the hemidesmosome bonds and detach from the basement membrane and then move rapidly across the wound until they reach the wound edge, then there is an increase in cell division and causes differentiated cells to form epithelial tissue back to normal. The lining of the oral mucosa is the non-keratinized stratified squamous epithelium. Oral mucosal epithelium under normal conditions has only a thin layer of keratin. Thickening of the nonkeratinized epithelium in the labial mucosa is thought to be a physiological response to irritants. Epithelial tissue turnover occurs on the surface of epithelial cells which are continuously undergoing cell death. Epithelium has high resistance to foreign objects or irritants that attack it[17].

The thickness of the epithelium in Gengigel® *Hyaluronic acid* spray has been formed but it is thinner compared to *Centella Asiatica* leaves, as mentioned in Puspita and Suharsini's research it appears that Gengigel® *Hyaluronic acid* spray can relieve inflammation and accelerate the wound healing process[12]. In previous studies, it was stated that *hyaluronic acid* has antibacterial and anti-

inflammatory effects, namely reducing inflammation associated with inflammatory lesions and oral ulcerative lesions. *Hyaluronic acid* binds to proteins scattered on the cell surface, extracellular matrix, cytoplasm, and nucleus which then form receptors on the cell surface, namely the transmembrane glycoprotein CD44, then CD44 interacts with hyaluronic acid causing proliferation of keratinocytes in reepithelialization[9]. This insignificant increase is because *Hyaluronic acid* only can control tissue hydration during the inflammatory process. Hyaluronic acid works by weakening the bonds of several tissue cells that are experiencing chronic inflammation so that they are easily released and replaced by new cell regeneration[12].

Based on this discussion, *Centella Asiatica* extracts gel spray 25% can be recommended as a natural ingredient that can be used in healing oral mucositis due to chemotherapy.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the administration of spray gel extract of *Centella Asiatica* leaves 25% can increase the thickness of the epithelium thicker than Gengigel spray in healing oral mucositis.

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