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## THE EFFECT OF SEMENDO COFFEE ON THE NUMBER OF FIBROBLAST CELLS IN GINGIVA WOUND OF RATS

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### KEYWORDS

semendo coffee, gingiva,  
fibroblast, Wistar rat

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### ABSTRACT

**Introduction:** Gingiva is a keratinized epithelium tissue that surrounds the tooth and protects the tissue beneath it, which is often wounded. Sumatran robusta coffee, semendo coffee, consists of active compounds such as polyphenol, alkaloid, and saponin that can increase fibroblast number during the wound healing process. This study aims to determine the effect of semendo coffee (*Coffea canephora*) extract on fibroblast number on gingiva wound of *Rattus norvegicus*. **Methods:** The true experimental study with a post-test-only control group design was confirmed in this study. This study used twenty-four rats (*Rattus norvegicus*) divided into 4 groups. The gingival injury was performed on mandible gingiva using a punch biopsy method with a 2mm diameter. The gel was given according to the treatment group 2 times a day for 7 days. Semendo coffee extract at 5%, 20%, and 40%, was applied to the wound treatment groups, while placebo gel was applied to the control group. Euthanasia was delivered on the 8th day, then histological preparation was made. The number of fibroblasts was analyzed by Olympus software. **Result:** The results showed that the Semendo coffee extract could significantly improve the number of fibroblast cells compared to the control groups. The highest fibroblast number was found in the group with Semendo coffee extract at a concentration of 40%. **Conclusion:** Semendo coffee extract at 5%, 20%, and 40% increased the number of fibroblast on the gingival wound in *Rattus norvegicus*.

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### INTRODUCTION

Gingiva is one part of the mouth that is layered by keratinized epithelium tissue.<sup>1</sup> Gingiva is located at the outermost and has the function to protect the underlying tissue.<sup>2</sup> Gingiva is one part of the oral mucosa that tends to get wounded.<sup>3</sup> A wound is a discontinuity of various anatomical structures of the body that causes disruption or damage to the physiology and

anatomical structure of the body.<sup>4,5</sup> Wound is caused by physical contact, a result of medical treatment, and physiological change.<sup>4</sup> The body will react when there is a wound, which is the wound healing process.<sup>6</sup> The wound healing process consists of three phases which are the inflammation phase, proliferation phase, and maturation (remodelling) phase.<sup>7</sup> In order to stop the bleeding, the blood clotting process will

begin to start when a wound appears.<sup>8</sup> The inflammation phase aims to kill bacteria that contaminate the wound. The inflammation phase is characterized by cardinal symptoms which are rubor, calor, dolor, and functio laesa. In this phase, neutrophils will get out and the monocyte will migrate to the wound area and then differentiate to be macrophages.<sup>9</sup>

The next phase is the proliferation phase which plays a role in new tissue forming. The cell that plays an important role in this phase is the fibroblast cell. Macrophages will synthesize growth factors that will stimulate fibroblasts to proliferate, migrate, and form an extracellular matrix. Fibroblast produces extracellular matrix to fill the cavity wound and keratinocyte migration to re-epithelization.<sup>10</sup> Maturation phase aims to maximize the integrity and strength of the new tissue.<sup>5</sup>

A fibroblast cell is a cell that has an important role in the proliferation phase. Fibroblast originated from undifferentiated mesenchymal cells. Fibroblast will produce collagen fiber that will close the wound and accelerate the wound healing process. Fibroblast forms a new connective tissue that gives strength and integrity to the wound.<sup>11,12</sup>

The herbal plant is safer to use compared to drugs that contain the chemical substance.<sup>11</sup> One of the herbal plants that can be used to heal a wound is coffee.<sup>13</sup> Three types of coffee are widely known in Indonesia, including Arabica, Robusta, and Liberica.

Robusta coffee is one kind of dominant coffee plantation in Indonesia.<sup>14</sup> In 2011, Indonesia became the third main coffee producer after Vietnam and Brazil with a coffee plantation area of 1.292.965 ha and production results of around 633.991 ton.<sup>15</sup>

Robusta coffee (*Coffea robusta*) contains active compounds, which are polyphenol, caffeic acid, chlorogenic acid, alkaloid, and saponin which function as anti-inflammatory, antibacterial, and antioxidant.<sup>16</sup> A study by Kenisa et al stated that robusta coffee (*Coffea canephora*) with a concentration of 22,5% and 45% can increase the number of fibroblast in the wound healing process.<sup>17</sup>

Semendo robusta coffee is a type of robusta coffee that originated in the South Sumatra province. South Sumatra produces an average of 137.780 tons of coffee per year.<sup>18</sup> This is a type of coffee with an affordable price, easy to find, and able to use as a local product from South Sumatra as an alternative material for wound healing on the gingiva. This present study aims to determine the effect of semendo coffee (*Coffea canephora*) on the number of fibroblast (in vivo study) in gingiva wounds on Wistar (*Rattus norvegicus*) rats.

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## **METHODS**

This study constitutes an experimental laboratory study (truly experimental) using a post-test-only control group design and has received approval from the Medical and Health Research Ethics Committee Faculty

of Medicine, Sriwijaya University with ethical certificate number 054-2021.

### **Research Subject**

This study used 24 male rats (*Rattus norvegicus*) aged 2-2.5 months old and weighing 250-300 grams, which were divided randomly into treatment groups (Semendo coffee extract concentration at 5%, 20%, and 40%) and control group (placebo).

### **The Making of Gel of Semendo Coffee Extract**

This study used Semendo coffee from Desa Segamit, Muara Enim, South Sumatera. The extract of semendo coffee was made by the maceration method.

The extract of Semendo next process in the making of gel base that began with 3-gram Na-CMC that developed in 0,3 L hot water with a temperature of 70°C in mortar for 15 minutes until it gets expanded. Then, stir it until a clear transparent gel is made. Then, add 5 grams of glycerin into the gel then stir until it gets homogeneous and a gel mass is formed. Robusta coffee extract is mixed with glycol protein and stirred evenly, then mix it into the gel base and stir until it gets homogeneous. The obtained robusta coffee extract with a concentration of 5%, 20%, and 40% was used in this study.<sup>16</sup>

### **Treatment on Rats**

The gingival injury was performed on mandible gingival rats using a punch biopsy method with a 2mm diameter. Before the gingiva wound was made, the anesthesia process on Wistar rats was done to give a sedation effect. All rats then were wounded using punch biopsy with 2mm diameter on mandible gingiva beneath central incisive mandible.<sup>3</sup>

In the treatment groups, Semendo coffee extract gel was smeared and in the control group, a placebo gel was smeared. Gel applications were done using a cotton pellet for 30 seconds twice a day in the morning and evening for 7 days.

### **Euthanasia**

Euthanasia was delivered on the 8th day then the tissue around the wound was cut using a scalpel and put into a fixated solution (10% formalin buffer).

### **The Making of Histological Preparation**

Tissue processing was started from the dehydration phase gradually into alcohol with a concentration of 70% until 100% for 15 minutes respectively. Then, a purification with xylol was done 2 times for 30 minutes respectively, hence the tissue became clear and transparent.

The next phase was immersing tissue into liquid paraffin 1 with a melting point of 56°C for 2½ hours, then putting it into liquid paraffin 2 for 4 hours. Then, the tissue was

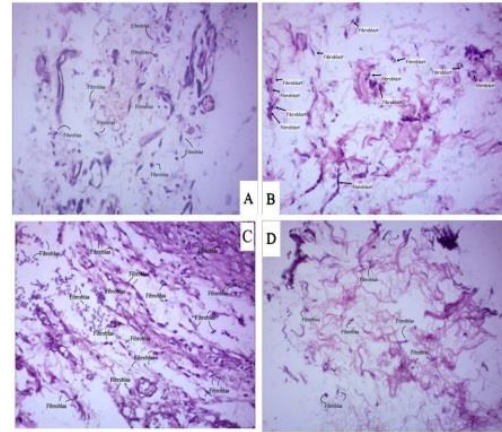
put into a paraffin box. Furthermore, the tissue was cut using a rotary microtome with a temperature of 5-10°C. The block then was moved into a water bath with a concentration of 37-40°C and let rest some time until the paraffin box got expanded. The paraffin box was put above the hotplate, then let rest for some hours. Preparat coloring was done to see the number of fibroblasts using Hematoxylin Eosin (HE).

**Tissue Observation**

The tissue was observed under a microscope with a magnification of 400x in 3 fields of view. The observed structure was fibroblast. Photo of the tissue was taken from an observation using a Micro-ocular MD 130 electron eyepiece that was connected with Olympus software. That photo was then analyzed to determine the number of fibroblast.

**RESULTS**

The observation in this study was seen microscopically on connective tissue using a microscope. The fibroblast cell was found the most on connective tissue, extracellular matrix, such as collagen, elastin, and reticular that have a function to form a tissue.<sup>8</sup> The tissue was measured on 3 fields of view with a magnification of 400x. Based on the content of Table 1, the highest mean of fibroblast was found in Semendo coffee extract at 40%.



**Figure 1.** Microscopic photo of the number of fibroblast on gingiva wound healing (400x): (1) Gel of semendo coffee extract 5%, (b) Gel of semendo coffee extract 20%, (c) Gel of semendo coffee extract 40%, (d) Placebo gel.

**Table 1.** The average value of the number of fibroblasts

Treatment Group	Average value ± SD The number of fibroblast
Gel of Semendo Coffee Extract 5%	8,50 ± 3,46
Gel of Semendo Coffee Extract 20%	9.38 ± 2,69
Gel of Semendo Coffee Extract 40%	21,77 ± 4,80
Placebo gel	7,94 ± 2,98

**Table 2.** The result of the one-way ANOVA test

	F	Sig.
Between groups	20,442	0,000*

\* Showed a significant difference (p<0,05)

One-way ANOVA statistical analysis showed a significance value of p<0.05. it can be stated that there was a significant difference in the number of fibroblast between the gel of Semendo coffee extract treatment groups towards the control group.

The results of post hoc analysis showed that there was a significant difference (p<0.05) between Semendo coffee at 40% towards Semendo coffee extract at concentrations of 5%, 20%, and control group.

**Table 3.** Post Hoc test between each group

	Gel of Semendo Coffee Extract 5%	Gel of Semendo Coffee Extract 20%	Gel of Semendo Coffee Extract 40%	Placebo Gel
Gel of Semendo Coffee Extract 5%		0,973	0,000*	0,993
Gel of Semendo Coffee Extract 20%	0,973		0,000*	0,897
Gel of Semendo Coffee Extract 40%	0,000*	0,000*		0,000*
Placebo Gel	0,993	0,897	0,000*	

\* Showed a significant difference (p<0,05)

## DISCUSSION

Semendo coffee has active compounds such as polyphenol, cafeic acid, chlorogenic acid, alkaloid, and saponin that works as anti-inflammatory, antibacterial, and anti-oxidant.<sup>20</sup> A study by Fadlil stated that chlorogenic acid and caffeine in robusta coffee contains antioxidant that works to prevent free radical activity that occurred during the inflammation phase. Chlorogenic acid also works as an anti-inflammatory that can reduce capillary permeability increase during the inflammation phase and also reduce edema. Saponin works to synthesize fibroblast growth factors.<sup>16</sup>

This study showed that Semendo coffee extract at concentrations of 5%, 20%, and 40% increased the number of fibroblasts in gingiva wounds on *Rattus norvegicus* in the

control group. Fibroblasts are the main cells that play a role in the wound healing process. Wound healing is enhanced by the presence of Reactive Oxygen Species (ROS), when the production of ROS too much, it can cause DNA and cellular damage. Caffeic acid and chlorogenic acid which is contained in Semendo coffee extract act as an antioxidant to neutralize ROS and protect tissues from oxidative damage. A study by Kenisa *et al* reported that robusta coffee (*Coffea canephora*) with a concentration of 22,5% and 45% could increase the number of fibroblasts during the wound healing process.<sup>17</sup> A study by Dewanti also stated that robusta coffee could increase the number of fibroblasts on wound healing of Wistar rats pulp for 7 days.<sup>21</sup> This study showed that Semendo coffee extract at a concentration of 40% has the highest number of fibroblasts. It can be assumed that the higher the concentration extract of Semendo coffee, the more active substances contain it so that more ROS can be neutralized. Besides that, caffeic acid plays a role in the healing process by stimulating the synthesis of the collagen-like polymer by fibroblast.<sup>17</sup>

The wound healing process aims to restore the function and shape of the tissue to its normal form with minimal complications.<sup>22</sup> Fibroblasts are actively moving from the network into the wound area, proliferate and issue some substances such as collagen, elastin, hyaluronic acid, fibronectin, and



proteoglycan, that play role in forming new tissue.<sup>22</sup>

Overall, the treatment group has a higher number of fibroblasts compared to the control group because of the active substance contains it, whereas control groups with placebo gel only contains Na-CMC, glycerin, and propylene glycol which are gel base materials and didn't have active substance to increase of the number of fibroblasts.

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## CONCLUSION

It can be concluded that there was an increase in the number of fibroblast cells in the gingival wound healing process induced by Semendo coffee extract.

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## ACKNOWLEDGMENT

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