

Research Report

Effect of Robusta coffee beans ointment on full thickness wound healing

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ABSTRACT

Background: Traumatic lesions, whether chemical, physical, or thermal in nature, are among the most common lesion in the mouth. Wound healing is essential for the maintenance of normal structure, function, and survival of organisms. Experiments of Robusta coffee powder on rat-induced alloxan incision wound, clinically demonstrated similar healing rate with the povidone iodine 10%. No studies that look directly the effect of coffee extract in ointment form when viewed in terms of histopathology. Robusta coffee bean (*Coffea canephora*) consists of chlorogenic acid (CGA) and caffeic acid which are believed to act as antioxidant and take part in wound healing process. **Purpose:** The aim of this study was to identify the enhancement of healing process of full-thickness skin wound after Robusta coffee beans extract ointment application. **Methods:** Sample consisted of 20 *Cavia cabaya* treated with full-thickness with wounds and was given Robusta coffee beans extract ointment concentration range of 22.5%, 45%, and 90% except the control group which was given ointment base material. Animals were then harvested on the fourth day and made for histopathological preparations. Data were calculated and compared by one-way ANOVA test and LSD test. **Results:** The study showed that Robusta coffee bean extract ointment can increase the number of lymphocytes, plasma cells, macrophages, fibroblasts, and blood vessels by the presence of chlorogenic acid (CGA) and Caffeic acid. **Conclusion:** In conclusion Robusta coffee bean extract ointment enhance the healing process of full-thickness skin wound of *Cavia cabaya*.

Key words: Robusta coffee bean extract, the healing process, chlorogenic acid, caffeic acid

ABSTRAK

Latar belakang: Lesi traumatik, baik akibat rangsang kimia, fisik, atau termal, merupakan lesi yang paling umum terjadi di dalam rongga mulut. Penyembuhan luka yang terjadi ini penting untuk pemeliharaan struktur normal, fungsi, dan kelangsungan hidup organisme. Percobaan pemberian bubuk kopi Robusta terhadap luka sayatan pada tikus yang diinduksi aloksan, secara klinis menunjukkan tingkat penyembuhan yang sama dengan povidone iodine 10%. Namun belum ada penelitian yang melihat secara langsung pengaruh ekstrak kopi dalam bentuk salep bila dilihat dari segi histopatologi. Biji kopi Robusta (*Coffea canephora*) terdiri dari chlorogenic acid (CGA) dan caffeic acid yang dipercaya berperan sebagai antioksidan dan mengambil bagian dalam proses penyembuhan luka. **Tujuan:** Tujuan penelitian ini adalah untuk mengidentifikasi peningkatan proses penyembuhan luka full-thickness pada kulit setelah pengaplikasian salep ekstrak biji kopi Robusta. **Metode:** Sampel terdiri dari 20 *Cavia cabaya* yang diberi perlakuan berupa luka sayat full-thickness pada kulit punggung dan diberi salep ekstrak biji kopi Robusta dengan beberapa konsentrasi, yaitu 22,5%, 45%, dan 90%, sedangkan kelompok kontrol hanya diberi bahan dasar salep. Binatang coba kemudian dieksekusi pada hari keempat dan dibuat sediaan histopatologinya. Data dihitung dan dibandingkan dengan uji One-Way ANOVA dan uji LSD. **Hasil:** Hasil penelitian menunjukkan bahwa salep ekstrak biji kopi Robusta dapat meningkatkan jumlah limfosit, sel plasma, makrofag, fibroblas, dan pembuluh darah yang dipengaruhi oleh chlorogenic acid (CGA) dan caffeic acid. **Kesimpulan:** Disimpulkan bahwa salep ekstrak biji kopi Robusta memiliki efek dapat meningkatkan proses penyembuhan luka full-thickness pada kulit *Cavia cabaya*.

Kata kunci: Salep ekstrak biji kopi Robusta, proses penyembuhan, chlorogenic acid, caffeic acid

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INTRODUCTION

Wound can be defined as a disability or injury of living tissue caused by physical or thermal disturbance arising both pathologically and physiologically.¹ Traumatic lesions, whether chemical, physical, or thermal in nature, are among the most common in the mouth. This lesion to oral-sot tissue can occur due to accidental, iatrogenic, and factitious traumas. They may present as burns, ulcerations, and gingival recession.² Bastone *et al.*,³ also described the aetiology of dental trauma from national and international studies as well as the different classifications currently used to report dental injuries. An English study determined the incidence of trauma to permanent incisors and related soft tissues as four cases/100 children/15 months, which was almost twice the incidence of Australian study. Based on those literatures, wound healing is essential for the maintenance of normal structure, function, and survival of organisms.⁴

Wound healing is a complicated pathophysiological process. Although mucosal wounds demonstrate accelerated healing compared to cutaneous wounds, both cutaneous and mucosal wound healing proceed through the same stages.⁵ Wound healing consists of several stages, namely stage of acute inflammation, cell proliferation, and maturation. At the stage of proliferation, cell proliferative activity of fibroblasts in the lesion has a central role to begin the wound healing process. Increasing number of fibroblasts in the dermal showed the healing ability.⁶ Wound healing process may be hampered by the presence of reactive oxygen stress (ROS) produced by microbes or neutrophils in the wound area, through mechanisms that lead to DNA damage. This fact strengthens the opinion that the existence of local antioxidants in wound area became crucial factors that have promoted the acceleration of the healing process.⁷⁻⁹

Several studies conducting the process of wound healing using natural materials have been widely applied. The use of natural materials done because it is easy to use, inexpensive, and has an adequate bactericidal or bacteriostatic effect.¹⁰ In addition, natural materials rarely cause adverse side effects compared with synthetic materials.¹¹

One of these natural materials is Robusta coffee beans. Robusta coffee is widely spread on the island of Java, Sumatra, and Sulawesi. Price of this coffee is cheaper than other types of coffee and more resistant to diseases that attack the coffee plants. Robusta coffee contains various compounds including 42.3% sugars (polysaccharides), 7.5% protein, 11% lipid, 2.4% caffeine, and 6.4% acids.¹² Also reported that the application powder of raw Robusta coffee on rat-induced alloxan incision wound, showed clinical cure rate similar to the application of povidone iodine 10%.¹³

The polyphenols of coffee, caffeic acid and chlorogenic acid (CGA), is believed to promote wound healing. Robusta coffee beans have higher number of these polyphenols than arabica coffee beans.¹⁴ Chlorogenic acid and caffeic acid have antioxidant properties that are significantly more potent than vitamin C and E.¹⁵ In addition to having antioxidant potential, Robusta coffee has also been investigated to have antibacterial ability against methicillin-resistant staphylococcus aureus that can cause opportunistic infections on the injured area. Phenolic compounds in coffee also have been studied to reduce the effects of histamine, bradykinin, and leukotrienes as well as to reduce the activity of the complement system.¹⁶

Research on the potential ointment of Robusta coffee bean extract in dosage form in wound healing has not been reported. The extraction is done so that the active substances are needed can be taken optimally. The purpose of this study was to determine the potential of Robusta coffee bean extract ointment on the healing process of full-thickness wounds on the skin of male guinea pigs (*Cavia cabaya*) which was evaluated histopathologically.

MATERIALS AND METHODS

This research is an experimental research laboratory. The material used is the ointment of Robusta coffee bean extract with a range of concentration of 22.5%, 45%, and 90%. Robusta coffee beans that have been roasted and used as a powder, then extracted using ethanol solvent. The extract was mixed with an ointment base material (PEG 400 and PEG 4000) and is based on the required concentration. This research used 20 male guinea pigs (*Cavia cabaya*), aged 2–3 months, and weighing 200–300 mg. Research subjects were divided into 4 groups each consisted of 5 guinea pigs. Incision wound of 2.5 cm long with a depth of 2 mm was created on the back skin of each guinea pigs using number 11 scalpel under the effect of 10% ether anesthesia by inhalation. Each treatment group was given ointment of Robusta coffee bean extract and a control group given only simple ointment base using a syringe at a quantity of 2 cc. Then treated in a closed wound with sterile gauze and plaster bandages. All guinea pigs in each group were harvested on the fourth day using 10% ether as sedation. Back skin biopsies and subsequent histopathological preparations was done using haematoxylin eosin (HE) staining. Then calculation of chronic inflammatory cells (macrophages, lymphocytes, plasma cells), capillary blood vessels, and fibroblasts were done.

The data obtained from histopathological examination is quantitative data obtained by calculating the number of cells and capillary blood vessels under light microscopy

Table 1. Distribution of mean and standard deviation of lymphocytes and plasma cells, macrophages, fibroblasts, and capillary blood vessels on the fourth day after treatment

Groups	$\bar{X} \pm SD$				
	Lymphocytes	Plasma cells	Macrophages	Fibroblasts	Capillary blood vessels
Control	13.60 ± 8.735*	2.60 ± 1.817	39.80 ± 21.394	148.20 ± 22.928*	206.00 ± 83.896
G1 (22.5%)	14.40 ± 3.435*	6.00 ± 4.899	19.20 ± 11,189	217.60 ± 57.051*	213.20 ± 68.766
G2 (45%)	25.60 ± 8.649*	8.80 ± 5.020	27.20 ± 8.927	271.00 ± 94.557*	219.80 ± 134.908
G3 (90%)	34.20 ± 19.136*	7.60 ± 2.702	27.80 ± 6.099	173.00 ± 22.226*	238.00 ± 63.075

Note: *: significant difference between groups

performed on five different fields of view with 1000× magnification. These research data were analyzed with statistical tests of One-Way ANOVA and LSD.¹⁷

RESULTS

The largest number of lymphocytes present in the sample group which were given ointment of 90% Robusta coffee beans extract, while the smallest number found in the control group. The largest amount of plasma cells present in the sample group which were given ointment of Robusta coffee beans extract concentration of 45%, while the smallest number found in the control group (Table 1).

The largest number of macrophages present in the control group, while the smallest number of groups present in concentrations of 22.5%. The largest number of fibroblasts present in the sample group which were given ointment of Robusta coffee bean extract concentration of 45%, while the smallest number found in the control group. The largest number of capillaries present in the sample group which were given ointment of Robusta coffee bean extract concentration of 90%, while the smallest number found in the control group (Table 1).

Obtaining data on the number of cells and capillary blood vessels in each group performed One-Way ANOVA test. Before the One-Way ANOVA test, this study shows that the data are normally distributed after the Kolmogorov-

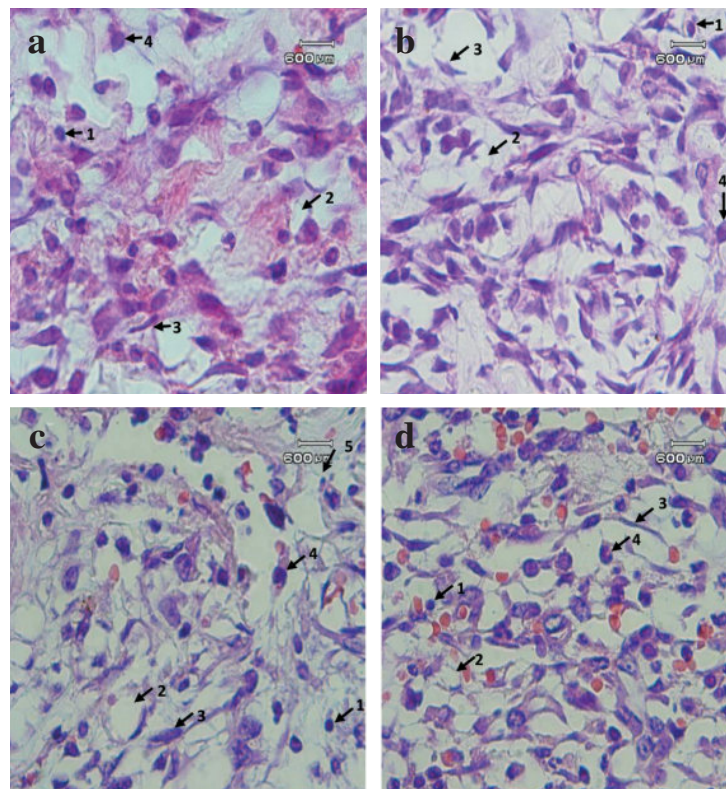


Figure 1. Histopathological image on the group: A) control group, B) group 1 (22.5%), c) group 2 (45%), group 3 (90%).

Note: 1) lymphocytes, 2) capillary blood vessels, 3) fibroblast, 4) macrophages, 5) plasma cells. (HE staining; magnification 1000×; Olympus BX-50 microscope. Pentax optio 230; Digital Camera 2.0 megapixels).

Smirnov test statistic and homogeneous after Levene test. One-Way ANOVA test showed significant values ($p < 0.05$) in lymphocytes and fibroblasts. The average value of lymphocytes and fibroblasts in the treatment group differed significantly, whereas the other dependent variables such as plasma cells, macrophages, and capillaries found no significant difference.

Table 2. LSD statistical test of significance figures on the number of lymphocytes and fibroblasts between groups

Concentrations of ointments	Compared concentrations	Sig. (Lymphocytes)	Sig. (Fibroblasts)
Control	22,5%	0.697	0.074
	45%	0.133	0.004*
	90%	0.012*	0.505
22,5%	Control	0.697	0.074
	45%	0.065	0.161
	90%	0.015*	0.238
45%	Control	0.133	0.004*
	22,5%	0.065	0.161
	90%	0.273	0.016*
90%	Control	0.012*	0.505
	22,5%	0.015*	0.238
	45%	0.273	0.016*

* the mean difference or significance value smaller than 0.05 ($p < 0.05$)

To determine the effect of differences in test conducted further Post Hoc Test LSD. Significant differences in this table are expressed with an asterisk ‘*’ on the mean difference or significance value smaller than 0.05 ($p < 0.05$). In lymphocytes, the data showed significant mean differences in comparisons between the control group with group 3 and group 1 with group 3. Whereas in fibroblasts, a significant mean differences found in comparisons between the control group with group 2 and between group 2 with group 3.

DISCUSSION

Regeneration process can be seen from the cells that play a role during the wound healing process such as polymorpho nuclear (PMN) cells, lymphocytes, macrophages, plasma cells, fibroblasts, and capillary blood vessels. Observation of the results of this study was done the fourth day after treatment for acute inflammatory cells such as PMN, especially neutrophils which will soon be replaced by macrophages on the third day and granulation tissue which enter the slit incision. Gap is filled with granulation tissue and maximum vascularization on the fifth day. The observation of the results on this study was conducted on the fourth day so that all cells needed can be seen.⁴

This study used a range of 22.5%, 45%, and 90% concentration and is a preliminary study using ointment of Robusta coffee bean extract. Extraction of coffee was done

so the active substances can be taken optimally. Ethanol was used as extraction solvent because it can withdraw the amount of phenolic acids higher than methanol and pure water.¹⁸ The ointment used are made of poly ethylene glycol (PEG) because PEG is chemically stable. Both PEG 400 and PEG 4000 used in this study are soluble in ethanol. PEG does not irritate skin and easy to clean by washing.¹⁹

Wound healing involves several mechanisms, such as inflammatory phase, proliferation, and maturation. In the inflammatory phase, the objectives are to stop the bleeding and clean the wound area of foreign bodies, dead cells, and bacteria to prepare for the start of healing process. PMN cells migrate into the interstitial area to perform phagocytosis of foreign bodies and bacteria. However, wound healing is enhanced by the presence of stress ROS produced by PMN or microbial infection. If ROS are produced too much, it can cause cellular and DNA damage. CGA and caffeic acid are contained in Robusta coffee beans extract act as antioxidants to neutralize ROS which is the free radicals produced in the process of wound healing. ROS can increase lipid peroxidation which is a major cause of damage to the cell membrane so that it can damage the cell structure and function.²⁰ Antioxidants have been reported to have a significant role in the process of wound healing and protect tissues from oxidative damage.²¹ Antioxidant mechanism is expected to protect cells that play a role in the process of wound healing. CGA and caffeic acid as antioxidants convert free radicals into stable products. The neutralized free radicals can not react on polyunsaturated fatty acids (PUFAs) which generate alcoxyl and peroxy radicals that responsible for the basic process of membrane cell lipid peroxidation.²² In the initial adhesion process, PMN adhere to the endothelium through the interaction of specific molecules such as selectin and glycosylated protein so that PMN able to exit the endothelial transmigration as it is called an acute inflammatory process.²³

This phase continues as chronic inflammatory cells into the injured area. Table 1 showed that the mean number of lymphocytes in group 3 is higher than the control group, group 1, and group 2. According to Hung *et al.*,²⁴ CGA was shown to increase lymphocytes proliferation.²⁴ It can be seen from the mean number of increased lymphocytes until the highest (90%). LSD test on lymphocytes showed that there were significant mean differences in comparisons between the control group with group 3 and group 1 with group 3 (Table 2). While results for the plasma cells in table 1 showed mean number of the highest plasma cells found in group 2 which did not differ significantly group 3. Active substances contained in coffee, namely CGA, has been mentioned to have a role in increasing proliferation of lymphocytes. This is probably an indirect effect of plasma cells as these cells is the end product of activation of B lymphocytes activation that have differentiated, then plasma cell produce direct antibody against antigens in inflammation.

Results of the next calculation is the amount of macrophage cells in control group which showed a higher

mean than group 1, group 2, and group 3. This is presumably due to the treatment group, the phase of chronic inflammation will soon ends characterized by the declining number of macrophages and the beginning phase of proliferation. Increasing the mean number of macrophages seen in the treated group. Group 3 has the highest mean followed by group 2 and group 1. This is because CGA stimulates the mobilization of macrophages, may indirectly increase the ability of macrophage phagocytosis because it affects the secretion of IFN γ that act as macrophage activators.^{22,24} T lymphocytes which are activated by interaction with macrophages that present antigen fragments on the surface of cells can produce IFN γ . These cytokines may activate macrophages so that macrophages release other cytokines to activate lymphocytes and causes inflammation where there is a focus of both these cells stimulate each other to destroy the antigen.

The next phase is the proliferative phase which involves the proliferation of fibroblasts, collagen synthesis, angiogenesis, granulation tissue formation, and epithelisation.²⁵ An important first step in this phase is the improvement of microcirculation to supply oxygen and nutrients needed to fill the metabolic needs of tissue repair. Regeneration of new blood vessels (angiogenesis) is stimulated by hypoxic injury condition as well as several growth factors, particularly VEGF-A, FGF-2, TNF- β . At the same time, fibroblasts migrate into the wound in response to cytokines and growth factors produced by inflammatory cells, among which are macrophage.²⁶ That activated macrophages can stimulate growth factors and cytokines (TGF- α , TGF- β , PDGF, VEGF -A, and IL-1) on the injured area. TGF- β plays a role in angiogenesis, reepithelisation, and connective tissue regeneration. TGF- β which are dominant in cutaneous wound healing is TGF- β 1. TGF- β works by activating its receptor on the cell surface and transducing signal on target genes. Binding of a TGF- β to its type II receptor in concert with a type I receptor leads to formation of a receptor complex and phosphorylation of type I receptor. Thus activated, the type I receptor subsequently phosphorylates a receptor-regulated SMAD (R-Smad), allowing this protein to associate with Smad4 (Co-Smad) and move into nucleus. In the nucleus, the SMAD complex associate with a DNA-binding partner (Fast-1) and this complex binds to a specific enhancers in target genes so that it can activate the gene transcription.^{27,28} In the injured tissues, extracellular matrix molecules (ECM), namely tenascin-C, expressed during the process of tissue repair. Tenascin-C plays a role in proliferation and migration of fibroblasts. This molecule can induce phosphorylation of epidermal growth factor receptor (EGFR) and stimulates activation of mitogenic activated protein (MAP) kinase and mitogenesis of fibroblasts. In addition, tenascin-C can induce migration of fibroblasts through the activation of PLC γ and m-calpain.²⁹ Those growth factors and molecules play a role in cell proliferation and migration of fibroblasts so that the process of wound healing can be achieved. The counting results in graph 2 showed the mean number of

fibroblasts in group 2 which is higher than the control group, group 1, and group 3. LSD test on fibroblast cells showed that there were significant mean differences in comparisons between the control group with group 2 and group 2 with group 3 (Table 2). The number of decreased fibroblasts in group 3 caused by the proliferation of fibroblasts cells which have reached the optimum effect at a concentration of 45%. The mean results of capillary blood vessels showed that the number of capillaries in group 3 is higher than the control group, group 1 and group 2. An increasing number of these occurred with increasing concentrations of the ointment. This occurs indirectly as the influence of several growth factors such as VEGF-A, FGF-2, TNF- β that are produced both by macrophages and fibroblasts. TGF- β 1 produced by macrophages can also induce up-regulation of growth factor for angiogenesis such as VEGF.²⁷

Fibroblasts are actively moving from the network around the wound into the wound area, proliferate and issue some substances (collagen, elastin, hyaluronic acid, fibronectin, and proteoglycans) that play a role in forming new tissue. Collagen is a protein substance that increase the surface tension of the wound.²⁵ Other phenolic compounds in coffee, namely caffeic acid, has also been studied to play a role in the healing process by stimulating the synthesis of collage-like polymer by fibroblasts.⁶ Increased amount of collagen that add strength to the wound surface can avoid the possibility of opened wound.²⁶

In the results of data analysis, the highest levels of Robusta coffee bean extract ointment (90%) showed the highest value on the mean number of lymphocytes, macrophages, and capillary blood vessels, but not in plasma cells and fibroblasts, although it is higher than the concentration of 22.5%. While coffee bean extract 45% concentration ointment showed the highest value on the average number of plasma cells and fibroblasts compared to ointment of coffee bean extract 90%. Overall, Robusta coffee bean extract 45% ointment can give a good effect on wound healing process because at this concentration the number of fibroblasts increased significantly compared with the control group. It can be concluded that ointment of Robusta coffee bean extract could enhance skin wound healing process of *Cavia cabaya*.

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