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Exploring the Potential of Green Coffee Extract for Wound Healing Treatment

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Abstract. Roasted coffee powder is used as wound dressing in Indonesian traditional medicine. Coffee bean have several properties, such as chlorogenic acid, that can facilitate the wound healing. Green coffee has a higher chlorogenic acid. This study is to exploring the effect of green coffee beans extract for wound healing in rats. Extraction of green Robusta coffee used ethanol 70% with ratio 1:3 and dried by rotary evaporation. There were 6 male rats in each group. The incision wound were held in back with diameter size 1.5 centimeter under lidocaine anesthetic. Treatment group were given extract every day for 15 days. Control group were not given treatment. Animal were sacrificed at day 16th for histophalology assessment of fibroblast and collagen using hematoxylin eosin stain. Percentage of complete wound closuring on day 16 in treatment group was 83%, whereas control group were only 66%. There were not significant different in fibroblast and collagen scoring between two groups (p> 0.05). Green coffee extract can accelerate wound healing.

1. Introduction
Coffee has phenolic compounds and their derivatives (such as chlorogenic acids), alkaloid (especially caffeine), diterpenoid alcohols (such as cafestol and kahweol). Chlorogenic acids has been shown to accelerate the skin wound healing and burn healing due to its antioxidant, anti-inflammatory, antiulcerogenic and analgesic properties. Chlorogenic acids amount in green coffee are higher than roasting coffee beans. Coffee beans powder has been used as traditional medicine in wounds healing. Many local health provides reported coffee powder sprinkled on wound does not cause infection.

Wound healing is a complex proses. Collagen is a key component in wound healing. Collagen is the major protein of the extracellular matrix and it acts as a structural scaffold in the tissues that giving structural support in wound healing. Collagen also have chemotactic role. It attracts cells such as fibroblast and keratinocytes to the wound. Fibroblasts are also a key component in supporting normal wound healing. They migrate to the wound area, proliferate and carry out a number of key activities of regulation of injury mediated factors and changing environment of the healing wound. Myofibroblasts are responsible for wound contraction.

Extraction is a method to extract essential bioactive compounds for pharmaceutical purpose. The proper extraction procedure has been considered, such as extraction time, temperature, solvent, solvent to feed ratio, the number of repeated extractions of the sample. Phenolics can be extracted from fresh,
frozen or dried plant samples. Better solvent of extraction of antioxidant and polyphenols in plant is ethanol: water as solvent.

This study is to determine the effect of extract green coffee on wound healing process based on duration of complete wound closing and histopathology of collagen and fibroblast parameters.

2. Methods

2.1 Preparation of Extracts
We used Robusta green coffee beans (Coffea canephora pierre ex a froehner). The coffee bean was dried at oven 70°C for 24 hours. The dried beans were powdered by using grinder. Coffee powder was macerated in ethanol 70% with ratio 1 :3 for 24 hours. The liquid filtrate was collated and evaporated using rotary evaporator to obtain semisolid extracts. The extract was stored in airtight container in refrigerator 4°C.

2.2 Qualitative Phytochemical Screening
The extracts of green coffee were subjected to qualitative test for the presences of some phytochemical constituents such as alkaloids, flavonoids, polyphenols, tannin, saponin, triterpenoids and steroid based on other studies.

2.3 Animals Models
Male Sprague dawney rats, weighing 200-250 gram were obtained from laboratory of Faculty of Medicine and Health Sciences Jambi University. The animals were acclimated for a period of 7 days under standard environmental conditions. All the animals were fed with rodent pellet diet and water ad libitum. Ethical clearance for performing the experiments on animals was obtained from Ethics Committee Faculty of Medicine and Health Sciences Jambi University.

Rats were divided into two groups each containing six rats. Incision were made in front back with diameter size 1,5 centimeter under lidocaine anesthetic. Group I as control groups were not received treatment. Groups II received green coffee extract on the surface of wound every day without debridement for 15 days. On day 16th, calculation of rats that had have complete wound closing were held, then animals were sacrificed. Skin wounds removed and were processed for histological evaluation.

2.4 Histological Evaluation
Skin wounds were fixated, dehydrated, embedded, cutted and stained. Section were stained using Hematoxylin Eosin. Semi qualitative method was used to evaluate fibroblast and collagen by one independent observers with microscopic with x10 and x40 magnification lenses. The parameter were fibroblast and collagen. The parameters were classified according to their intensity, as weak, moderate and high.

2.5 Statistical Analysis
Fisher’s exact test was used to compare proportions. The level of significance was set at p < 0.05.

3. Findings

3.1 Phytochemical Screening
Phytochemical analysing revealed that the extract have polyphenols, flavonoids, tanin, saponin, triterphenoid and didn’t have alkaloids and steroid.

Polyphenols were a groups of phenolic acid, flavonoids, coumarins, lignans, stilbenes and tannins. Flavonoids and phenolic acids are of great abundance in coffee. Polyphenols have effective scavenging ability of reactive oxygen species (ROS) to the skin. Polyphenols affects skin protection, neutralizing
free radicals, antioxidant activity and reduce inflammatory conditions of the skin. Chlorogenic acid as the main components of the phenolic fraction of green coffee beans, up to 14%. Component of chlorogenic acid of coffee beans varies considerably during fruit maturation. Compare to arabica coffee, Robusta coffee have higher chlorogenic acid compound. Chlorogenic acids have potent antioxidant activity.

3.2 Percentage of Complete Wound Closure

Figure 1. Percentage of complete wound closure in each group

Complete wound closing was calculated from day of incisions to the day of eschar falling and there was no raw wound behind. Percentage of complete wound closing on day 16th in treatment group with green coffee extract was higher than control group (83% vs 66%). Green coffee extract can accelerate the wound healing. The methods of extraction that was used in this study can extract chlorogenic acid, a compound that accelerate wound healing. Other studies also found that coffee can accelerate healing of various wound.

Figure 2. Wound on day 1 (left), complete wound closure (middle) Uncomplete wound closure on day 16th (right).

3.3 Histological Analysis

The histological results from the rats euthanized on days 16th are presented in table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Groups I)</th>
<th>Coffee extract (Group II)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>NE</td>
</tr>
<tr>
<td>Moderate</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0,455</td>
</tr>
<tr>
<td>Moderate</td>
<td>0 (0%)</td>
<td>2 (0%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>6 (100%)</td>
<td>4 (80%)</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s exact test. NE : not evaluable

Histological analysis for fibroblast and collagen between two groups were not significant different. In the proliferative stage of wound healing, fibroblast produce type III collagen. Myofibroblast, component of fibroblast, have contractive activities and contract the lesion’s border toward the center.

Thereafter, final stage of wound healing is remodeling stage that begins two to three weeks after the onset of the lesion. This stage form scar tissue. Type I collagen is the main component of mature connective tissue scar. Collagen fibers become thicker and are placed in parallel resulting in an enhanced
tensile strength for the tissue. If too much collagen is deposited in the wound, normal anatomical structure is lost, function is compromised and fibrosis occurs. Conversely, if an insufficient amount of collagen is deposited, the wound is weak and may dehisce.

4. Conclusion

Extraction of green coffee beans used ethannel 70% with ratio 1:3 result an extract that have ability to accelerate wound healing on rats. Further study is needed to determine active compound of green coffee beans that responsible for its effect in wound healing.

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