The Effect of Ethanol Extract Of Sarang Semut Plant (Hypnophytum Formicarum) to Granulation Tissue for Wound Healing After Teeth Extraction
Experimental Research on Marmot (Cavia cobaya)

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Abstract
Tooth extraction is a common thing, more often after extraction patients have any complication, to prevent it, physician provide some drugs from chemicals that often give adverse effects, so that required for a safe substitute medicine, which derived from herbs. One of the herbs are often used by people in the Mentawai Islands is a sarang semut's root species of Hypnophytum formicarum jack. This study aimed to determine the effect of sarang semut’s extract orally for granulation tissue formation in wound healing after tooth extraction. This research is experimental with posttest only control group design. Subjects were 48-animal guinea pigs, were divided into 4 groups: group I (control 0.5% CMC, treatment group II, III, IV with 4.65mg, and 9.3 mg 6.2 mg dose tuber ethanol extract dissolved anthill in CMC 0.5%), were given 3 ml orally, 3 times a day until the day of decapitation. Early research conducted identification and phytochemical screening, this plant belongs to the species Hypnophytum formicarum jack, contain flavonoids, phenols, triterpenoids and tannins. The data was analyzed with non-parametric Kruskal-Wallis, to see the significance between doses used Mann-Whitney. The results showed that the extract of sarang semut effect (p < 0.05) on the formation of granulation system in wound healing after tooth extraction. The most effective concentration was 4.65 mg

Keywords: granulation tissue, sarang semut (Hypnophytum formicarum), tooth extraction, wound healing after tooth extraction

Introduction
Tooth extraction is the most frequently performed in clinics, it may cause injury. Wounds can be healed normally, but sometimes cause some complications that would make the healing slower.¹,² Wound healing after tooth extraction healing process is similar to other tissues, consists of the inflammatory phase, the proliferative phase and remodeling phase.²,³ In the proliferative phase the formation of granulation tissue, which is consist of inflammatory cells dominated by macrophages, fibroblasts and vascular/angiogenesis. Granulation tissues formation begin on day 4 and lasted until day 21.⁵,⁶ Macrophages are cells phagocytosis, which also secrete cytokines and growth factors including platelet growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), vascular growth factor (VEGF), which plays an important role in the wound healing process. Macrophages recruited fibroblasts, keratinocytes, and endothelial cells for tissues repair. Fibroblasts proliferate and synthesize extracellular matrix components by secreting some substance collagen, reticulate, elastin, fibronectin and proteoglycans were act in the reconstruction of new tissues while the blood vessels carrying oxygen and nutrients that necessary for tissues survival.⁷,⁸,⁹ Wound healing after tooth extraction can be accelerated with many attempts, including by giving some medicines. Medicine which often used is antiseptic that can kill bacteria and prevent bacteremia, beside the fact that antiseptics have many adverses.¹⁰ Therefore, there are several researches to find substitute medicine, by switching it to herbs. The root of Sarang Semut (Hypnophytum formicarum) is one of the herbs, Mentawai Islands communities often use root of this plant to treats many diseases.

According to the phytochemical test, sarang semut’s root extract contains flavonoids, tannins, compounds, saponins and alkaloids.¹¹,¹² Flavonoids as antibacterial, anti-inflammatory and antioxidant, antibacterial saponins, tannins as a hemostatic and astringent and alkaloids as an analgesic.¹³,¹⁴

The aim of this study was to determine the effect of ethanol extract of the root of sarang semut as formulation of granulation tissue on wound healing after tooth extraction.
Methods

This study was purely experimental design with posttest only control group design. This research was carried out at: Laboratorium Herbarium Universitas Andalas, this herb belongs to Family: Rubiaceae with species: Hydrophytum formicarum jack. (SK No. 037 / K-ID / YOU / II / 2016. Preparation of extracts and phytochemical test was performed at Laboratorium Kimia Universitas Padjajaran and this extract’s herb was known containing flavonoids, triterpenoids, phenols, and tannins. Teeth extraction, coloring, and histological observations were performed in LPPT Unit IV, and Laboratorium Patologi Anatomi Fakultas Kedokteran, Universitas Gajah Mada. This study starts from April to July 2016. The study declared eligible conduct by Komisi Etik Penelitian Fakultas Kedokteran Universitas Andalas (SK No. 073 / KEP / FK / 2016).

The extract of sarang semut’s root was obtained by peeling the root, sliced thinly (3–5 mm), dried in oven (temp500C) to obtain the dry root and easily broken for, then blended up into a coarse powder escaped sieve no. 30.11 A total of 1000 g of dry powder sarang semut’s root was marinated with 70% ethanol with a ratio of 1: 5. Maceration was carried out by shaking the first 6 hours and placed for 18 hours. Maceration was performed over and over until the filtrate obtained discolored. Furthermore, the filtrate evaporated with a rotary evaporator (temp 500C), and put into the oven until all the water evaporates and becomes dry extract, to obtain a thick extract with fixed weights (not dripping).

Determination of dose

Dose of sarang semut’s extract was given to humans as powder as 1500-3000 mg / day (1-2 capsules @ 500 mg, three times daily) (Suryajaya, 2013). The dose used in this study is based on a conversion scale doses of human (70 kg) to guinea pigs weighing 400 grams, is 0.031.

Dose I: 4.65 mg / 400g mm / day Dose II: 6.2 mg / 400g mm / day Dose III: 9.3 mg / 400g mm / day.

Preparation of Animal Test and Tooth extraction

Population for this study is male guinea pigs. The sample is a part of the population that according to the inclusion and exclusion criteria. Inclusion criteria: male guinea pigs, weighing 250-400 grams, aged 6-8 weeks. Exclusion criteria: guinea pigs had ever sick and died during the treatment. The sample size is determined by the formula Federer (t - 1) (n - 1) ≥15. Because there are four groups (control, dose I, II, III) with observation time (days 3, 7, 14, and 21), the sample size used was 48-animal guinea pigs, consists of 3 guinea pigs respectively. The independent variables: sarang semut’s root extract and dependent variables: the formation of granulation tissues (macrophages, fibroblasts, and angiogenesis).

Guinea pigs have adapted for one week, then anesthetized IM with ketamine (0.1 ml) and zylazin (0.1 ml). After tooth extraction on control group (I) given CMC 0.5%, the treatment group was given a dose of the sarang semut’s root extraction I, II, and III 3 ml orally using a gastric’s explorer three times a day, until the day of decapitation. Socket tissues were taken and fixed in 10% buffered formalin, furthermore it processing for preparing for histological by HE coloring. Data obtained by assessing the formation of granulation tissue by counting the macrophages, fibroblasts and new blood vessels (angiogenesis) using a light microscope with 40x magnification, an area that is observed starting from the apex, medial, basal, and lateral using 400x magnification with this following criteria:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages and Angiogenesis (for each view)</td>
<td></td>
</tr>
<tr>
<td>Not finding</td>
<td>0</td>
</tr>
<tr>
<td>Less (local)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (rare diffusion)</td>
<td>2</td>
</tr>
<tr>
<td>Many (masif, diffusion, hard)</td>
<td>3</td>
</tr>
<tr>
<td>Fibroblast (for each view)</td>
<td></td>
</tr>
<tr>
<td>Not finding</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 10 %</td>
<td>1</td>
</tr>
<tr>
<td>10 - 50 %</td>
<td>2</td>
</tr>
<tr>
<td>50 s/d 90 %</td>
<td>3</td>
</tr>
<tr>
<td>90 s/d 100 %</td>
<td>4</td>
</tr>
</tbody>
</table>

Granulation tissue formation assessed by professional anatomical pathologist and researchers. All data was tabulated by group then calculated the average for each group. The statistical test was used by Kruskal-Wallis and to see the significance between dose used Mann-Whitney. The difference is expressed significant when $p \leq 0.05$.

Research result

1. Results of phytochemical test

Results of phytochemical test for sarang semut’s root (Hydrophytum formicarum jack) of the Mentawai Islands, is containing phenols, flavonoids, triterpenoids and tannins.

2. The formation of granulation tissue

The formation of granulation tissues was assessed by observing total of macrophages, fibroblasts and angiogenesis.

Figure 1. Average of Macrophages Amount
On day 3, the average of Macrophages amount from all group, most of them are on control group (CMC) and 4.65 are on dose group (the same score = 2) compared with other groups. On day 7, 14 and 21 total of macrophages for all the treatment group were decreased, while the control group from day 7 to day 14 are relatively stable, and after the 14th day started having decreased.

**Figure 2. Average of Fibroblast amount**

The highest averages of fibroblasts from all groups at a dose of 4.65 mg, which is an increase on days 3 and height at the 7th day. The control group and 6.2 mg dose groups but these stable from the beginning to the end of the day of observation, (the average of fibroblasts in CMC group = 1, the group of 6.2 mg = 2) while the 9.2 mg dose group the average number of macrophages stable from day 3rd to the 7th day, after that there is an increase and the height at day-21.

**Figure 3. Average of angiogenesis**

The most total average number of angiogenesis is 6.2 mg dose group, but the numbers were stable from day 3 to day 21, 4.65 dose group average numbers of macrophages had increased on day 3 and the height at the 7th day. On day 14 decreased and the number is relatively stable until the 21st day.

Then, data were analyzed with the Shapiro-Wilk normality test and homogeneity test with Levene Test. Obtained from the second test p-value < 0.05. That means data not normally distributed and was not homogeneous, then to see the effect of ethanol extract of sarang semut’s root extract (Hydnophytum formicarum jack) to the granulation tissue we had performed nonparametric Kruskal-Wallis test

<table>
<thead>
<tr>
<th></th>
<th>Sig Border</th>
<th>Sig Value (p-value)</th>
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</thead>
<tbody>
<tr>
<td>Makrofag</td>
<td></td>
<td>0.037</td>
</tr>
<tr>
<td>Fibroblas</td>
<td>0.05</td>
<td>0.041</td>
</tr>
<tr>
<td>Angiogenesi</td>
<td></td>
<td>0.037</td>
</tr>
</tbody>
</table>

Based on the table, we can make a conclusion there is the influence of ethanol extract of sarang semut’s root (Hydnophytum formicarum jack) to total of macrophages, fibroblasts and angiogenesis. To see the difference between the doses, this analysis followed by Mann-Whitney test and founded:

The total of macrophages was significantly different only at day 14, which is between the control group (CMC) at a dose of 4.65 mg, 6.2 mg dose (p 0081, respectively) and the 9.3 mg dose (p = 0.017).

The total of fibroblasts on day 3 was significantly different CMC with all groups, doses of 4.65 mg, 6.2 mg (p = 0.003) and 9.3 mg dose (p = 0.002). On day 7 CMC significant difference at a dose of 4.65 mg (p = 0.004) and 6.2 mg dose (p = 0.017). On the 14th day CMC significant difference at a dose of 4.65 mg (p = 0.002), 6.2 mg, and 9.3 mg (p = 0.003). On day 21 CMC significantly different with the 6.2 mg dose (p = 0.0017) and the 9.3 mg dose (p = 0.004).

Angiogenesis on days 3 and 7, CMC looks significantly different with a dose of 4.65 mg and 6.2 mg (p = 0.022 and 0.003), and a dose of 4.65 mg to 9.3 mg dose (p = 0.022). As well as, between the dose of 6.2 mg to 9.3 mg dose (p = 0.003). On day 14 and 24 was look significantly different CMC at a dose of 6.2 mg (0.003), between the dose of 4.65 mg doses of 6.2 mg and 6.2 mg doses at a dose of 9.3 mg (p = 0.003).

**Discussion**

Observation of wound healing after tooth extraction was performed histologically by comparing the total of macrophages, fibroblasts and angiogenesis between the control group and the treatment group (by the ethanol extract of the sarang semut (Hydnophytum formicarum jack) at a dose of 4.65 mg, 6.2 mg and 9.3 mg. Observations were made histologically on days 3, 7, 14 and 21.

**Macrophages**

On graphic one has shown on day three the average score of macrophages at most was the control group and 4.65 mg dose group. On the 14th day and the 21th-dose group 4.65 the total of macrophages was decreased (process is similar to the normal resolution). It showed that the extract of sarang semut 4.65 mg dose has no effect in stimulating macrophage formation. Macrophages in significant amount can accelerate wound healing, because it may eliminate the bacteria, debris and necrotic tissue so
that the tissue regeneration process occurs rapidly. In addition to the macrophages also release cytokines and growth factors PDGF, TGF-β, EGF and VEGF which stimulates the fibroblasts, keratinocytes, and endothelial cells to synthesize the collagen, epithelium and new blood vessels. The control group the average number of macrophages until day 14 remained relatively stable, this indicating that groups are remaining inflammation.

**Flavonoids as anti-inflammatory mediators to restrict the inflammatory and oxidative reactions.** Flavonoids have anti-inflammatory capabilities, which can inhibit the growth and replication of pathogenic bacteria by deploying a wide range of products including IFN-γ (interferon gamma) that activate macrophages. Macrophages are active phagocytosis, produce cytokines, tissue repair (Fibroblast Stimulating Factor, fibronecrtin, collagenase), and produces growth factor. Tannin content of tuber extract sarang semut associated with the process of formation of collagen, which can accelerate the formation of collagen tannins thereby accelerating wound healing.

**References**

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