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RESEARCH ARTICLE

Irritation Test in Periodontitis Wistar Rat Case after Sarang Semut Kalimantan Extract Application

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Abstract
Introduction: Infected periodontal pocket by microorganisms plays an important role in the occurrence of periodontal tissue necrosis and the formation of periapical abnormalities. Therefore, periodontal pocket treatment should be carried out with the aim of eliminating or reducing the microbial population and biomechanically removing necrotic tissue which is a medium for microbial growth and preventing re-infection. One of the medicinal plants from the Kalimantan Forest that has great potential is Sarang Semut (*Myrmecodia pendens*). **Objective:** This study aims to determine the response of gingival healing in the case of periodontitis in the Wistar rat model after the application of Sarang Semut (Myrmecodia pendens) extract. **Material and Methods:** Sarang Semut (*Myrmecodia pendens*) extract dilution to concentration of 2.5%, 5% and 10% was carried. This research is a laboratory experimental research and used post-test with control group design. **Results:** There are three concentrations that have been given: 2.5%, 5%, and 10%. The smallest concentration provides a healing area. **Discussion:** The results showed that ethanol extract of Sarang Semut can increase the healing response of the gingiva that has been induced by periodontitis bacteria. **Conclusion:** Gingival healing response in periodontitis case of Wistar rat model after application of Sarang Semut extract (*Myrmecodia pendens*) showed results similar to positive control on 2.5% Sarang Semut extract application. Clinical signs on the rat's gingiva experienced discoloration, changing countours and bleeding, but on the 3rd day, the gingiva gradually showed improvement and on the 7th day there was no pocket depth.
Keywords: Gingival healing, *Myrmecodia pendens,* Periodontitis, Sarang Semut, Wistar rats

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INTRODUCTION

Infected periodontal pocket by microorganisms plays an important role in the occurrence of periodontal tissue necrosis and the formation of periapical abnormalities such as abscesses, granulomas and cysts.[1] Therefore, periodontal pocket treatment should be carried out with the aim of eliminating or reducing the microbial population, biomechanically removing necrotic tissue, and preventing re-infection.[2] Necrotic tissue is a medium for microbial growth.[3]

Indonesia is a tropical country that has a high diversity of plants that must be utilized properly, one of which is widely used as a dental material.[4,5] One of the medicinal plants from the Kalimantan forest that has great potential is Sarang Semut (*Myrmecodia pendens*).[6] Its epiphytic nature is beneficial for its use as a medicinal plant because its exploitation does not harm the ecosystem. Due to the high failure rate of periodontal treatment caused by the activity of several anaerobic and aerobic bacteria and there has been no research on Sarang Semut plants used as materials for periodontal pocket irrigation and mouthwash, the researchers are interested in conducting research on gingiva healing response in the periodontitis case of Wistar rat model after the application of Sarang Semut (*Myrmecodia pendens*) extract.

MATERIAL AND METHODS

This research is a laboratory experimental study with post-test and control group design. The study was conducted in October – November 2020. Sarang Semut (Myrmecodia pendens) extract diluted to concentrations of 2.5%, 5% and 10% respectively. The study was carried out at the Microbiology Laboratory of the Hasanuddin University Medical Faculty. The extract of Sarang Semut is shown in figure 1. This research has been approved for ethical feasibility by the ethics committee of the Faculty of Medicine, Mulawarman University. The approval number is NO.60/KEPK-FK/XII/2020.

Figure 1. Sarang Semut extract (Source: photograph by researcher)

experimental animal cages before treatment. No criteria were set for animal selection. Experimental animal cages were made of rectangular plastic basins and used wire mesh as a cover. The Wistar rats were placed in a clean cage with good ventilation with a length of 50 cm, a width of 40 cm, a height of 40 cm with a temperature of 25-27oC, humidity of 70-75% and lighting for 12 hours of light and 12 hours of darkness. Bedding in experimental animals using husks was replaced every three days. The rats were fed according to standard ADH pellets and drinking was libitum (unlimited). Monitoring the health of the environment and experimental animals is carried out every day.

There were 3 (three) stages for induction of periodontitis in Wistar rats in this research. The first stage was preparing bacterial colonies. Preparing bacterial colonies was carried out at the Microbiology Laboratory of the Hasanuddin University Medical Faculty. The bacterial

colony to be induced in wistar rats is A. actinomycetemcomitans ATCC 33277. Culture media per liter was prepared: 5 g yeast extract, 5 g peptone, 200 ml goat blood fill up to 1 liter. Then in the autoclave for 15 minutes. After the cold media was inoculated with *A. actinomycetemcommitans* and incubated at 35oC, with a population density of 2 x 108 CFU/ml.

The second stage was doing anesthesia and induction of periodontitis. Anesthesia in rats was performed using a 0.2 ml/kgBW 10% Ketamine HCL solution intramuscularly in the thigh muscle using a 1 cc syringe. After 10-15 minutes, the rats began to limp and their movements slowed down, the gingival sulcus into the sulcus was measured using a periodontal probe. Next, a silk ligature on the lower anterior teeth. Silk ligatures are tied firmly in the cervical region of the teeth by wrapping them around the anterior teeth, so they don't come off easily. Then silk ligature was inserted and pushed into the gingival sulcus with the help of a probe. Subsequently, the bacteria *Aggregatibacter actinomycetemcomitans* was inserted by infiltration injection using a 1 cc syringe needle on the labial portion of the mandibular anterior teeth as much as 0.25 ml. The injection was only administered once, at the start of the trial. The third stage was observation. Observing the clinical condition of the oral cavity and changes in the rat's movements were carried out daily until periodontitis developed.

An Sarang Semut (*Myrmecodia pendens*) weighing 500-800 grams is blended by cutting the Sarang Semut into a size of 3x2 cm to facilitate the blender process then weighed 100 grams each using a digital scale then mixed with 100 ml of sterile distilled water and blended until a smooth consistency is obtained and stored in a plastic container. Sarang Semut *(Myrmecodia pendens*) were mixed with 1500 ml of sterile distilled water. After that, the procedure for making crude extract was carried out using the freeze dry method. Making Sarang Semut (*Myrmecodia pendens*) extract is shown in figure 2.

Figure 2. Making Sarang Semut extract (Source: Photograph by researcher)

The Sarang Semut (Myrmecodia pendens) extract were dissolved using 97% ethanol solvent indicator and soaked (maceration process) for 24 hours then filtered with filter paper. The filtered liquid is formed in three kinds of conditions, namely concentrated, semi-concentrated and not concentrated. Furthermore, the filtering results are evaporated using a rotary evaporator at a temperature of 50ºC so that the solvents are separated. The first evaporation is in the non-concentrated liquid so that the filtered liquid runs out and is continued with the semi-concentrated then the concentrated one. The evaporation results are cooled to produce Sarang Semut (*Myrmecodia pendens*) extract.

This research was carried out in 8 steps. A sample of 20 rats, divided into 5 groups, namely the Sarang Semut (*Myrmecodia pendens*) extract treatment group 2.5%, 5%, 10%, the positive control group using CHX and the last group the negative control using distilled water. Where each group contains 5 Wistar rats. General anesthesia was applied to Wistar rats, then disinfected in the lower labial area using povidine iodine, then wire was given to bind the two anterior teeth. Ketamine 0,3-0,5 ml was used for anesthesia. The determination of 2.5%, 5%, and 10% concentrations in the study was done by trying to take the smallest concentration to minimize the side effects of this herbal plant.

A silk ligature was used the following day to tie the two anterior teeth. *Aggregatibacter actinomycetemcomitans* bacteria were induced and the periodontal pocket depth was measured. Observations were made again 1x24 hours. On the next day, clinical observations were made and if there were signs of inflammation, the periodontal pocket depth was measured. The treatment group was then applied with 1 ml of Sarang Semut (Myrmecodia pendens) extract into the periodontal pocket using a 1 ml dropper pipette and the control group was applied with CHX (+) and aquades (-). Applications are made once for 3 days.

On the third day, the rats' gingiva was observed in each group. Mice sacrificed on days 3 and 7 days after application and observed for clinical gingival signs and periodontal pocket depth. For each Wistar rat that was sacrificed, the periodontal pocket was measured using a periodontal probe, the gingival tissue was cut, and put into a sample cup which had been filled with 10% formalin solution.

RESULTS

We conducted the study on twenty Wistar rats which were divided into 5 groups, where in each group contained four 4 female Wistar rats weighing between 160-220 g, age 11 months old. No exclusion and confounders in this research. The results of observations on the gingiva of Wistar rats are in the following Table 1. Blinding was not used because the animal sampling and the extract of Sarang Semut (*Myrmecodia pendens*) is homogen. There is no difference specific from the sample animal and extract of Sarang Semut (*Myrmecodia pendens*).

Tabel 1. The effect of Sarang Semut Extract Application on healing response

Healing response		**Before**	**Day 3**	**Day 7**
Bleeding (n = 10)	Yes	8	5	10
	No	2	5	0
Hyperemia (n = 10)	Yes	10	2	8
	No	0	8	2
Edema (n = 10)	Yes	10	5	10
	No	0	5	0
Pain (n = 10)	Yes	8	3	5
	No	2	7	5
Color change (n = 10)	Red	2	3	4
	Red bluish	3	2	3
	Blue	5	0	0
	Pink coral	0	5	3

Table 1 shows clinical signs of bleeding in the gingiva before application as many as 8 Wistar rats and no bleeding as much as 2 Wistar rats, then clinical signs of bleeding in the gingiva as a healing response, during observation 1 gingival bleeding was 5 Wistar rats and no

bleeding 5 Wistar rats while during observation 2 gingival bleeding occurred in all samples, namely 10 Wistar rats. Clinical signs of hyperaemia in the gingiva before application occurred in all samples, namely 10 Wistar rats, then clinical signs of hyperaemia in the gingiva as one of the healing responses, when observing 1 hyperaemia in the gingiva as many as 2 Wistar rats and not hyperaemic as many as 8 Wistar rats while during observation 2 hyperaemia of the gingiva as many as 8 Wistar rats and 2 Wistar rats that are not hyperaemic.

Clinical signs of edema on the gingiva during application occurred in all samples, namely as many as 10 Wistar rats, then clinical signs of edema in the gingiva as a healing response, during observation 1, edema on the gingiva was 5 Wistar rats and 5 rats were not edematous, while during observation 2 There was gingival edema in all samples as many as 10 Wistar rats.

Clinical signs of pain in the gingiva before as many as 8 Wistar rats and not sick as many as 2 Wistar rats, then clinical signs of pain in the gingiva as a healing response, during observation 1 pain in the gingiva as many as 3 Wistar rats and not sick 7 Wistar rats while during observation 2 pain in the gingiva as many as 5 Wistar rats and no pain in 5 samples. Observation of pain in Wistar rats was carried out by making observations related to excessive movements made by rats such as foot and hand movements, occasionally scratching the abdomen. in addition to movement, rats also make typical sounds such as hissing. Clinical signs of bright red color on the gingiva before as many as 2 Wistar rats, red bluish as many as 3 rats Wistar, and blue were 5 Wistar rats, then clinical signs of color on the gingiva as one of the healing responses, during observation 1 bright red color was 3 Wistar rats, blueish red was 2 Wistar rats, and pink coral was 5 Wistar rats while during observation there were 4 Wistar rats bright red, bluish red 3 Wistar rats, and pink coral 3 Wistar rats. For pocket depth examination, before treatment on the rat's gingiva there were no pockets (0 mm) in the periodontal, then at the time of bacterial induction there was a pocket of 2 mm. After the intervention/treatment was given to each group, the periodontal pocket depth was measured on 3rd day and 7th day (Table 2).

Table 2. Periodontal pocket depth measurement results of each group

Group	Periodontal pocket depth	
	Day 3	Day 7
Group 1 (2.5%)	1.5 mm	0.5 mm
Group 2 (5%)	2 mm	1.5 mm
Group 3 (10%)	2 mm	1.5 mm
Positive control group (CHX)	1 mm	0 mm
Negative control group	2,5 mm	4.0 mm

DISCUSSION

Previous study has found many uses of *Myrmecodia pendens* in dentistry scope. The ethanol extract of *Myrmecodia pendens* can assist wound healing from tooth extraction by increasing the expression of TGF-B1 and the number of osteoblast cell.[7] *Myrmecodia pendens* recorded to have potential antibacterial-active phytochemical compounds for pathogenic oral disease.[8] Expected alternative for dental therapy stated because *Myrmecodia pendens* extract has inhibitory effects against *S. sanguinis* and *T. denticola*.[9]

Wounds are damage to the anatomical structure and disruption of the body's physiological functions. The presence of a wound causes a biological response in the form of wound healing. Wound healing has several phases, namely, hemostasis phase, inflammatory phase, proliferative phase, and remodeling phase. Right after the injury there will be vasoconstriction followed by vasodilation thereby increasing the supply of blood in the wound area. In addition, platelets will degranulate and secrete Platelet Derived Growth Factor (PDGF) to attract polymorphonuclear

leukocytes (PMN) to the wound area and initiate the inflammatory phase. The inflammatory response lasted for 4 days which was dominated by PMN leukocytes and macrophages. After the inflammatory phase, there will be a proliferative phase characterized by the formation of new blood vessels and the synthesis of collagen. Angiogenesis or the formation of new blood vessels is the key to the wound healing process. This process plays a role in providing oxygen supply, nutrients, inflammatory cells, and eliminating tissue that is experiencing necrosis.[10]

Wound healing is a natural restorative response to damage and is a dynamic process that optimally restores tissue function and integrity which is the interaction of complex cellular steps that promote closure and reorganization, as well as restoration of the tensile strength of injured tissue. Re-epithelialization is the proliferative phase of the wound healing process that begins 24 hours after the wound occurs. When re-epithelialization is complete, the mucosal epithelium returns to its original shape, and new desmosome bonds form with other epithelial cells and hemidesmosome bonds to the repaired basement membrane.[10] *Myrmecodia pendens* ethanol extract was found effective in healing the inflamed pulp by reducing inflammatory cells and recover blood vessels within the pulp.[11]

Several previous studies have found that Myrmecodia pendens has antimicrobial effects against *Enterococcus faecalis, Fusobacterium nucleatum, Porphyromonas gingivalis, Staphylococcus aureus*, and *Agregatibacter actinomycetemcomitans*.[12] There is a relationship between 25% concentration of Sarang Semut plant extract and 50% concentration as root canal irrigation material that can inhibit the growth of *Enterococcus faecalis*.[13] The growth of *Fusobacterium nucleatum* was inhibited by 20%, 40%, 60%, and 80% of the ethanol extract of the Sarang Semut plant. [14] Sarang Semut extract of *Myrmecodia pendens* with a concentration of 25% and a concentration of 50% also effective in inhibiting the bacteria *Porphyromonas gingivalis*.[15] Sarang Semut ethanol extract also has anti-caries potential against *Staphylococcus aureus* which has been resistant to commercial mouthwashes containing hyaluronic acid and essential oils.[16] In a study that tested the acute toxicity of ethanol extract of Sarang semut (*Myrmecodia pendes*) in male rats (*Rattus norvegicus*), the formulation of medicinal plants with *Myrmecodia pendens* extract is recommended to be studied at low doses such as 0.1 g/kg BW because after 7 days it showed minimal toxic effects after application, with minimal inflammatory cell infiltration seen histologically from liver and liver parts.[17] *Hypnophytum, Myrecodia pendens, and Myrmecodia tuberosa* Jack are Sarang Semut species that are known to have been utilized as medicinal plants. Especially in research by Astuti (2023) who has compared the antibacterial effectiveness against *Porphyromonas gingivalis* bacteria, both have the same effectiveness at concentrations of 1.95 mg/mL-125 mg/mL but there is no significant difference between the two.[18]

Based on the phytochemical analysis, Sarang Semut (*Myrmecodia pendens*) contains chemical compounds from the flavonoid and tannin. Other bioactive ingredients that act as antimicrobials in Sarang Semut (*Myrmecodia pendens*) include saponins, alkaloids, phenolics, triterpenoids, and glycosides.[12] The content of flavonoids, saponins and tannins are antimicrobial so they may play a role in the wound healing process by reducing the length of the inflammatory phase. Flavonoids and tannins also have astringent properties that cause wound shrinkage, resulting in a narrower surface area that must be covered by the epithelium. Saponins have an effect on stimulating TGF-β which is important in the migration and proliferation of epithelial cells and the production of fibronectin and integrins. Tannins, saponins, flavonoids and ascorbic acid are useful in stimulating the growth of new cells in wounds. Flavonoids are known to play an important role in wound contraction and increase the rate of re-epithelialization. Flavonoids are thought to have functions as anti-inflammatory and moderator of type III collagen synthesis,

saponins function as antibacterial and can stimulate angiogenesis, while tannins function to limit secondary infection.[19] Previous study stated the flavonoid effect of the *Myrmecodia pendens* fraction has been found to have anticancer potential through Akt signaling and inhibition of NF-KB in SP-C1 tongue cancer cells, supress the growth of plaque bacteria and have potential to support healing of damaged or wounded tissue by increasing the body's immune system.[20,21]

CONCLUSION

Gingival healing response in periodontitis case of Wistar rat model after application of Sarang Semut (*Myrmecodia pendens*) extract showed results similar to positive control on 2.5% Sarang Semut (*Myrmecodia pendens*) extract application. Clinical signs on the rat's gingiva that experienced discoloration, counturs and bleeding on the 3rd day gradually improved and on the 7th day there was no pocket depth. The limitation of this study is the need for further research on ther irritation test and acute toxicity test of Sarang Semut Plant.

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CONFLICT OF INTEREST

The authors have declared that no competing interest exist.

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