

The Effect of *Myrmecodia pendans* Ethanol Extract on Blood Vessels Within Pulpitis (Study on *Sprague Dawley* Rats)

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ABSTRACT

Sarang semut (*Myrmecodia pendans*) is one of traditional herbs used to treat various diseases. *Myrmecodia pendans* contain chemical compound flavonoid which has anti inflammation effects. Pulpitis is a dental pulp inflammation that can be caused by bacteria invasion, physical trauma or iatrogenic factor which affecting blood vessels in dental pulp. This research is an in vivo laboratory experimental pre and post treatment with control. The purpose of this study is to find out the effects of *Myrmecodia pendans* ethanol extract on blood vessels appearance of *Sprague Dawley* rats' dental pulps which had been inducted with 0,01ml *Porphyromonas gingivalis* for 48 hours. The subjects were divided into 3 groups with Group I representing the negative control, group II representing treated group with *Myrmecodia pendans* ethanol extract, and group III representing positive control with Ca(OH)₂. Group II and III were divided into subgroups based on the treatment periods of 48, 96, 168, 336 hours. The statistic results showed that there were significant differences ($p < 0.05$) among the pulpitis, negative control, positive control Ca(OH)₂ and the *Myrmecodia pendans* treatment subgroups of 48, 96, 168, 336 hours. *Myrmecodia pendans* ethanol extract has comparable anti inflammation effect with Ca(OH)₂ which is used to pulpitis commercial medicament.

Key words: *Myrmecodia pendans*, pulpitis, blood vessel appearance, anti inflammation.

INTRODUCTION

Inflammatory process is a human defense mechanism to neutralize and clean harmful agent at the damage site in order to provide the environment of tissue repair. Inflammation is a respond to chemical, mechanical, infection, bacterial and viral pathogen.^{1,2,3} Vascular respond within inflammation in form of cellular and humoral extravasation is leucocytes and chemical mediators collected at damage site in order to neutralize, remove the harmful materials and repair the damage tissue.¹ The signs of inflammation include micro vascular destruction, capillary permeability increase, leucocytes immigration toward inflamed area.¹ Pulpitis is an inflamed respond of the dental pulp caused of cariogenic bacteria.⁴

Medical world recently used traditional medicine through the usage of medicinal plants. One of this potential plants is Sarang semut (*Myrmecodia pendans*).^{5,6} Sarang semut plant (*Myrmecodia pendans*) distributed throughout the equator countries.⁵ This plant occupied by variety of ants especially *Ochetellus* sp. The stable temperature within the tuber plant make the ant colonized that the natural chemical reaction occurred between ant' secret and plant

tuber. This reason assumed that why Sarang semut plant able to recover various diseases.⁵ This plant used empirically as anti tumor, anti cancer and diabetes remedies.⁵ This traditional remedy by boiling the plant within water used to treat ulcer, hemorrhoid, epistaxis, low back pain, allergy, uric acid problem, stroke, heart coronary disease, cancer.⁶

Today, medicinal plant widely used such as *Myrmecodia pendans* that has phytochemical compounds such as flavonoid, triterpenoid/steroid, and saponin.^{5,6} Previous studies revealed that Sarang semut contain a lot of phenolic compound, tannin, phenolic, glucoside, and terpenoid.⁷ Flavonoid has potency as anti bacterial, anti fungal, prevent of free radical, immune regulator.^{5,7}

There was also in vitro study that revealed the n-hexane fraction of *Myrmecodia pendans* inhibits cell survival and proliferation in colon cancer cell line.⁸ Ethanol extract of *Myrmecodia pendans* has cytotoxic effect on cervix cancer cell line.⁹ Some genus of *Rubiaceae* family, such as *Myrmecodia* was known to have pharmacology potency like flavonoid as anti inflammation. Ethanol extract of this plant has anti inflammation.¹⁰ Recently, the

therapeutic effect of flavonoid has already been used in dentistry such as anti inflammation within paradontosis, oral surgical lesion wound healing¹¹, direct pulp capping material¹² and anti-cariogenic bacteria *Streptococcus mutans*.¹³ Therefore this research was conducted to evaluate anti-inflammatory effect of *Sarang semut* (*Myrmecodia pendans*) ethanol extract respond especially on the features of blood vessel within tooth pulp of *Sprague Dawley* rat.

MATERIALS AND METHODS

This study was given Ethical Approval No.35-2016 ACUC RSHP FKH-IPB by Animal Care and Use Committee (ACUC) Veterinary Teaching Hospital, Animal Medical Faculty, Bogor Agricultural University. This pre and post treatment study used male *Sprague Dawley* rats of 28-32 weeks and 200-300 gram body weight from Bogor Agriculture University (IPB). *Sarang semut* (*Myrmecodia pendans*) plant originated from Tomohon, North Sulawesi. The total sample is 27 that divided into 3 groups, negative control, treatment group with *Sarang semut* ethanol extract, and treatment group with Ca(OH)₂ as positive control. *Sarang semut* and Ca(OH)₂ groups were divided into 96, 168, and 336 hour group. Cavity class I Black was performed on upper molar occlusal surface used round bur diameter 0.10 mm up to pulp chamber under ketamine and xylazine HCL intra peritoneal anesthesia (Figure 1).

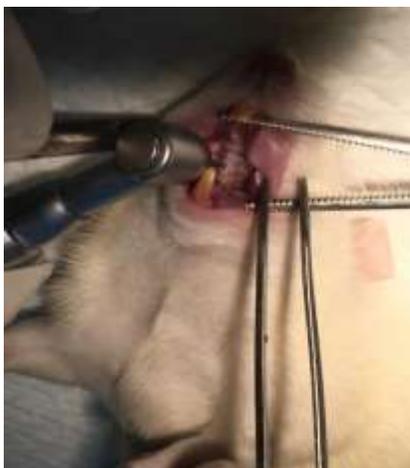


Figure 1. Class 1 cavity preparation on upper first molar.

Irrigation procedure was done used NaCl solution during the cavity preparation. Induced the pulpitis by injected 0.01 mL *Porphyromonas gingivalis* (3x 10⁸ CFU) using tuberculin syringe and temporary filling with GIC Fuji IX for 48 hours. At the 48 hours, open the temporary filing and applied the *Sarang semut* ethanol extract and Ca(OH)₂ paste within treatment and positive control group respectively. The same procedure was done on 96, 168, and 336 hours for *Sarang semut* treatment group and positive control group. The specimen stained with hematoxylin eosin for histopathological evaluation was derived from 48, 96, 168, and 336 hours specimen of each group. The scoring based on microscopic features of blood vessel within pulp. Score 1: normal pulp, score 2: hyperemia and congestion dominated, score 3: less or none of blood vessel.

RESULTS AND DISCUSSION

Most of pulpitis group on day 2 showed decrease of body weight that might due to loss of appetite. This condition disappeared within treatment group with *Sarang semut* and positive control of Ca(OH)₂ on day 7 and 14 following the healing process. The histopathological evaluation was done on HE staining based on the blood vessels appearance that divided into score 1 for normal condition, score 2 for hyperemia or blood vessel congestion, and score 3 for less vascularisation marked by necrotic tissue. Kruskal Wallis test showed significant differences between negative control, treatment group with *Sarang semut* and positive control with Ca(OH)₂ on day 4 (p=0.000<0.05), day 7 (p=0.000<0.05) and day 14 (p=<0.000<0.05). After pulpitis induction by *P.gingivalis*, on day 2, there was increase vascular supply in form of dilated blood vessel or hyperemia within pulp tissue as inflamed respond. Bacterial invasion produced virulent factors caused direct or indirect tissue destruction that compensated by increase blood supply or hyperemia.^{1,3,4} This feature shown in this study on Figure 2.

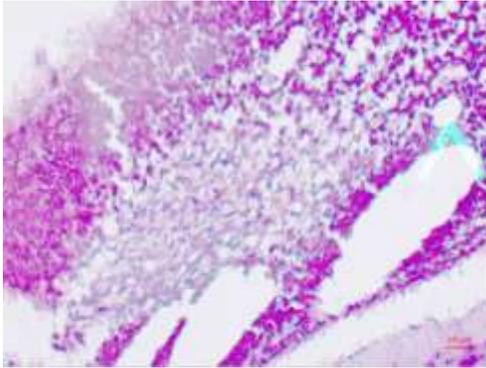


Figure 2. Score 2: there was increase vascular supply with hyperemia within pulp tissue (magnification 10x40).

On day 4, there was significant difference ($p=0.000<0.05$) between treatment groups whereas the *Sarang semut* group showed higher anti inflammation effect than those of Ca(OH)_2 (Figure 3a and 3b). This might be caused of anti oxidant effect of phytochemical content within *Sarang semut* such as flavonoid and tannin. Flavonoid is known as an effective compound to repair and protect cell structure outside of its potency to inhibit neutrophil degranulation, leucocytes accumulation and inflammatory chemical mediator production. This mechanism supported by tocopherol, saponin, and mineral such as magnesium within *Sarang semut* plant. In this study, these phytochemical compounds within *Sarang semut* ethanol extract influence the healing process within the inflamed pulp tissue.

On day 7, there was significant differences ($p=0.000<0.05$) between treatment groups whereas Ca(OH)_2 group showed higher anti inflammation effect than those of *Sarang semut* plant as shown on Figure 4a and 4b. This suggested that highly pH of Ca(OH)_2 increase alkali phosphatase activity followed by increase mineralization beside the pathogen microbe killer effect at apical site resulted in facilitating the reparative dentin formation. Alkali condition is also decrease osteoclast cells activity therefore able to continue the healing process well.

On day 14, there was significant differences ($p=0.000<0.05$) between treatment groups (either positive control Ca(OH)_2 or *Sarang semut*) with day 2, pulpitis group with the equal anti inflammation effect of *Sarang semut* with those of positive control Ca(OH)_2 group (Figure 5a and 5b).

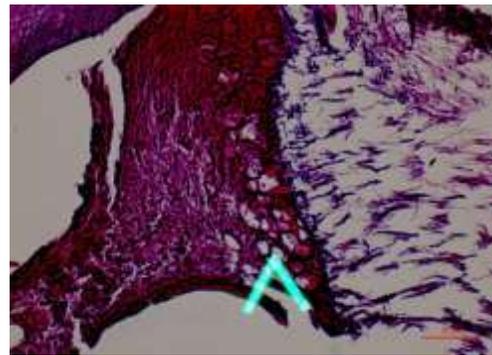


Figure 3a. Day 4, there was increase vascular supply with hyperemia of blood vessels (score 2) on treatment group with *Sarang semut* (magnification 10x40).



Figure 3b. Day 4, there was necrotic tissue (score 3) on treatment group with Ca(OH)_2 as positive control (magnification 10x40).

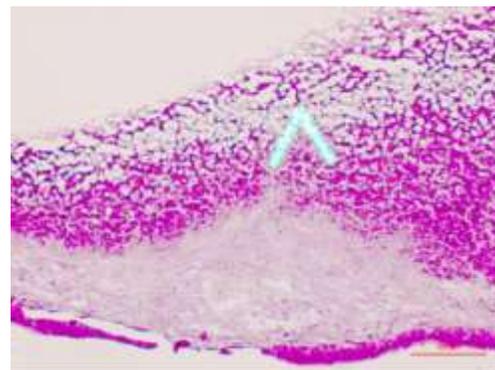


Figure 4a. Day 7, there was hyperemia of blood vessels (score 2) on treatment group with *Sarang semut* (magnification 10x40).

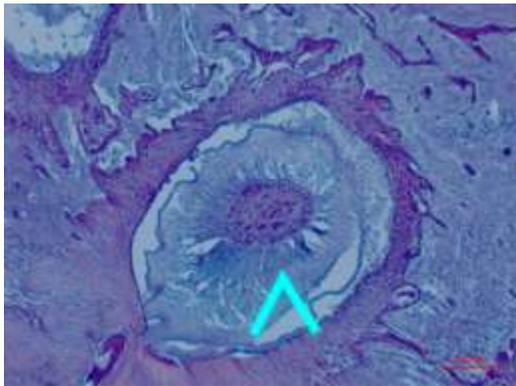


Figure 4b. Day 7, there was odontoblast cells at the peripheral of normal pulp (score 1) on positive control or treatment group with Ca(OH)₂ (magnification 10x40).

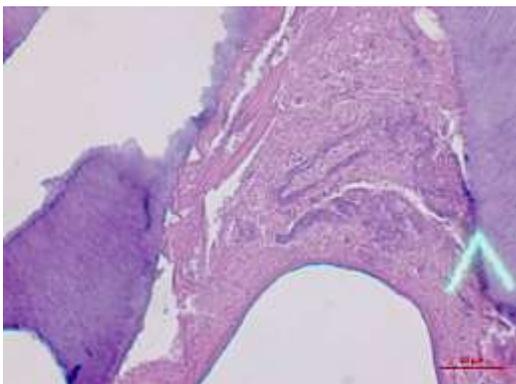


Figure 5a. Day 14, normal pulp (score 1) on treatment group with Sarang semut (magnification 10x40).



Figure 5b. Day 14, normal pulp with odontoblast cells (score 1) on positive control or treatment group with Ca(OH)₂ (magnification 10x40).

CONCLUSION

Ethanol extract Sarang semut (*Myrmecodia pendans*) has anti inflammation effect proved by pulp tissue healing process based on blood

vessel microscopic aspect. Ethanol extract Sarang semut has anti inflammation potency equal to those of Ca(OH)₂ especially on day 14.

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