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Chinese Petai Leaf Extract Gel (*Leucaena glauca*, Benth) decreasing neutrophil number in gingival inflammation animal model of Wistar Rats

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ABSTRACT

When damage signals occur, neutrophils are the first cells to migrate from blood vessels as a natural response of the body. However, in chronic inflammation, neutrophils contribute to tissue injury and potentiate the immune response. This study aimed to determine the number of neutrophil cells in gingiva of rats induced with inflammation after the administration of Chinese petai leaf extract gel. The study was conducted using true experiment with a posttest-only controlled group design in vivo. Male Wistar rats were induced with Porphyromonas gingivalis ATCC®33277TM bacteria on the cervical gingiva and kept for 4x24 hours until inflammation occurred. Inflammed gingiva in rats was treated in several groups, including gel containing Chinese petai leaf extract of 6 and 15%, Gengigel®, and distilled water. On days 3 and 5, a dissection of each treatment group was conducted to determine the number of neutrophil cells in rats using an Olympus CX31 microscope at 400x magnification. *One-way ANOVA* analysis revealed that the 15% concentration significantly reduced the neutrophil count on days 3 and 5 ($p \leq 0.05$). In conclusion, Chinese petai leaf extract gel 15% reduced the number of neutrophils in rats with gingivitis on days 3 and 5. This signified a normal transition from the inflammatory phase, which was considered beneficial for rapid wound healing. Future research is necessary to explore the content of Chinese *petai* leaves at different concentrations.

Keywords: Inflammation, Chinese petai leaf, Neutrophils, *Porphyromonas gingivalis*, Gingivitis

ABSTRAK

Sel neutrofil adalah sel pertama yang bermigrasi dari pembuluh darah sebagai respons alami tubuh ketika terjadi sinyal kerusakan. Namun, pada peradangan kronis, neutrofil dapat berkontribusi terhadap kerusakan jaringan dan memperburuk respons imun. Penelitian ini bertujuan untuk menentukan jumlah sel neutrofil pada gingiva tikus yang mengalami peradangan setelah pemberian gel ekstrak daun petai cina. Metode yang digunakan adalah true experiment dengan desain post-test only controlled group.Tikus Wistar jantan diinduksi dengan bakteri Porphyromonas gingivalis ATCC®33277™ pada gingiva servikal dan dibiarkan selama 4x24 jam hingga terjadi peradangan. Gingiva tikus yang mengalami peradangan kemudian diberi perlakuan dalam beberapa kelompok, yaitu gel ekstrak daun petai cina dengan konsentrasi 6% dan 15%, gengigel, serta air suling. Pada hari ke-3 dan ke-5, dilakukan diseksi pada masing-masing kelompok perlakuan untuk menghitung jumlah sel neutrofil menggunakan mikroskop Olympus CX31 dengan perbesaran 400x. Hasil uji *One-way ANOVA*
menunjukkan bahwa gel ekstrak daun petai cina dengan konsentrasi 15% secara signifikan mengurangi jumlah neutrofil pada hari ke-3 dan ke-5 ($p \leq 0,05$). Dapat disimpulkan bahwa gel ekstrak daun petai cina 15% menurunkan jumlah neutrofil pada tikus yang mengalami gingivitis pada hari ke-3 dan ke-5, yang menandakan transisi normal dari fase inflamasi, sehingga bermanfaat untuk mempercepat penyembuhan luka. Penelitian lebih lanjut diperlukan untuk mengeksplorasi kandungan ekstrak daun petai cina pada konsentrasi yang berbeda.

Kata kunci: Peradangan, Daun petai cina, Neutrofil, *Porphyromonas gingivalis*, Gingivitis

1. Introduction

Gingivitis is the most common oral disease experienced by more than 75% of people in the world after dental caries. According to World Health Organization (WHO) survey, gingivitis is experienced by 90% of the global population.[1] Gingivitis is an inflammatory process that occurs in the gingival tissue without extending to the alveolar bone, periodontal ligament, or cementum. Neutrophils are the immune system of gingivitis pathogens in inflammatory cells. These increase in number to form the initial defense during inflammation in the early stages of gingivitis.[2] Neutrophils help phagocytose bacteria and control disease. The Gram-negative anaerobic bacterium *Porphyromonas gingivalis* causes gingivitis by colonizing the oral cavity and reproducing in subgingival biofilms.[3,4] In treating periodontal disease, the gold standard can be achieved through scaling and root planning as the first step. Additional treatment using medicines such as Gengigel® is needed to increase the healing speed of gingivitis.[5] Gengigel® is a gingival gel containing 0.2% hyaluronic acid, xylitol, and excipients. Hyaluronic acid can weaken the bonds between cells in tissues that are continuously inflamed, allowing the formation of new cells.[6] However, Gengigel® currently has an expensive price and is not evenly distributed across Indonesia in nearby pharmacies.[7]

Therefore, alternative solutions using efficient and easily accessible materials are needed. Because herbal medicines as traditional medicines have fewer side effects than chemical medicines, the use of herbal medicines derived from natural ingredients is considered safer than chemical medicines.[8] One natural ingredient that is often found in Indonesia is the Chinese petai plant. For a long time, the seeds of is plant have been known as an anthelmintic and the stems have been used as tool materials. The leaves themselves are currently not used optimally and only end up as organic waste. Veronica et al. reported that a topical gel formulation containing 6% Chinese petai leaf extract demonstrated notable efficacy in promoting cutaneous wound healing.[9] In a separate study, Fitrian et al. observed that a 15% concentration of the same extract significantly induced angiogenic activity in incisional wound models in rats.[10] Based on phytochemical results, petai cina leaves contain flavonoids, phenolics, saponins, terpenoids, tannins [11] and alkaloids [12] which are useful as antibacterial, anti-inflammatory, antihyperglycemic, analgesic, antioxidant and larvicide[13]. In light of previous research findings, the present study aims to compare the efficacy and biocompatibility of ethanol extracts of *Leucaena glauca,* Benth leaves at concentrations of 6% and 15%, in order to identify the optimal concentration for the therapeutic management of gingivitis.

2. Materials and Methods

The study was conducted using a posttest-only controlled group design. Chinese petai leaves *(Leucaena glauca,* Benth) were collected from Baringan Gardens, Tebing Tinggi City. Gel preparation and evaluation were performed at Faculty of Pharmacy, Universitas Sumatera Utara (USU). The in vivo study using male Wistar rats was carried out at Faculty of Medicine, USU. First of all, Chinese petai leaf were extracted to get a thick extract through multi-stage maceration using 96% ethanol as a solvent, and thickening using a rotary evaporator. Furthermore, the thick extract was added to the mixture of carbopol 960, triethanolamine (TEA), propylene glycol, distilled water, and methylparaben, which were homogenized resulting in homogenous gel containing 6 and 15% extract of Chinese petai leaf (namely CPLEG). Ethical approval was received from the Health Research Ethics Committee (KEPK) of Universitas Sumatera Utara (number 870/KEPK/USU/2023) before conducting the animal study. The animals used were 40 male Wistar rats divided into 8 groups. Inclusion criteria included rats that were healthy with no abnormalities, 2-3 months old, and weighing 160-200 g. Rats were induced by the bacteria Porphyromonas gingivalis ATCC®33277™ on the cervical gingiva and observed for 4x24 hours until inflammation occurred, marked by redness, swelling, and slight bleeding. After the occurrence of gingivitis, the gums of rats were smeared with treatments from each group.

The treatment groups used were Chinese petai leaf extract gel concentrations of 6 and 15%, Gengigel (positive control group), and distilled water (-negative control group). Rat gingiva was smeared using a microbrush 2 timesdaily and treated for 5 days. On day 3, the jaws of 20 rats were surgically removed and the number of neutrophils were checked after treatment. On day 5, surgery was performed for 20 rats again to count the number of neutrophils using an Olympus CX31 microscope with 400x magnification. Statistical analysis was conducted using one-way ANOVA to determine significant differences in the administration of Chinese petai leaf extract gel on the number of gingiva neutrophils in rats induced by inflammation with the bacteria *Porphyromonas gingivalis* ATCC®33277™ on days 3 and 5.

3. Results

Neutrophil normality for each group was determined using the Shapiro-Wilk test and homogeneity was evaluated through the Levene test, while observations were conducted on days 3 and 5. The normality and homogeneity tests of data on the number of neutrophils in the inflamed gingiva of rats showed normal and homogeneous distribution ($p>0.05$).

Table 1. The Number of Neutrophils with 6% Concentration in Gingivitic rats after 3 and 5-day treatment

	Concentration 6% (Number of Cells)	Gengigel (+) (Number of Cells)	Aquades(-) Number of Cells)
Day 3 (Repetition 1)	3.00	1.00	5.67
Day 3 (Repetition 2)	5.00	1.33	6.33
Day 3 (Repetition 3)	2.00	1.00	6.00
Day 3 (Repetition 4)	3.67	1.00	3.00
Day 3 (Repetition 5)	2.33	1.13	2.33
Mean±SD	3.20±1.194	1.13±0.181	4.67±1.857
Day 5 (Repetition 1)	1.33	0.67	1.67
Day 5 (Repetition 2)	1.33	0.67	1.67
Day 5 (Repetition 3)	0.67	0.33	2.00
Day 5 (Repetition 4)	1.00	0.00	1.33
Day 5 (Repetition 5)	1.00	0.00	2.00
Mean±SD	1.07±0.276	0.33±0.335	1.73±0.280

One-way ANOVA test results in Table 1 showed that the mean and standard deviation of the number of neutrophil cells in the 6% concentration, Gengigel, and distilled water groups on day 3 were 3.20 ± 1.194, 1.13 ± 0.181, and 4.67 ± 1.857. On day 5, the number of neutrophil cells decreased to 1.07 ± 0.276 and 0.33 ± 0.335 in the 6% concentration and Gengigel groups, respectively, but increased in the distilled water group by 1.73 ± 0.280. Based on the results of the *one-way ANOVA* test, the table shows that the 6% concentration group exhibited a reduction in neutrophil count, and there was a significant difference in the mean number of neutrophils on days 3 and 5 among all groups.

The content of Chinese petai leaf has an anti-inflammatory function which inhibits the migration of more neutrophils to the inflammatory area. This corresponds with the study by Rahmadania showing that horsewhip root extract gel containing saponins, flavonoids, tannins, terpenoids, and alkaloids at a concentration of 6% can reduce the number of neutrophils on day 3.[14] The content of petai cina leaves found in petai cina leaves has an anti-inflammatory function which can inhibit the migration of more neutrophils to the inflammatory area.

According to study by Leoni et al., neutrophils are the initial responders that infiltrate the wound area within the first 12 hours and are subsequently phagocytosed by macrophages on the third day. Although neutrophils play a crucial role in preventing infection during the inflammatory process, their prolonged presence in the inflamed area may lead to tissue damage. Flavonoids, one of the active compounds found in Chinese petai leaf extract gel, possess anti-inflammatory properties that can inhibit the excessive migration of neutrophils to the site of inflammation.[15]

The Chinese petai leaf extract gel exhibits antibacterial and anti-inflammatory properties, thereby accelerating the healing process of gingivitis in rats. This effect is attributed to the flavonoid content in the leaves. This finding is consistent with the study by Atik Kurniawati, which reported a decrease in the number

of neutrophils over time. The group treated with the flavonoid-containing extract gel showed a lower neutrophil count compared to the control group that did not receive any treatment.[16]

Table 2 The Number of Neutrophils with 15% Concentration in Gingivitic rats after 3 and 5-day treatment

	Concentration 15% (Number of Cells)	Gengigel (+) (Number of Cells)	Aquades(-) Number of Cells)
Day 3 (Repetition 1)	4.67	1.00	5.67
Day 3 (Repetition 2)	2.33	1.33	6.33
Day 3 (Repetition 3)	2.00	1.00	6.00
Day 3 (Repetition 4)	2.33	1.00	3.00
Day 3 (Repetition 5)	2.33	1.13	2.33
Mean±SD	2.80±1.046	1.13±0.181	4.67±1.857
Day 5 (Repetition 1)	1.67	0.67	1.67
Day 5 (Repetition 2)	0.33	0.67	1.67
Day 5 (Repetition 3)	0.67	0.33	2.00
Day 5 (Repetition 4)	1.67	0.00	1.33
Day 5 (Repetition 5)	0.00	0.00	2.00
Mean±SD	0,87±0,769	0.33±0.335	1.73±0.280

One-way ANOVA test results in Table 2 showed that the mean and standard deviation f the number of neutrophil cells in the 15% concentration, Gengigel, and distilled water groups on day 3 were 2.80 ± 1.046, 1.13 ± 0.181, and 4.67± 1.857, respectively. On day 5, the number of neutrophil cells decreased to 0.87 ± 0.769 and 0.33 ± 0.335 in the 15% concentration and Gengigel groups, respectively, but increased in the distilled water group by 1.73 ± 0.280. Based on the results of the one-way ANOVA test, the table shows that the 15% concentration group demonstrated a reduction in neutrophil count, with a significant difference in the mean neutrophil count observed on days 3 and 5 among all groups ($p \leq 0.05$).

Chinese petai leaf extract gel contains flavonoid which is capable of reducing the number of neutrophils. Similarly, Nindya reported that a 15% concentration of Jerang resin decreased the number of neutrophils in rats experiencing inflammation due to flavonoid content with anti-inflammatory pharmacological activity.[17]

The number of neutrophils decreased in the 15% concentration group, compared to the 6% and distilled water groups because higher gel extract concentration leads to greater antibacterial and anti-inflammatory impact. A similar previous study reported that neutrophils could decrease over time and become increased by the presence of certain ingredients such as flavonoids in Chinese petai leaf.

Based on the results of the Post Hoc LSD test to assess the comparison between the 6% and 15% concentration groups on day 3 had a p-value of 0.723, suggesting no significant difference in the number of neutrophils ($p>0.05$). Furthermore, the comparison between the 6% and 15% concentration groups on day 5 had a p-value of 0.509, suggesting no significant difference in the number of neutrophils ($p>0.05$).

Aoethpah et al. reported that least significant difference (LSD) results from 7%, 9%, and 11% acacia leaf extract gel did not differ significantly but the three concentrations had the same effectiveness in the process of healing cuts in rats.[18] In this study, Chinese petai leaf extract gel concentrations of 6% and 15% with no significant differencestill decreased the number of neutrophils effectively in Wistar rats experiencing inflammation, compared to the negative control treated using only distilled water.

The results of this study demonstrate the effect of Chinese petai leaf extract on gingival neutrophil count in Wistar rats with induced inflammation. The highest number of neutrophil cells was observed in the negative control group, as this group only received treatment with distilled water (aquadest). In contrast, the lowest neutrophil count was found in the positive control group. Meanwhile, the treatment groups receiving 6% and 15% concentrations of the extract showed a reduction in neutrophil count compared to the negative control group. These findings suggest that the flavonoid content of Chinese petai leaf leaf extract exhibits anti-inflammatory effectiveness by decreasing neutrophil infiltration in the gingiva of inflamed rats.

The results are consistent with the study by Blezeinsky showing that the administration of extracts containing flavonoid can reduce the number of neutrophil cells.[19] The presence of flavonoid inhibits the synthesis of eicosanoids, which decreases arachidonic acid concentration in phospholipid cell membrane tissue, thereby preventing the release of various inflammatory mediators.[20]

4. Discussion

This study found that the administration of Chinese petai leaf extract gel at concentrations of 6% and 15% had a significant effect on reducing the number of neutrophils in rats experiencing gingivitis compared to the distilled water control group on days 3 and 5. Additionally, there was a difference in the decreased number of neutrophils between the administration of Chinese petai leaf extract gel at concentrations of 6% and 15% on days 3 and 5 as a wound healing parameter in the inflammatory process.

5. Conclusion

The findings of this study offer significant insights for healthcare professionals and the broader scientific community regarding the potential application of Chinese petai leaf extract in dental practice. The results may serve as a foundational reference for future investigations exploring the extract's influence on neutrophil count in gingival inflammation. Furthermore, this study contributes to the identification of an optimal healing timeline for the effective management of gingival inflammatory conditions.

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7. Conflict of Interest

The authors declare no conflicts of interest concerning this study.

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Appendix

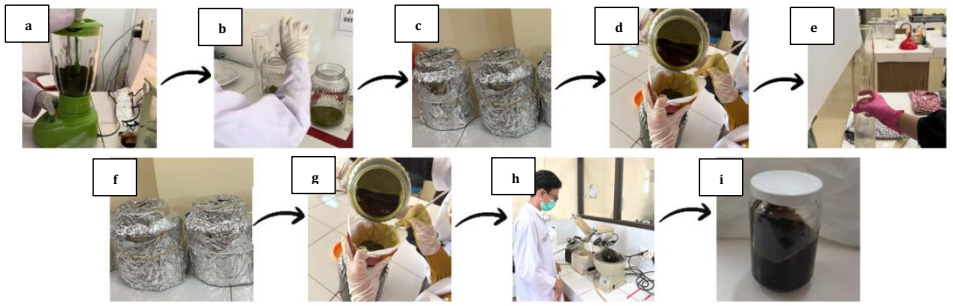

Figure 1. The extraction procedure for Petai Cina leaves: (a) Blend the simplicial; (b) Add 96% ethanol; (c) Let it stand; (d) Filter; (e) Add more ethanol; (f) Let it stand ; (g) Filter again; (h) Concentrate; (i) Thick extract.

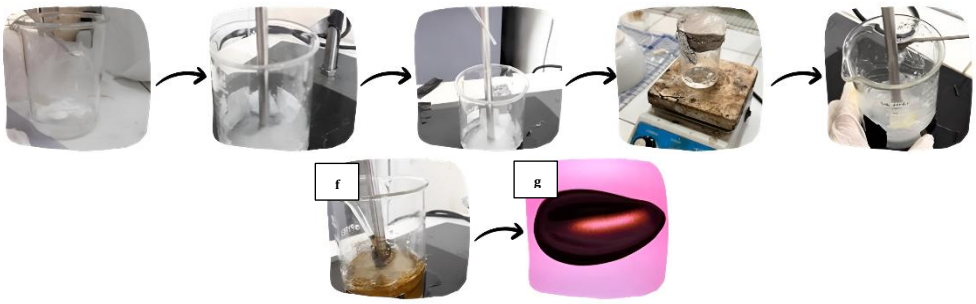

Figure 2. The procedure for preparing Petai Cina leaf extract gel: (a) Swell carbopol; (b) Add triethanolamine; (c) Add propylene glycol; (d) Dissolve methylparaben; (e) Homogenize; (f) Add thick extract; (g) Petai Cina leaf extract gel.

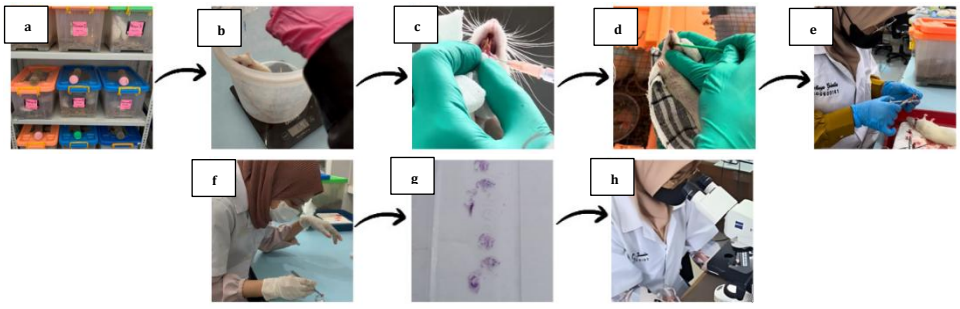

Figure 3. The animal testing procedure: (a) Acclimatization; (b) Weigh the rats; (c) Bacterial induction; (d) Administer treatment; (e) Dissection; (f) Place in Formaldehyde; (g) Prepare Slides; (h) Count Cells.

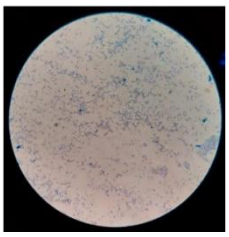

Figure 4. Microscopic view of *Porphyromonas gingivalis* ATCC® 33277™.

Figure 5. (a) 6% Group, (b) 15% Group, (c) Hyaluronic Acid 0,2 % Group, (d) Aquades Group.