Computer Engineering
ISSN: 10003428
Volume 15 | Issue 3 | Year 2026
https://journalofcomputerengineering.com/

Investigating the Anti-Inflammatory Properties of Sida rhombifolia L. in a Rat Periapical Lesion Model

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ABSTRACT

Sidaguri (*S. rhombifolia* L) is one of the most important species of medicinal part in Indonesia as anti-inflammation. To clarify this, we investigated the anti-inflammatory effect of the root of *S. rhombifolia* on rat periapical lesion model. The incisivus teeth were drilled to expose the dental pulp to the oral cavity and apical lesions were induced with LPS isolated from Porphyromonas gingivalis. Pour plate method was used to determine the bacterial CFU of gingival crevicular fluid (GCF) and C-reactive protein (CRP) serum was examined by enzyme-linked immunosorbent assay. The results showed that the root of *S. rhombifolia* could not reduce the total bacteria of GCF; however, it could reduce the level of CRP compared to negative control ($p<0.05$). In conclusion, *S. rhombifolia* has anti-inflammatory potency on rat periapical lesion.

Keywords: anti-inflammation, *Sida rhombifolia*, periapical lesion.

INTRODUCTION

According to data of Indonesian Health Profile year 2009, pulpal and periapical diseases were the 8th rank of the 10th outpatients at hospital in Indonesia. This situation increased and become the 7th in year 2010[1,2]. Pulpal and periapical diseases are inflammatory diseases caused by colonized bacteria in the root canal system. Mixed bacteria are usually found in the root canal such as *Peptostreptococcus micros* (35%), *Fusobacterium necrophorum* (23.3%), *Fusobacterium nucleatum* (11.7%), *Prevotella intermedia/nigrescens* (16.7%), *Porphyromonas gingivalis* (6.7%) and *Porphyromonas endodontalis* (5%)[3]. Inflammation of pulpal and periapical diseases is a pathophysiological response to irritants that leads to accumulation of plasmatic fluid and immune cells in the apical area of teeth. Complex events and mediators involved in the inflammatory reaction can be induced which maintain or aggravate the diseases[4]. Currently available anti-inflammatory drugs have problems during their clinical use and development of newer antiinflammatory drugs with lesser side effects is necessary[5,6]. In recent years, Indonesian government promotes various herbs for the treatment as part of traditional medicines which has been applied in some primary health care since year 2009. For dental treatment, a wide variety of plants are commonly used by people as analgesics; clove oil, ginger, and *Andrographis paniculata*, as antibacterial; onion, curcuma, as anti-inflammatory; lemongrass, ginger, noni[7]. Some other plants have also been used for tooth ache such as jatropha, gambir, and sidaguri[7-9], however Sidaguri has not been further explored. Sidaguri (*Sida rhombifolia*), a genus of flowering plants of mallow family Malvaceae, has been studied, and each part of the plants could be used for relieving various symptoms of the body. However only some people use the root of Sidaguri for tooth ache[10]. Previous study using agar diffusion method showed the ethanol extracts of roots of Sidaguri has an antibacterial activity against *Enterococcus faecalis*, while no inhibition zone against *Actinomyces* spp was noted. In addition, anti-inflammation test using plethysmometer on carrageenan-induced rat of the ethanol extracts of root of Sidaguri showed significant anti-inflammatory effect compared to negative control[11]. Further studies on animal model are recommended for optimal results. This study aimed to examine the anti-inflammatory effect of root of Sidaguri on rat periapical lesion model.

MATERIALS AND METHODS

Plant collection and extraction

The roots of Sidaguri were collected from their natural habitat in Bone, South Sulawesi. Firstly, they were identified and determined, then the roots were cleaned, sun-dried, grounded into powder and extracted using reflux method with ethanol 96%. The extracts were evaporated using rotary evaporator and dried extracts were kept in vacuum desiccator.

Animal model

Healthy male Wistar rats of 2-3 month-age were chosen (180-200 gr) and housed in the same environment, adapted for one week prior to treatment, fasting for 18 hours, water and food *et libitum*. The rats were intra-peritonealanesthetized with Ketamine-HCl (Pfizer) (80mg/kgBW)[12], fixed at retraction board, and the lower tooth was drilled with steel bur ¼ (Emeco, Vanadium, Germany) to expose

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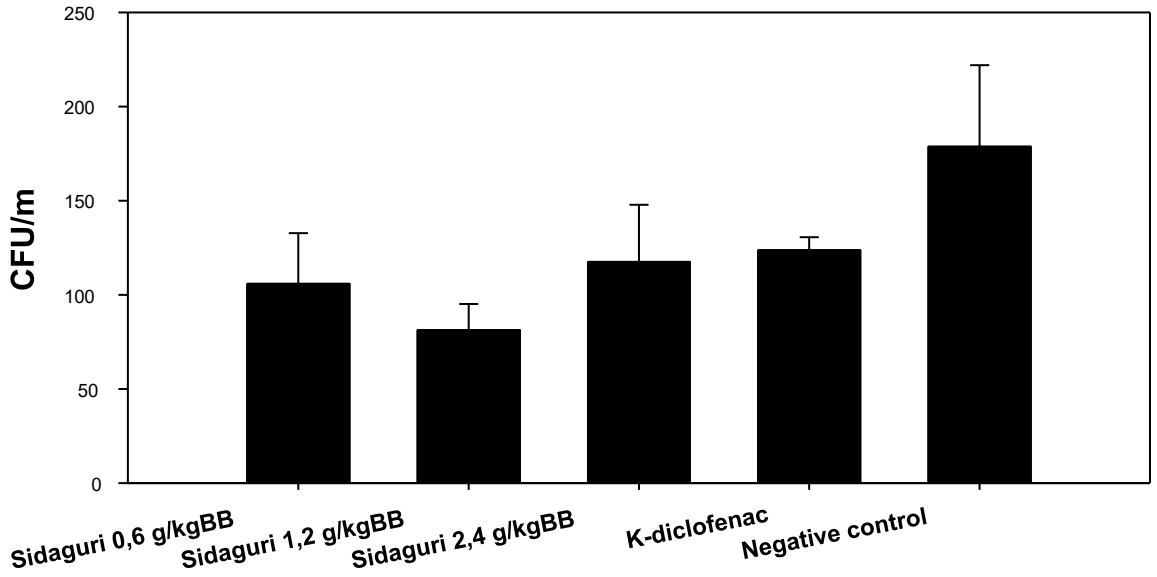

Figure 1: Number of cultured bacteria on animal model following treatment with Sidaguri extracts in various concentration.

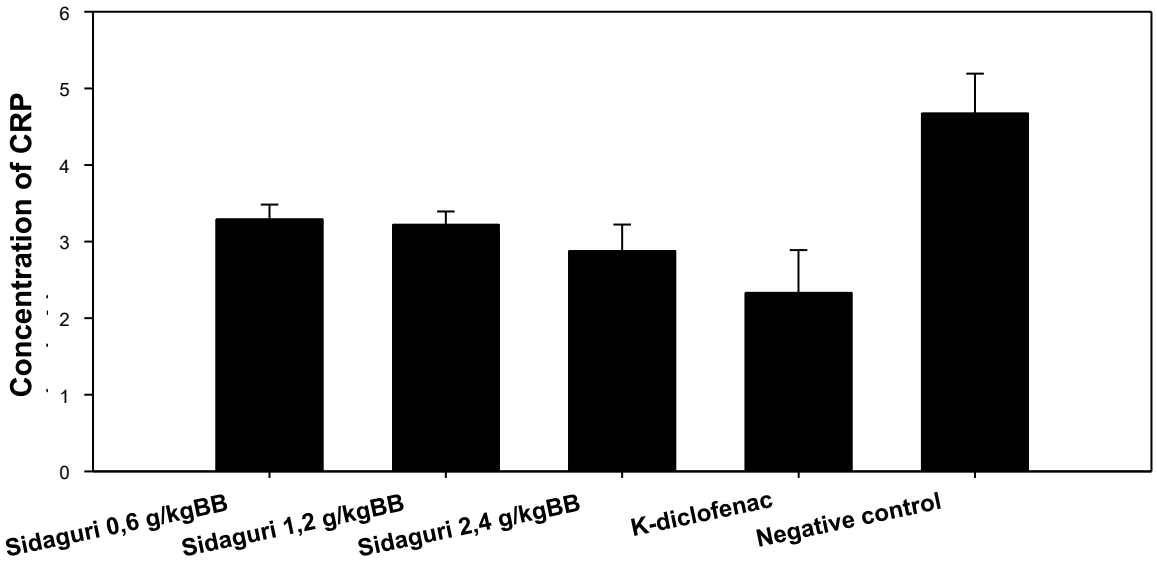

Figure 2: Anti-inflammatory activities of various dosage of root extracts of Sidaguri on animal model following 14day treatment.

the dental pulp to the oral cavity. Periapical inflammation was induced with LPS isolated from *Porphyromonas gingivalis*. To enhance the inflammation, LPS was injected through the cavity entrance and sulcular gingival. The rats were kept for 3 weeks following evaluation.

Treatment

Suspension of Sidaguri extracts in Na-CMC 1% was evenly distributed. The extracts were given orally using oral cannula with each of doses 0.6; 1.2; and 2.4 g/kgBW, every day for 3 weeks. The body weight, quantity of meal consumed, and the activities were examined every day. *Determination of CFU bacteria with pour plate method* Number of bacterial load (CFU) on animal model was evaluated using pour plate method. The samples with bacterial contained, mixed with agar medium and colony of bacteria in teeth of animal model was taken with cotton button, then inserted into the tube.

Evaluation of CRP level

CRP level of the collected blood was determined using ELISA method according to the procedures in manual kit.

Statistical Analysis

All the grouped data were statistically evaluated, hypothesis testing methods included one-way analysis of variance followed by least significant difference test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for five experiments in each.

RESULTS AND DISCUSSION

Malvaceae is a cosmopolitan family of herbs, shrub and trees revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds like *S. rhombifolia*, locally

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known as Sidaguri in Indonesia. It has a long history for its medicinal value in traditional medicine for the treatment of gout, hypertension, diabetes, anti-tuberculosis agent, diarrhea, and indigestion disorder[13,14]. This research focus to the roots as anti-inflammation on rat periapical lesion model induced by LPS. A model of this periapical lesion model LPS-induced could be valuable for improving the understanding of disease etiology and progression and effective treatments. There are many reports regarding the induction of periapical lesions in animals but rat models are the most frequently used models for such studies: the morphology of a rat molar is similar to that of a human molar, and the genetic background of these animals is clear, with practically no individual specificity[15,16]. *Antibacterial activities of Sidaguri extracts on animal model periapical inflammation induced with LPS, Pour Plate method*

The results in Figure 1 showed the extracts of root of Sidaguri has an effect to decrease the number of bacterial load compared to negative control. Dosage provided is comparable to the effect resulted although the difference is little. Statistical analysis showed (CI: 95%) all treated groups, either extracted Sidaguri group or positive control group showed significant difference compared to negative control group. It can be concluded that the extracts of roots of Sidaguri could inhibit the growth of bacteria on animal model periapical inflammation induced with LPS in optimal dosage 0,6 g/kgBW.

The antimicrobial activity showed that the Sidaguri has ability to decreases the microorganisms in GCF. Some authors have reported the antibacterial activities of the petroleum ether, chloroform, ethyl-acetate extracts of Sidaguri on Gram-positive and Gram-negative bacteria[17], the antimicrobial activity attributed to the presence of various bio-actives components such as tannins, polyphenols, alkaloids, glycosides ((phenyl-Ethyl-Dglucopyranoside and phytoécdysteroide), flavonoids, steroids and saponins[18,19]. But in another research reported that Sidaguri showed weak antibacterial activity against both Gram-positive and Gram-negative test organisms[20] correlated with this result that activity no dependent doses.

Anti inflammatory activities of Sidaguri extracts on animal model periapical inflammation Induced with LPS Porphyromonas

Periapical lesion is a local inflammatory process mediating the destruction, triggered by bacterial or its toxic such as LPS. Immunological marker of acute phase response is CRP, refers to local inflammation response[21]. Consequently, CRP level relationship to periodontal disease. The level of CRP in blood rats of animal model was evaluated using ELISA method, as seen in Figure 2. The results of this study showed the extracts of roots of Sidaguri have an anti-inflammatory activities and the higher the dosage, the higher anti-inflammatory effect, marked by decreased level of CRP. Statistical analysis (CI:95%) showed all treated groups, either extracted Sidaguri group or positive control group showed significant difference compared to negative control group. Group of dosage 2,4 g/kg BW differs significantly to the group of dosage 1.2 g/kgBW and 0,6 g/kg BW. It can be concluded that optimum dosage for anti-inflammatory to inhibit CRP was 2.4 g/kgBW.

Inflammation is the first step of disease proses, inhibit this proses can inhibit the pathogenesis of disease. Using Sidaguri as inhibitor more possible. The stems and roots can inhibit arthritic on rats[22]. The roots have scavenging activity on 1,1-diphenyl-2picrylhydrazyl and, antiinflammatory on NF-Kb cell line inflammation model[23].

CONCLUSION

Under the limitation of this study, it can be concluded that the ethanol extracts of roots of Sidaguri has potent antiinflammatory activities but lack of antibacterial effects in periapical inflammation induced with LPS Porphyromonas gingivalis. Future studies need to be elucidated on the active components of the roots of Sidaguri.

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