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Research Article

Anti-inflammatory Activity of Water Extract of *Luvunga sarmentosa* (BI.) Kurz Stem in the Animal Models

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Abstract

The study was aimed to determine the anti-inflammatory activity of water extract of the *Luvunga sarmentosa* stem in an animal model. Twenty-five Wistar rats were divided into five groups (n=5). Group 1 was administered 0.9% normal saline (negative control), group 2 was administered 150 mg/kg diclofenac sodium (positive control), and groups 3 to 5 were administered 50, 300, and 550 mg/kg BW of *L. sarmentosa* extract, respectively. Carrageenan was injected subcutaneously into each rat's subplantar region of the left hind paw. The paw volume was measured using a plethysmometer. The results showed that the water extract of *L. sarmentosa* stem (doses of 50, 300, and 550 mg/kg BW) significantly reduced the paw edema volume from the 4th to 5th hour compared to the negative control. The percent inhibition of edema at the 5th hour is 47.45; 46.95; 50.39%. The first phase of the edema (1st and 2nd hour) was not affected by the extract. Meanwhile, diclofenac sodium decreased paw edema volume from the 1st to 5th hour with a percent inhibition of 95.90% at the 5th hour. The histopathology result is relevant to the percentage inhibition of edema. Treatment with *L. sarmentosa* extract showed slight improvement, destruction of epidermal tissue, hyperkeratotic skin, and subepidermal edema. Meanwhile, positive control showed no inflammatory signs with normal keratin, subepidermal, and subcutaneous layers. The water extract of *L. sarmentosa* stem has anti-inflammatory activity. This extract effectively reduces the paw edema volume in the late phase with decreased neutrophil infiltration.

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INTRODUCTION

Inflammation is the body's normal response to wounds, injuries, microbial infections, allergies, and other harmful factors[1,2]. Symptoms of inflammation are pain, swelling, redness, heat, fever, and loss of body tissue function[3]. These symptoms are caused by inflammatory mediators and chemical agents such as prostaglandins (PG), serotonin, histamine, bradykinin, nitric oxide, and leukotrienes[4]. Inflammation plays a vital role in the physiological process. However, if the inflammatory process is prolonged and the offending agent persists, the intended protective process tends to be destructive that can damage the cell and cause various diseases[5-7].

Steroid and non-steroidal anti-inflammatory drugs (NSAIDs) are often used to treat pain and manage inflammatory conditions. The NSAIDs inhibit cyclooxygenase enzymes (COX-1 and COX-2), decreasing prostaglandin production[8,9]. The use of such drugs causes severe side effects, including severe gastrointestinal toxicities such as gastric ulcers and bleeding. Therefore, this instigates the development of effective, safe, and economic anti-inflammatory drugs[10].

Natural products from medicinal plants have been considered a potential alternative source of pharmacological substances with minimal adverse effects[11]. The plant represents a significant natural source of valuable compounds that might lead to novel drugs. World Health Organization (WHO) reported that about 70–80% of the world's population relies mainly on plant-based drugs. Its demand is increasing daily in developing countries[12-14]. Accordingly, there is a renewed interest in medicinal plant research to identify alternate agents that may be cheaper and have fewer adverse effects[15].

Luvunga sarmentosa (Bl.) Kurz, known as saluang belum in Uut Murung district, Central Kalimantan[16]. This plant is one of the endemic plants of Borneo Island, often used by local ethnic groups to increase male vitality[17]. The ethanolic extract of *L. sarmentosa* increased the number of spermatocytes and spermatid cells and showed aphrodisiac activity in male albino Wistar rats[18]. Several studies have reported compounds from *L. sarmentosa*. Flavonoids, steroids, and tannin have been isolated from the plants' roots[18]. Apotirucallane triterpenoids named luvungins A–G and 1a-acetoxyluvungin A (apotirucallane triterpenoids) were isolated from leaves[19].

The Dayak community uses a combination of *L. sarmentosa* and pasak bumi (*Eurycoma longifolia*) to increase stamina, sexual arousal, and male fertility by drinking root boiled water once a day. These plants are often used in a mix and prescribed root or stem, but the majority are used by the public, especially the root. Therefore, more attention is needed to avoid experiencing scarcity in nature, such as using stem parts instead of roots[16]. The use of mixed plants possibly aimed to obtain a synergism effect, in which *E. longifolia* was reported to have anti-inflammatory activity[20]. However, the effect of anti-inflammatory on *L. sarmentosa* has not been investigated. This study aims to determine the anti-inflammatory activity of water extract of *L. sarmentosa* (Bl.) Kurtz stem. This study's results could be used as supporting data on the utility of *L. sarmentosa* water extract in traditional medications.

MATERIALS AND METHODS

Materials

The stems of *L sarmentosa* was collected from traditional healers in the Pager, Rakumpit district, Palangka Raya City, Central Kalimantan, Indonesia on September 2019 (**Figure 1**). A licensed botanist made authentification and plant identification at Purwodadi Botanical Garden, East Java, Indonesia, with voucher specimen number No.1048/IPH.06/HM/IX/2019.

Figure 1. *Luvunga sarmentosa* stem simplicia

Methods

Plant extraction

The stem of *L. sarmentosa* was shade dried and powdered mechanically. The dried powdered (400 g) was extracted in water at 40-50°C for approximately 30 minutes. The extract was then filtered and concentrated with a vacuum evaporator and then dried with a freeze dryer to obtain a dry extract.

Experimental animal

Male Wistar rats (250-300 g) were obtained from the Laboratory Animal of the Department of Pharmacology, Faculty of Medicine, Universitas Airlangga. They were housed at a temperature of 25 ± 1°C, 12-hour light/dark cycles, and fed a standard rodent diet with water *ad libitum*. All the animals were acclimatized to the laboratory conditions before experimentation for seven days. Permission and approval for animal studies were obtained from the Faculty of Veterinary Medicine, Universitas Airlangga, with approval number KE.026.03.2021.

Anti-inflammatory activity by carrageenan induction

The carrageenan-induced paw edema model was used to evaluate the anti-inflammatory effect of *L. sarmentosa* extract (400 g). The initial paw volume was recorded using a plethysmometer (UGO Basile® 7140, Italy). Twenty-five male rats were selected and randomly divided into five groups (n=5). The negative control group was administered 0.9% normal saline (G1). The positive control group was administered 150 mg/kg of sodium diclofenac (G2), and the three test groups were administered 10, 40, and 80 g of simplicia *L. sarmentosa*, which is equal to extract doses of 50, 300, and 550 mg/kg BW, respectively (G3-G5). All drugs were administered an hour orally before the delivery of carrageenan injection. Carrageenan (0.1 mL of 1.5% w/v) was injected subcutaneously into the subplantar region of the left hind paw of each rat. The right hind paw was not treated and taken as a comparison. The paw volume was measured at 0, 30 minutes, 1, 2, 3, 4, and 5 hours following carrageenan injection using a plethysmometer[20-22]. The formula for calculating the percentage of inhibition was presented in equation [**1**], in which A was the mean paw volume for the test group and B was the mean paw volume for the control group.

$$Inhibition\ percentage = \frac{A-B}{B} \times 100\% \quad \ldots [1]$$

Histopathological analysis of paw tissue

The left hind paw of each rat was collected five hours after carrageenan was injected. The entire paw tissue sections (5 mm) were fixed by immersion in 10% formalin solution at room temperature. Paraffin-embedded paw tissue sections were stained with hematoxylin and eosin (H&E). Observation of structural abnormality and photographed under a light microscope (Olympus CKX41 microscope equipped with a digital camera). The observation was conducted at the Department of Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, to analyze the severity of paw tissue inflammation.

Data analysis

The results were presented in mean ± SEM, in which each value represents a minimum of five rats (n=5). The rise in paw volume data was tested for one-way analysis of variance (ANOVA) using GraphPad version 9.0 for Windows Software, followed by Dunnett's multiple comparison tests. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The extraction of the *L. sarmentosa* stem was carried out using water as a solvent at 40-50°C. The extraction yielded 5.5% w/w dry matter and was light brown. In this study, the water extract evaluated the anti-inflammatory activity induced by carrageenan. The carrageenan induction of rat paw edema is a suitable test for evaluating the anti-inflammatory activity of natural products[22,23]. Carrageenan-induced inflammation is acute, non-immune, well researched, and highly reproducible[24]. Carrageenan is used as a phlogistic agent, a substance that causes inflammation or edema[25].

The anti-inflammatory effect of water extract of *L. sarmentosa* stem on carrageenan-induced edema in rat's hind paws is presented in **Tables I** and **II**. Extract and sodium diclofenac significantly reduced the paw edema hours after carrageenan injection. For the control, swelling increased progressively to a maximum volume of 3.61±0.95 at five hours after carrageenan injection (**Figure 2**).

The first phase of the edema (1st and 2nd hour) was not affected by the water extract of *L. sarmentosa*. Administration of 50, 300, and 550 mg/kg extract significantly reduced the paw edema volume from the 4th to 5th hour compared to the negative control. Inhibition percentage of edema at the 5th hour of extract doses 50, 300, and 550 mg/kg showed no significant difference (47.45; 46.95; 50.39%), so we suggest using 50 mg/kg doses of the extract. This is to minimize the toxicity that may arise from the extract. On the other hand, 150 mg/kg of sodium diclofenac substantially decreased paw edema volume from the 1st to 5th hour compared to the negative control. Maximum percent inhibition of edema (95.90%) was estimated at the 5th hour after the carrageenan administration. This result confirms that sodium diclofenac has higher inhibition against inflammation than water extract of *L. sarmentosa*.

Table I. Average paw size of a rat in all groups after carrageenan injection

Groups	Dose (mg/kg)	Average paw size (mL)						
		0 minute	30 minutes	1 hour	2 hours	3 hours	4 hours	5 hours
Negatif control	-	3.67±0.29	4.83±0.27	5.40±0.30	5.92±0.53	6.40±0.90	6.93±1.08	7.28±1.09
Positive control	150	4.10±0.45	4.27±0.47	4.28±0.58*	4.20±0.37****	4.28±0.62****	4.33±0.53****	4.25±0.43****
Luvunga sarmentosa water extract	50	3.78±0.14	5.50±0.30	5.63±0.82	5.94±0.60	6.02±0.41	5.88±0.32*	5.68±0.25**
	300	4.05±0.40	5.90±0.37*	6.08±0.37	6.23±0.44	6.60±0.34	6.19±0.13	5.97±0.47*
	550	4.18±0.76	5.48±0.85	6.07±0.51	6.29±0.53	6.48±0.40	6.04±0.55	5.97±0.51*

Data were reported as mean±SD; n = 5. One-way ANOVA was carried out using Dunnett's multiple comparison test. Symbols represent statistically significant: *p <0.05 **p <0.01 ***p <0.001 ****p <0.0001

Table II. Percentage inhibition of inflammation in all groups after carrageenan injection

Groups	Dose (mg/kg)	Inhibition of edema (%)			
		2 hours	3 hours	4 hours	5 hours
Negative control	-	-	-	-	-
Positive control	150	95.47	93.56	92.87	95.90
Luvunga sarmentosa water extract	50	4.26	18.16	35.50	47.45
	300	3.29	6.88	34.40	46.95
	550	6.22	15.59	42.75	50.39

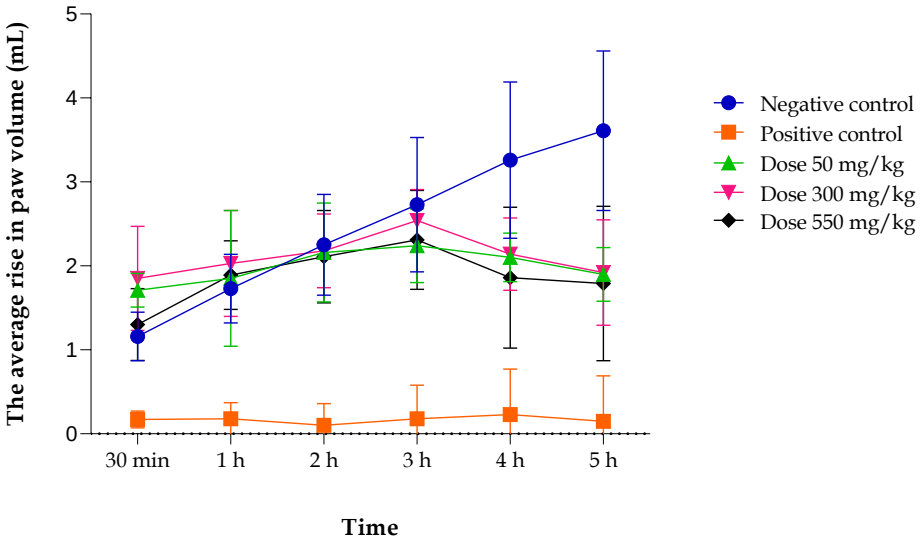

Figure 2. The average rise in paw volume in all groups after carrageenan injection (n=5)

Histopathology analysis of paw tissue showed a massive influx of inflammatory cell infiltration, proliferated collagen, keratinization was decreased dermis, and subepidermal edema in the negative control. Treatment with *L. sarmentosa* extract showed slight improvement, destruction of epidermal tissue, hyperkeratotic skin, and subepidermal edema. Meanwhile, positive control showed no inflammatory signs with normal keratin, subepidermal, and subcutaneous layer. The histopathology result was relevant to the inhibition percentage of edema (**Figure 3**).

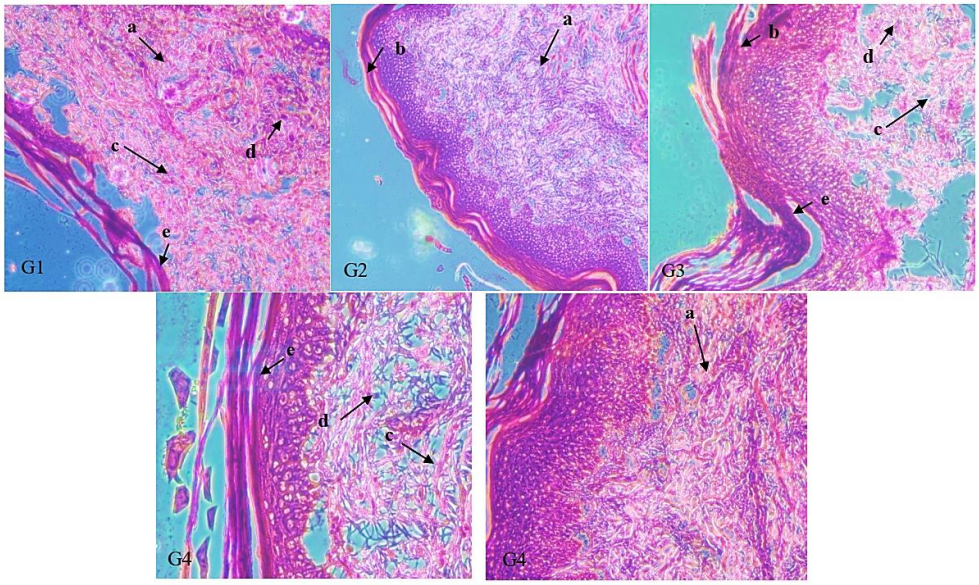

Figure 3. Histology of rat paw tissue after five hours injected with carrageenan. **G1**: negative control; **G2**: positive control; **G3**: dose 50 mg/kg BW; **G4**: dose 300 mg/kg BW; **G5**: dose 550 mg/kg BW of *L. sarmentosa* water extract with H&E staining and 400x magnification. **a**: dermis; **b**: epidermis; **c**: subepidermal edema; **d**: inflammatory cell infiltration (ICI); **e**: creatine

Carrageenan injection given subplantar will increase the rat paw's swelling, consisting of a relatively fast initial phase (up to 3 hours), followed by a late phase (3-5 hours)[2]. The initial phase was the release of histamine, serotonin, bradykinin, and a small number of prostaglandins produced by the COX enzyme. The late phase was associated with neutrophil infiltration, releasing free radicals, nitric oxide, pro-inflammatory cytokines, and continued prostaglandins[26]. We suggest that the administration of *L. sarmentosa* extract is effective in the late phase with decreased neutrophil infiltration.

CONCLUSION

The water extract of *L. sarmentosa* stem has anti-inflammatory activity, which effectively reduces the paw edema volume in the late phase.

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AUTHORS' CONTRIBUTION

Sabar Deyulita: Extraction, anti-inflammatory test, data analysis, and article writing. **Hilkatul Ilmi**: anti-inflammatory test, data analysis, and article writing. **Hanifah Khairun Nisa**: histopathological examination and data analysis. **Lidya Tumewu**: Extraction and article writing. **Aty Widyawaruyanti**: Supervision, conceptualization, validation of methods, writing review & editing. **Achmad Fuad Hafid**: Supervision, conceptualization, validation of methods, writing review & editing.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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