Intradermal Irritation Assessment of Ethyl Acetate Extract of Sarang Semut (Myrmecordia pendans) on Rat Skin: An In-Depth In Vivo Study

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Abstract

Bone defects, arising from conditions such as periodontitis and fractures, present formidable challenges due to their complex and intricate healing processes, often leading to failures or delays. Consequently, there is a pressing need for innovative strategies to expedite this healing trajectory. One promising avenue lies in the exploration of medicinal plants indigenous to specific regions, such as sarang semut (*Myrmecodia pendans*) from Indonesia.

This study conducted sensitivity and irritation tests on male Wistar rats, segregated into test and control groups. The group received 1 cc of physiological NaCl or 1 cc of the test material, namely, ethyl acetate extract of sarang semut containing flavonoids. Skin changes, including edema, erythema, and edema area measurements, were monitored over a span of 24 hours. Remarkably, both groups exhibited no signs of erythema. Regarding edema, the control group scored zero at 12 hours, while the test group reached this at 24 hours, indicating slight irritation (PII index < 2 mm).

Crucially, statistical analyses revealed no significant differences in edema diameter between the groups. This evidence underscores that the ethyl acetate extract of sarang semut, enriched with flavonoids, does not induce irritation. Hence, it emerges as a promising avenue for further exploration in subsequent scholarly investigations, offering potential solutions for challenging bone defect scenarios.

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Introduction

A bone defect is a disorder in which some of the bone's tissue is lost; a fracture, trauma, or infection like osteomyelitis can bring it on. Bone defects are repaired in the same way as bone fractures as long, flat, or even alveolar bones. These are all lengthy and intricate procedures.[1] The size of the defect region affects the healing of bone defects greatly; if it surpasses a critical size, delays or even poor healing outcomes frequently occur. According to several research studies, a bone defect is considered important if its length does not exceed 2 cm and it does not cause the bone to lose mass by more than 50% of its circumference.[2] To overcome this problem, various types of treatment have been developed to shorten the healing process and prevent failure at the end of healing. One type of treatment that is currently being developed is herbal treatment. This treatment is expected to speed up and complete the healing process.

In the rich biodiversity of Indonesia, the sarang semut (*Myrmecodia pendans*) plants, originating from the island of Papua, has emerged as a traditional herbal remedy. First introduced in 2006, this plant has garnered attention due to its healing properties.[2,3,4] Based on indigenous knowledge and observational practices, it has been integrated into traditional medicine. According to prevalent beliefs, this

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plant has the capacity to treat a spectrum of illnesses, including cancer and infectious diseases. Subsequently, numerous studies have been initiated. According to the findings from several conducted studies, it is claimed that *Myrmecodia pendans* can reduce inflammatory responses and expedite the healing process of diseases.[3,4] The healing power of this plant is attributed to its chemical components.[4]

Myrmecodia pendans, belonging to the Rubiaceae family, shares a unique symbiotic relationship with ants. This plant is attached to a large tree where the lower part of the trunk is swollen, containing a cavity occupied by ants of a certain type, living at an altitude of 2400m above sea level.[4] Chemical analyses have unveiled the presence of flavonoids, tannins, polyphenols, and triterpenoids within the plants.[5] Flavonoid compounds in this plants, including kaempferol, luteolin, routine, quercetin, and apigenin, as well as phenolic acids, were identified as robust antioxidants.[3,6] These compounds play a crucial role in neutralizing and eliminating free radicals, highlighting their significance in cellular protection.[6] In addition to their antioxidant properties, flavonoids offer various health benefits, exhibiting antiproliferative, anticarcinogenic, antibacterial, anti-inflammatory, anti-allergic, and antiviral effects, thus showcasing their potential therapeutic applications.[7,8]

Before integrating herbal remedies into medicinal practices, thorough evaluations are crucial to ensure safety and effectiveness. Sensitivity or irritability testing, typically conducted in vivo on experimental animals, provides vital insights into potential allergic responses. This study focused on the ethyl acetate extract of *Myrmecodia pendans*, rich in flavonoids. The primary goal was to assess its impact on rat skin, determining its suitability for medicinal use. Irritation, in this context, signifies the body's response to introduced substances, indicated by inflammatory signs like edema and erythema. The ultimate aim remains establishing the material's safety and efficacy for potential medicinal applications.[11]

Materials and methods

All procedures in this study were in accordance with the ethical standards. This research has received ethical permission from the Animal Ethics Committee Faculty of Veterinary Medicine Bogor Agricultural University with reference number 023/KEH/SKE/XII/2020.

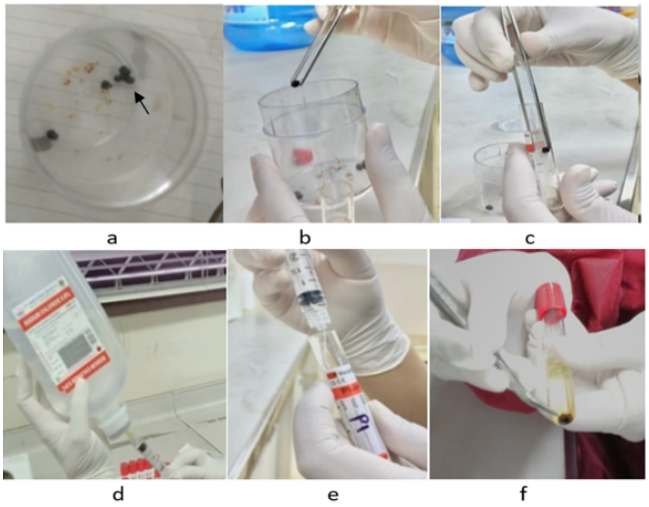

Figure 1. Procedure for preparing test materials: a, b. The ethyl acetate of *Myrmecodia pendans* extract containing flavonoids, formed into spheres weighing 2 mg each; c to f. Preparation of materials, 1cc of NaCl is prepared, and NaCl is put into the tube, five tubes for each group. For the test group, the test material is added the ethyl acetate of *Myrmecodia pendans* extract containing flavonoids.

The test material used was the ethyl acetate extract of *Myrmecodia pendans*, containing flavonoids in paste form. This extract originated from the Sorong area of the Ayawai Mountains in Papua and was analyzed at the Biology Laboratory, Padjadjaran University, Bandung, Indonesia. The paste was shaped into 2 mg small balls, which were then dissolved in 1cc of physiological NaCl, resulting in five doses corresponding to the number of test animals. The mixture was left to stand for 2 x 24 hours at room temperature (36°C or 96.70°F) to ensure the complete dissolution of the extracted material in the solvent, in this case, physiological NaCl. The 2 mg dosage was determined based on the MTT assay test, demonstrating optimal growth effectiveness at this concentration (Figure 1 and 2). The experiments were conducted on male Wistar strain rats of the Rattus Norvegicus species, weighing 200-250 grams and aged 12 weeks. A total of 10 rats were divided into two groups: the control group received an injection of 1cc of buffer solution (Physiological NaCl), while the test group received an injection of 1cc of the test material solution. Each solution was

intradermally injected into the shaved skin on the rats' backs (Figure 3).

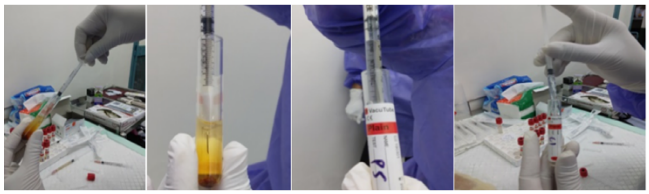

Figure 2. Preparation of the materials to be tested on animals.

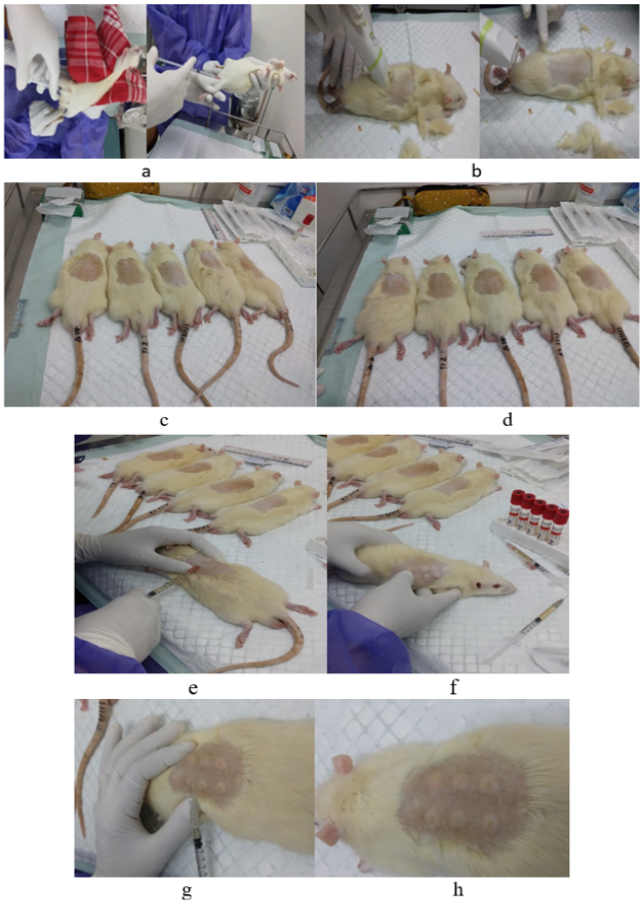

Figure 3. Preparation and testing of the animal subjects: a. Animals anesthesia procedures before the procedure was carried out. b. The process of shaving animal back hair. c and d, Two groups of animals that are ready for testing. e, f, g and h. The process of injecting the test material intradermally into the animal's back, 10 injections of 1cc each on the back of each animal subject.

Skin Reaction	Score	z
Erythema and Eschar Formation		
No erythema	0	
Very slight erythema	1	
Well-defined erythema	2	
Moderate to severe erythema	3	
Severe erythema with slight eschar formation	4	
Edema Performance		
No edema	1	
Very slight edema	2	
Slight edema with raised margin	3	
Moderate edema with a raised margin≈of 1 mm	4	
The interpretation of Scoring		
slight irritation	< 2	
moderately irritation	2 to 5	
severely irritation	>5	

Table 1. Primary skin irritation scoring[13,14,15,16].

The technical procedure for sensitivity test, employing a skin test or intradermal irritation test method, was conducted based on the modified Buehler method.[12] In this test, skin irritation and inflammation were assessed through indicators such as redness and edema, as outlined in Table 1.[13,14] Observations and recordings were made at 0, 1, 2, 3, 12, and 24-hours post-injection. These observations followed the administration of the test material. Three evaluators, including a researcher and two veterinarians, utilized the Primary Skin Scoring (PSI) method from the Organization for Economic Co-operation and Development 2002 for assessment. Scoring interpretation was determined using the Primary Intradermal Irritation Index (PII Index) established by Lansdown (1972), as detailed in Table 1.[13,14] The calculation of the Primary Intradermal Irritation Index involved adding the erythema and edema values, which were then divided by the total number of experimental animals.[15-18]

$$PII = \frac{\Sigma \text{ score Ed} + \Sigma \text{ score Er}}{\Sigma \text{ animals score}}$$

Subsequently, measurements were conducted for both the area and diameter of the swelling (Figure 4). This process involved assessing the swelling's overall area and its specific diameter. The area and diameter measurements were performed utilizing ImageJ software. All the gathered data were statistically analyzed through tests comparing differences

between two distinct groups and differences within the data from the same group.

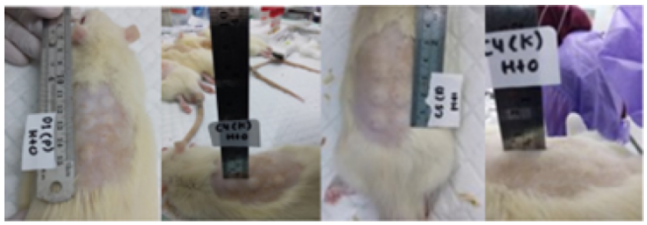

Figure 4. Measurement of area and diameter of swelling.

Score of Edema and Erythema				
Hour	Control Group		Test Group	
	Ed	Er	Ed	Er
0	4	0	4	0
1	3.73	0	3.73	0
2	2.33	0	2.67	0
3	1.47	0	1.47	0
12	0	0	0.07	0
24	0	0	0	0
Σ Data	11.53	0	11.94	0
Score of PII Index				
	Control Group		Test Group	
Score	1.15		1.19	
	Slight irritation			

Table 2. Score of edema, erythema and PII index. *Ed: Edema values, Er: Erythema values.

Results

The research results obtained regarding the edema, erythema and PII analysiscan be seen in Table 2. The control group exhibited an edema score of 4 at 0 hours (h0), which steadily decreased from h2 to h12, reaching a final score of 0. Similarly, the test group displayed a gradual decrease in edema score at h2, followed by rapid reduction after h3, ultimately reaching zero at h24. Both control and test groups registered zero for erythema score. There were indeed differences in the two groups where, in the control group, a score of zero was obtained more quickly compared to the group test. However, PII calculations for both groups categorized them as having slight irritation, with scores <2.

Table 3 presents the results of measuring the area and diameter of edema after the test. The table displays scores for the largest edema area and diameter, observed at h0, both of which decrease proportionally with time. In the control group, the value reached zero at h12, whereas in the test group, the edema disappeared by h24. Subsequently, the observation was stopped because there were no more areas of edema that could be assessed.

There was no significant difference in the area and diameter of the edema area between the control and test groups ($p<0.05$), indicating that the two groups had the similar means. The difference in the size of the area and diameter of the edema in the control group (between h0 to h24) apparently shows that the area variable has a difference between the values ($p<0.05$). In contrast, the diameter variable shows no difference ($p>0.05$). Meanwhile, in the test group, both area and diameter of edema showed statistical differences, where both showed $p<0.05$.

Group	Hour	Wide of Edema Area	Diameter of Edema Area
Control Group	0	64.48	8.96
	1	27.93	5.61
	2	14.54	4.28
	3	0.62	0.59
	12	0	0
	24	0	0
Test Group	0	73.9	10.53
	1	34.7	6.63
	2	16.19	4.56
	3	0.65	0.71
	12	0.04	0.1
	24	0	0

Table 3. Results of measuring the area and diameter of edema.

Discussion

In the context of medical advancements, the significance of herbal medicine cannot be overlooked. It offers a potential reduction in dependence on or supplementation to chemical medicines, providing a natural alternative for healing processes. The treatment of bone defects in dentistry, especially in cases of trauma or defects in the alveolar bone, necessitates breakthroughs for a more perfect healing process. *Myrmecodia pendans* plants are currently being studied, and it is imperative to subject them to a sensitivity test before utilization. Skin irritation assessment is crucial for evaluating the safety of medical products, with tests primarily conducted in animal models.[19,20]

Skin irritation assessment stands as a crucial step in evaluating the safety of medical products. Skin irritation refers to skin damage occurring after applying a test substance for up

to 4 hours. Typically, it is assessed based on a substance's potential to cause erythema or edema after a single topical application.[17,18,19] Skin sensitization indicates an allergic reaction to a particular irritant, manifesting as irritation, itching, or skin abnormalities.[17] Noor Farizah et al. investigated the effects of irritation on rabbits following the application of essential oil from Piper sarmentosum.[19] Similar research was conducted by Craig et al., studying acute skin toxicity with pure essential oils extracted from Juniperus occidentalis and Chamaecyparis lawoniana plants at concentrations of 0.5%, 5%, and 50% in albino subjects in New Zealand.[20] Other studies that use the same method are from Suresh Jain et al., where the test was carried out on mice with the test material Chitosan Containing Trazodone-HCl.[21] Similar methodologies have been adopted in various studies, albeit with different animal models.

Based on the results of sensitivity tests in this study, irritation tests were carried out on the skin on the backs of rats; it was proven that ethyl acetate extract from *Myrmecodia pendans* did not cause irritation. This is proven by the results of the erythema test, which, at the start of the test (h0 to h24), did not show any redness on the skin. Meanwhile, the edema test produced data that starting from the administration of the h0 material, there was mild irritation, and it continued to improve until the edema deflated and disappeared. Edema disappeared on h24, and correction 3 to 6 hours later did not show recurrent edema. Based on the PII index calculation, this condition can be categorized as slight irritation. The statistical results also explain that the variables of the area of edema and the diameter of the swelling starting from h0 to h24 showed statistically significant differences in ant nest ethyl acetate extract with flavonoid content, both of which did not show any difference.[22,23] The sensitivity test was conducted for 72 hours, but it was terminated 3 hours after the 24-hour mark due to the absence of visible signs of irritation. This forms the basis for further research on the use of ethyl acetate extract from ant nests containing flavonoids.[22]

Despite the promising results, this study still has some limitations. The sample size utilized for testing in this study was relatively small, and the specific flavonoid types present in the extract remain unidentified. Additionally, the variety of test methods employed could be broadened for a more comprehensive analysis. Addressing these limitations in subsequent research endeavors is paramount to enhancing the reliability and applicability of the findings. As a step toward innovative medical solutions, these results lay the foundation for further investigations into the therapeutic potential of *Myrmecodia pendans* in the realm of healing bone defects, offering a natural alternative for the advancement of medical science.[23]

Conclusions

The ethyl acetate of *Myrmecodia pendans* extract containing flavonoids, after a skin test was carried out on male rats, was proven not to cause irritation to slight irritation. Thus, this material can be continued as a test material for other tests and in this case, as a material to accelerate the healing process of bone defects in fractures or bone injuries.

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All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

Declaration of Interest

No conflicts of interest are disclosed by the authors.

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