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Original Research Article

Anti-Arthritic Activity of Combination of *Caesalpinia sappan* and *Zingiber officinale* Extracts in Complete Freund's Adjuvant- Induced Arthritic in Rats

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ABSTRACT

Several plant species, including sappan and red ginger, have been studied to treat inflammation. Each species has been tested for anti-arthritis, but a combination of the two species has never been done. This study was conducted to test the anti-arthritis potential of a combination of ethanol extract of sappan wood and red ginger using a rat model of Complete Freund's Adjuvant-induced rheumatism. The study involved several percentages of inhibition of rat paw edema volume (%), arthritis index, histopathological profile, and haematological evaluation. The test formula prepared included sappan wood single extract (ES), red ginger single extract (ERG), comparison of the combination of two extracts at doses F1 (1 ES: 1 ERG), F2 (2 ES: 1 ERG), and F3 (1 ES: 2 ERG). The results showed that the F2 formula had the best anti-arthritis effect with an inhibition percentage of rat paw edema volume of 86.72% by showing relatively mild inflammatory cell infiltration, the formation of connective tissue through histopathological observations of rat foot tissue. Haematological observations of platelet increase were highest in group F2 which functions to defend the body from toxic substances. There was no significant difference in the rat haematological parameter in each treatment group ($p>0.05$).

Keywords: Anti-arthritis, Complete Freund's Adjuvant, red ginger, sappan

Introduction

Rheumatoid arthritis (RA) is a persistent, inflammatory, systemic autoimmune disorder distinguished by inflammation of the synovial membrane, swelling, generation of antibodies, and impairment of cartilage and bone in the vicinity of the joints.[1] The user's text is already academic. In industrialized nations, there is a higher incidence of this particular ailment among males compared to females, particularly between the age range of 30 to 55 years. However, it is important to note that this condition can manifest at any age.[2] However, it is important to note that this condition can manifest at any age. The user's text is too short to be rewritten in an academic manner. The etiology of cartilage deterioration and joint destruction in rheumatoid arthritis can be attributed to an inflammatory response characterized by an upregulation of inflammatory mediators, including cytokines, chemokines, and reactive oxygen species (ROS), which are synthesized within the joint tissue. The involvement of proinflammatory cytokines, including interleukins IL-6, IL-1b, IL-1, and tumor necrosis factor (TNF-a), in the pathogenesis of arthritis has been well established.[3] The user's text is too short to be rewritten in an academic manner. Interleukin-6 (IL-6) facilitates the process of inflammation by inducing angiogenesis, which is the formation of new blood vessels. Interleukin-1 (IL-1) plays a role in facilitating bone resorption, cartilage breakdown, and subsequently modulates the synthesis of nitric oxide (NO) and prostaglandins (PGE2).

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Tumour necrosis factor-alpha (TNF-α) amplifies the inflammatory response by activating synovial fibroblasts, which express cellular adhesion molecules and promote the infiltration of leukocytes into the joints, ultimately leading to tissue destruction. Prostaglandin E2 (PGE2) has the ability to activate pain receptors and elicit a pyrogenic response.[4] In the context of osteoarthritis, the degenerative state of the cartilage matrix is further aggravated by the presence of oxidative stress, which arises from an excessive production of reactive oxygen species (ROS).[5] An imbalance between proinflammatory and anti-inflammatory circumstances can lead to inflammation of the synovial membrane and subsequent joint injury.[6]

The current treatment for rheumatoid arthritis (RA) encompasses a range of medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), tumour necrosis factor-alpha (TNF-a) antagonists, anti-interleukin receptor antibodies (IL-6), IL-1 receptor antagonists, steroid agents, and immunosuppressants/immunomodulators. These medications have been reported to effectively suppress inflammation and ameliorate symptoms associated with rheumathoid arthritis [7]. The suppression of proinflammatory cytokines has emerged as a critical approach for managing inflammation in contemporary time[8].

Nevertheless, the current medical interventions for rheumatoid arthritis have not yet yielded a definitive cure. Furthermore, it should be noted that a very high cost characterizes nonsteroidal anti-inflammatory medicines (NSAIDs), and their prolonged usage has been associated with the potential for significant adverse effects. These side effects include but are not limited to nausea, anorexia, gastritis, epigastric discomfort, stomach ulcers, flatulence, and diarrhoea, which have been reported in 15-60% of patients.[9] This medicine may also be associated with renal and cardiovascular problems, including acute renal failure, hypertension, and electrolyte abnormalities. The extended utilization of steroids has been found to be linked to various adverse effects, including hindered wound healing, osteoporosis, muscle weakness, cataracts, and gastrointestinal ulcers.[10]

The side effects caused by drug therapy have prompted many arthritis sufferers to turn to herbal medicine. Many herbal plants have been explored and researched for RA treatment alternatives. It was reported that herbal plant products that have a variety of phytonutrient

compositions have the potential to treat various diseases.[11] Several plant species have been studied for their potential in the treatment of inflammation, including sappan wood and red ginger which have an important role in reducing pain and inflammation associated with rheumathoid arthritis.[12,13]

Sappan is known to possess phytochemical constituents belonging to diverse structural categories of phenolic compounds, such as xanthones, coumarins, chalcones, flavone, homoisoflavonoids, and brazilin,[14] sappanone,[15] caesalpiniaphenols E and F,[16] sappanchalcone,[17] and brazilein. The main phytochemical constituent present in sappan wood is brazilin which has so far been reported to have pharmacological activities, namely vasorelaxant,[18] anticancer,[19] anti-inflammatory,[20] and also used in the treatment of rheumatoid arthritis.[12]

In contrast, it has been documented that red ginger possesses pharmacological properties, including analgesic, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, nephron protective, and antioxidant activities.[23] Additionally, red ginger has been found to be efficacious in alleviating symptoms associated with chronic inflammatory conditions. Red ginger is known to possess various bioactive chemicals, including gingerol and its derivatives (6-gingerol, 10-gingerol, etc); shagol and its derivatives (6-shogaol, 10-shogaol, etc); zingerone, 6-paradol, 10-dehydrogingerdione, 10-gingerdione.[21] The main pharmacological activity of red ginger appears to be due to gingerols and shogaols.[22] Given the involvement of oxidative stress and inflammatory responses in the pathogenesis of arthritis, antioxidant and anti-inflammatory substances derived from plants have potential in the treatment of rheumathoid arthritis.[24]

The utilization of a combination of the two plants as herbal medicinal preparations for the treatment of inflammatory illnesses is based on the documented anti-inflammatory action of these plants. Polyherbal formulations, which involve the utilization of extracts from multiple plant species, are believed to possess supplementary pharmacological properties that can interact synergistically and dynamically. This interaction is expected to result in the attainment of optimal therapeutic advantages while minimizing the occurrence of adverse effects.[25] Nevertheless, the investigation into the anti-arthritis properties of the two plant extracts has not yet been conducted. Hence, the present investigation was undertaken to assess the potential antiarthritic effects of a mixture of ethanol extracts derived from two plant species, employing rat models with arthritis produced by Complete Freund's Adjuvant. two plants using Complete Freund's Adjuvant-induced arthritis rat models.

Materials and Methods

Collection of Sappan Wood and Red Ginger

The specimens included in the study consisted of sappan wood (*Caesalpinia sappan*) and red ginger (*Zingiber officinale*) rhizome. The sappan wood was procured from the Rengganis Jamu Shop, located in Gresik at coordinates 7°09'06.7"S 112°39'25.0" E, on January 17, 2023. The red ginger was acquired on January 18, 2023, from the Wonokromo Market located in Surabaya at coordinates 7°18'07.8"S 112°44'17.1" E. The Sappan wood obtained was in a desiccated state. The rhizomes of red ginger were subjected to a washing process using running water, followed by thin slicing, and subsequent drying.

Preparation of Sappan Wood and Red Ginger Extracts

The ethanol extracts of *Caesalpinia sappan* wood and *Zingiber officinale var. rubrum* were acquired using the maceration technique. The Sappan wood simplicia powder and red ginger were individually measured to have a mass of 500 g each. Subsequently, they were immersed in 96% ethanol for a duration of 24 hours and subsequently subjected to filtration using a Buchner funnel. The activity as mentioned earlier was conducted on three separate occasions. The macerate underwent filtration and concentration by the utilization of a vacuum rotary evaporator, resulting in the acquisition of a dense extract. Additionally, each of these viscous extracts underwent a freeze-drying procedure in order to eliminate any residual water content.

Preparation of Experimental Animal

The study utilized male Wistar rats with a weight range of 150-200 g, who were housed in animal cages under controlled laboratory settings, including a temperature of 25°C and a humidity level of 60%. The animals were provided with unrestricted access to conventional animal pellets and water. The animals underwent a two-week acclimatization period to conventional laboratory settings before to the commencement of the experiment.[42] The experimental procedure employed in this work received ethical approval from the Ethics Committee of Airlangga University, Surabaya, with the reference number 147/EC/KEPK/FKUA/2022.

Research Preparation

Preparation of Sappan Wood Ethanol Extract Formula, Red Ginger, and Their Combination

The formula employed in this investigation consisted of an ethanol extract derived from sappan wood and an ethanol extract derived from red ginger, administered at a dosage of 30 mg/200 g BW/day for each extract. In the interim, the formula utilized for the amalgamation of the two extracts can be expressed as follows: The study consisted of three experimental groups: a) F1, which received a 1:1 combination of sappan wood extract (30 mg/200 g BW/day) and red ginger extract (30 mg/200 g BW/day), b) F2, which received a 2:1 combination of sappan wood extract (60 mg/200 g BW/day) and red ginger extract (30 mg/200 g BW/day), and c) F3, which received a 1:2 combination of sappan wood extract (30 mg/200 g BW/day) and red ginger extract (60 mg/200 g BW/day).

Preparation of Diclofenac Sodium Formula

The recommended dosage of diclofenac sodium for humans is 150 mg per day. In order to administer an equivalent dosage to rats, the conversion is made to 2.7 mg per 200 g of body weight. This dosage is then suspended in a solution containing 0.5% carboxymethylcellulose (CMC).[43]

Experiment Design

Forty male Wistar rats were randomly divided into 8 groups (n = 5 per group) with the following group details: 1) Normal healthy rats, 2) Rats induced arthritis received saline solution (negative control), 3) Arthritis induced rats treated with diclofenac sodium (positive control), 4) Arthriris induced rats administered with sappan wood ethanol extract solution (ES), 5) Arthriris induced rats administered with red ginger ethanol extract solution (ERG), 6) Arthritis induced rats induced F1, 7) Arthritis induced rats F2, and 8) F3-Arthriris induced rats.

Complete Freund's Adjuvant-Induced Rat Arthritis (Volume Edema and Arthritis Index)

Arthritis was induced in rats by sub-plantar administration of 0.1 mL of Complete Freund's Adjuvant (0.05% b/v *Mycobacterium butyricum*, which was weakened by heat in sterile paraffin oil) to the rats' right hind legs in all groups (except the normal group) on day 0 and measuring the volume of leg edema using a digital plethysmometer. On the 14th day after inoculation until the 28th day, the rats were treated as outlined in the experimental design. Respectively, the animals were observed for symptoms of rheumatism on days 14, 17, 20, 23, 26, and 29 based on the volume of rat leg edema as measured using a digital plethysmometer.

The anti-arthritis effect from the percentage inhibition of edema caused by Complete Freund's Adjuvant using the following formula:

$$\% \text{ edema volume inhibition} = 1 - \left[\frac{a-x}{b-y}\right] \times 100\%$$

Where:

a : average volume of rat leg ede.ma after induction of arthritis in each group of rats and given the extract or drug

x : average volume of leg edema before induction of arthritis in each group of rats and given the extract or drug.

b : average volume of rat leg edema after induction of arthritis in each group of rats that were not given the drug (negative control).

y : average volume of rat leg edema before induction of arthritis in each group of rats that were not given the drug (negative control).

The arthritis index was observed based on macroscopic score observations on a scale from 0 to 3 with the following details. Scale 0 means no signs of arthritis; scale 1 means there is light swelling and redness of the legs; scale 2 means there is medium swelling and redness of the legs; and scale 3 means there is severe swelling and redness of the legs. The arthritis index was calculated by adding the scores for each individual rat leg.[26]

Histopathological Profile of Rat Leg Tissue

The rat leg tissue was fixed by immersing it in a 10% formalin neutral buffer solution at room temperature. The rat limbs underwent a decalcification process using EDTA for a duration of 30 days, followed by their embedding within a paraffin block. The block was divided into sections with a thickness of 4 µm. The junction sections were then subjected to staining using Mayer's Hematoxylin solution for a duration of 8 minutes, followed by Eosin Y solution for 1 minute. These staining procedures were conducted at room temperature. The rat leg tissue was examined using a light microscope, with the complete field of view observed at magnifications of 20X, 40X, and 400X. The evaluation of the stained region is thereafter conducted using a numerical scale ranging from 0 to 3 in order to ascertain alterations in cell infiltration, synovitis, synovial proliferation, and cartilage or bone degradation. The scale ranging from 0 to 3 was interpreted and categorized in the following manner: The scoring system ranges from 0 to 3, with each score corresponding to specific pathological characteristics. A score of 0 indicates the absence of cell infiltration, synovitis, and damage to the synovial layer, cartilage, or bone. A score of 1 signifies a minimal presence of infiltrating cells, mild synovitis, limited pannus formation, and no evident damage to cartilage or bone. A score of 2 indicates a moderate density of infiltrating cells, moderate synovitis, medium pannus formation, and moderate damage to cartilage or bone. Lastly, a score of 3 denotes a substantial amount of cell infiltration, severe synovitis, extensive pannus formation, and significant damage to cartilage or bone.[28]

Rat Haematological Profile

Rats were sacrificed by administering ether anesthesia on day 29 and blood was taken through heart puncture and collected in a K-EDTA tube. Furthermore, blood was checked using a Hematology Analyzer based on the following parameters: hemoglobin level (HGB), total leukocyte count (WBC), and platelets (PLT) which were determined in the blood using anticoagulants with the usual standard laboratory methods.[27]

Statistical Analysis

The data was presented as mean ± standard error of the mean and tested by one-way analysis of variance followed by post hoct tukey test (SPSS). The results with $p < 0.05$, was considered statistically significant.[29] The statistical analysis was performed using the IBM SPSS Statistik for Windows software (version 25.0).

Results and Discussion

Rheumathoid arthritis is a chronic inflammatory (autoimmune) disease characterized by progressive joint damage. This study determined the anti-arthritis activity of a single extract of sappan wood, single extract of red ginger, and a combination of the two extracts (F1, F2, and F3) in inhibiting the volume increase in rat leg edema induced by Complete Freund's Adjuvant. Edema is a condition when the body experiences excess fluid which causes swelling in certain parts of the body. Complete Freund's Adjuvant is one of the inducers of arthritis that has been widely used as an experimental model to study the pathophysiology of this disease. Complete Freund's Adjuvant contains *Mycobacterium butyricum* which is attenuated so that arthritis occurs only locally in the induced area.[30] This bacterium is expected to stimulate inflammation in the soles of rats' feet. The presence of antigens in the form of bacteria will certainly stimulate the body to express the body's defenses in the form of mast cells and cytokines as inflammatory mediators. Activation of mast cells (an important cell of the immune system) in rheumathoid arthritis can cause the release of mast cell proteinases (tryptase and chymase), which are matrix metalloproteinnase (MMP) precursors that can degrade cartilage matrix.[31]

The experiment commenced by administering subplantar induction of Complete Freund's Adjuvant at a volume of 0.1 mL in the left leg of the rats belonging to each treatment group, with the exception of the normal control group. The severity of arthritis in each treatment group generated by Complete Freund's Adjuvant (CFA) was assessed over a period of 17 days. The evaluation was based on the extent of redness and swelling noticed on the soles, wrists, and toes of the rats. The severity of arthritis was assessed by assigning an arthritis index (IA) based on the symptoms observed following induction with Complete Freund's Adjuvant. The test animals were determined to have a positive diagnosis of arthritis if the index of arthritis (IA) was found to be more than 1. In this investigation, the manifestation of rheumatoid arthritis became apparent on the eighth day, as evidenced by the presence of swelling and redness observed on the soles and ankles of the experimental rats. By the 17th day, all treatment groups, with the exception of the normal control group, exhibited IA values greater than 1. This observation suggests that the rats in every treatment group exhibited symptoms of rheumatoid arthritis.

Moreover, the administration of the test formula was conducted in accordance with the predetermined treatment groups for a duration of 14 days. The objective of this study is to evaluate the potential anti-arthritis effects of sappan wood extract, red ginger extract, and a combination of both extracts on rats provoked with Complete Freund's Adjuvant, using the parameter of inflammatory arthritis (IA). A statistical analysis was conducted on the independent variable using the Kruskal-Wallis technique, resulting in a hypothesis with a significance level of less than 0.05 ($p < 0.05$). This implies that there exists a notable disparity in the observations of artificial intelligence (IA) between the several groups undergoing therapy. This finding demonstrates that the administration of varying doses of the formula in each treatment group results in the production of anti-arthritis action.

One of the distinguishing features of an inflammatory process in the soles, ankles, and toes of the test animals generated by Complete Freund's Adjuvant is the presence of symptoms such as redness and swelling. The phenomenon is distinguished by heightened circulation of blood that transports the body's immune agents to combat invading entities, specifically bacteria.[32] Angiogenesis (the process of forming blood vessels) will only occur to support the cell infiltration process as an initial pathological change in the synovium (tissue that is experiencing inflammation/inflammation).[33] Angiogenesis, the physiological process of blood vessel formation, is observed exclusively in response to the invasion of cells as an early pathological alteration in the synovium, a tissue undergoing inflammation. Cytokines implicated in the inflammatory process can also elicit localized capillary vasodilation, leading to heightened blood circulation and a consequent flushed appearance of the skin. The term "arise" refers to the act of coming into existence or becoming apparent[34]. Freund's Complete Adjuvant functions as an immunogenic substance that stimulates macrophages to generate proinflammatory cytokines, including IL-6, IL-2, and TNF-α. The immune system response can be stimulated by the upregulation of proinflammatory cytokines, leading to an overexpression of PGE-2 and subsequent inflammation[35].

Percentage Inhibition of Volume of Rat Leg Edema

The percentage of edema volume inhibition (%) of each treatment (sample) group is, shown in Table 1 and Figure 1. Data on decreasing edema volume in the positive control treatment, sappan wood ethanol extract, red ginger ethanol extract, and the combination of the two extracts in samples F1, F2, and F3 showed that the percentage inhibition of edema volume (%) increased until day 31 and reached each 80.73; 71.87; 59.37; 79.69; 86.72; and 80.00%. The anti-arthritis activity was in the order: F2 > positive control > F3 > F1 > ethanol extract of sappan wood > ethanol extract of red ginger.

The pharmacological effects of sappan wood extract and red ginger extract in treating arthritis can be linked to the phytochemical elements present in each extract. According to reports, sappan has been found to contain flavonoid components, including brazilin and brazilein, which exhibit anti-inflammatory effects. Similarly, red ginger is known to

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have phenolic compounds such as gingerol and shogaol, which also possess anti-inflammatory characteristics. Formula F2, which consisted of a mixture of sappan wood extract and red ginger, had the highest percentage of reduction in edoema volume (%) on the 31st day of the experiment. This implies that the F2 polyherbal formula exhibits pharmacological efficacy through the dynamic and synergistic interaction of its constituents, resulting in optimal therapeutic outcomes while minimizing adverse effects.[36]

Based on statistical analysis of data on the decrease in the volume of rat leg edema on days -17 to 31 it was shown that all treatment groups met the requirements of the normal distribution test ($p>0.05$) and were homogeneously distributed ($p>0.05$). Therefore, the next one-way ANOVA test was carried out and the results showed that there was no significant difference between the decrease in the volume of rat leg edema in each treatment group ($p> 0.05$). This means that there is no difference in anti-arthritis activity produced by the treatment of the sample (formula) in each treatment group. This may be due to the possibility that the multiple doses prepared are not large so that the resulting effect is less than optimal and the time for giving the sample treatment (formula) is not long.

Table 1: The percentage of inhibition of the average edema volume

Sample Groups	Percentage of average edema volume inhibition (%) measurement-					
	1st	2nd	3rd	4th	5th	6th
Positive control	31.282	36.076	48.125	70.370	79.444	80.729
Sappan Wood	33.538	36.203	41.500	40.889	58.667	71.875
Red Ginger	18.461	18.987	38.750	45.185	52.500	59.375
F1 (1:1)	33.128	36.203	42.000	49.037	68.667	79.688
F2 (2:1)	53.230	65.063	63.750	64.815	75.000	86.719
F3 (1:2)	36.410	36.709	55.500	54.815	71.330	80.000

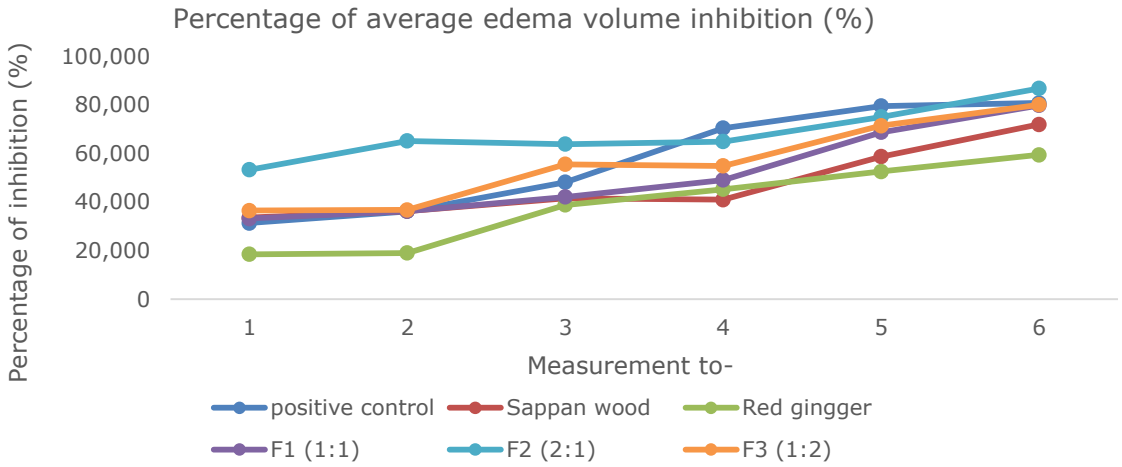

Figure 1: Percentage of average edema volume inhibition of rat leg

Table 2: Evaluation of the results of observations of inflammatory cell infiltration from histopathological preparations of rat leg tissue

Sample Groups	Category of inflammatory cell infiltration
Normal	0
Negative control	Heavy
Positive control	Heavy
Sappan wood	Medium
Red ginger	Light
F1 (1:1)	Medium
F2 (2:1)	Light
F3 (1:2)	Medium

Histopathological Examination of Rat Leg Tissue

The results of the histopathological examination are presented in Tables 2 and Table 3. In the normal control group treatment there was no visible inflammatory cell infiltration. In contrast to the negative controls which were classified as severe, there were visible inflammatory cells in large numbers compared to the other test groups because no drug therapy or test samples (formulas) were carried out. Meanwhile, in the treatment of the positive control group, the number of inflammatory cell infiltration was reduced, but it was still relatively severe compared to the other treatment groups. Less inflammatory cell infiltration was observed with the red ginger extract and F2 treatment group, F2 had less visible inflammatory cell infiltration and connective tissue. The formation of this connective tissue is the body's repair process after the inflammatory reaction stops. Formation of connective tissue involves proliferation of granulation tissue starting from areas adjacent to necrotic tissue extending into areas that have been destroyed by inflammatory reactions.[37] The histopathological presults of rat leg tissue, in the group treated with F2 showed reduce inflammation compared to the positive control, sappan wood extract, red ginger extract, F1, and F3. The results of histopathological examination of the leg tissue of these rats certainly support anti-arthritis activity related to the percentage reduction in edema volume (%) which also showed better results in the F2 combination treatment group, as shown in Table 3.

Rat Hematology Examination

The results of haematological examination of rats using parameters of hemoglobin level (HGB), total leukocyte count (WBC), and platelets (PLT) in the treatment group, as shown in Table 4. Complete Freund's

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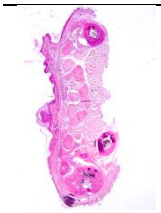
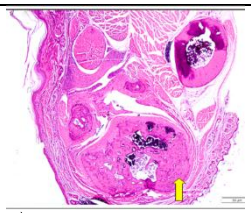
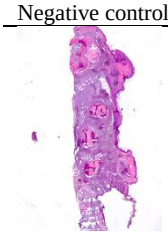
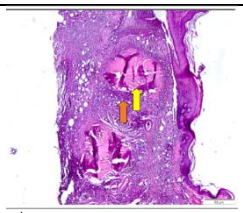
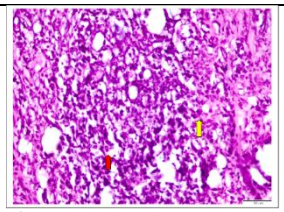
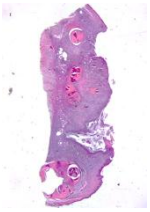
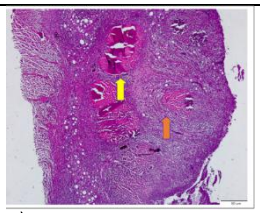
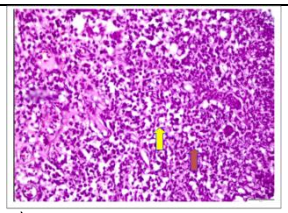
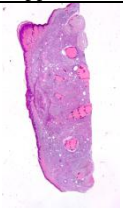
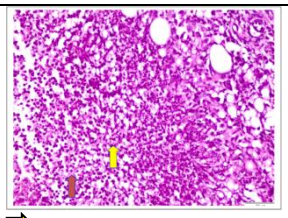
Adjuvant-induced haematological examination of test animals can be identified and differentiated from the parameters of a significant decrease in the number of HGB levels, the volume of packed cells with an increase in the number of WBC, and the amount of PLT. The value of rodentia hematology parameters are specific and cannot be generalized because they are influenced by geographical conditions, such as location, climate, temperature, humidity, altitude, and lighting.[38] Based on one-way ANOVA statistical analysis of data HGB, WBC, and PLT, it was shown that there was no significantly difference in the haematological examination of rats in each treatment (sample) group ($p>0.05$).

HGB is a protein-porphyrin complex containing iron. Hemoglobin is the most important part of the erythrocytes because this substance fills almost 32% after water, carbohydrates and fat. HGB plays an important role in binding, distributing and delivering oxygen to body tissues.[39] HGB levels in the test animals did not differ significantly between the treatment (sample) groups and the data from each treatment (sample) group were also not much different, as shown in Table 4. The sample groups of rats that were injected with Complete Freund's Adjuvant were then injected with various formulas (F1, F2, and F3) showed that extracts of sappan wood and red ginger which have various bioactive compounds can help maintain HGB levels in mice like normal rats. WBC (leukocytes) are cells that are responsible for the body's defense system. Leukocytes have a wide variety of total cell counts. The total WBC count in the test animals that were given the extract was not significantly different from the normal rat group. The important role of WBC in defending the body from infection and tissue damage can be seen from its immunostimulating properties which can be mediated through a stimulatory effect on WBC production in the red ginger extract group, the F2 and F3 combination groups. PLT is the first line of defense against bleeding, contributing to the process of thrombosis, inflammation, and neoplasia.[40] PLT contributes to host defense because PLT recognizes bacteria, recruits traditional immune cells to the site of infection and secretes bactericidal mediators.[41] The body tries to increase platelets for the purpose of defending the body against toxic substances. The increase in platelets in this study was highest in the F2 combination extract group.

Table 3: Results of histopathology observation of rat leg tissue using a light microscope

20x Magnification	**100x Magnification**	
Normal		
	 ⇨ : trabecular bone	
20x Magnification	40x Magnification	400x Magnification
Negative control		
	 ⇨ : trabecular bone ⇨ : inflammatory cell infiltration	 ⇨ : histiocyte cell ⇨ : lymphocyte
Positive control		
	 ⇨ : trabecular bone ⇨ : inflammatory cell infiltration	 ⇨ : histiocyte cell ⇨ : lymphocyte
Sappan wood		
	 ⇨ : trabecular bone ⇨ : inflammatory cell infiltration	 ⇨ : histiocyte cell ⇨ : lymphocyte
Red ginger		

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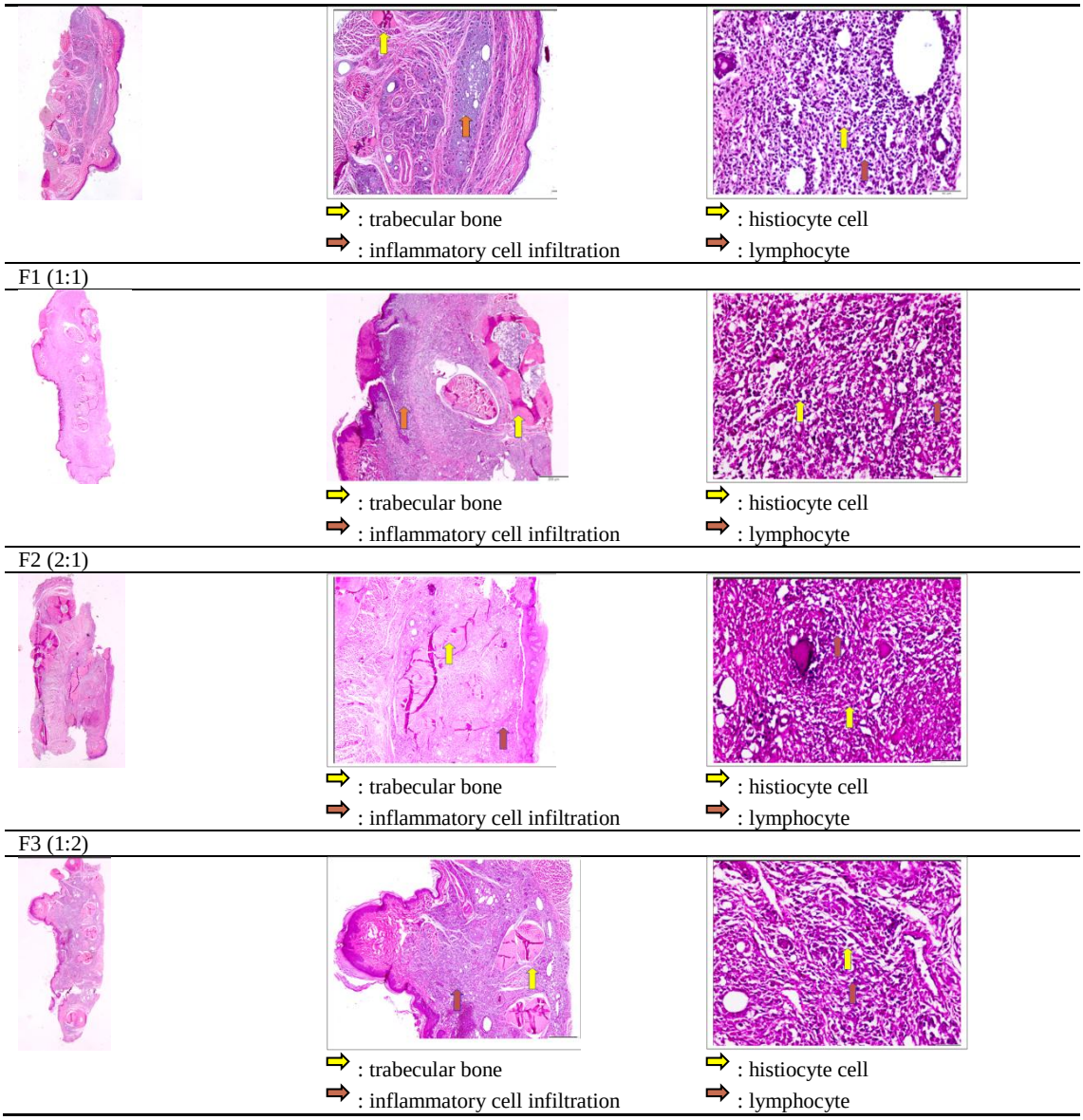

Table 2: Haematological examination of rats with HGB, WBC, and PLT parameters

Sample groups	HGB (g/dL)	WBC (10^3/µL)	PLT (10^3/µL)
Normal	12,87	22,30	851,00
Negative control	12,40	16,73	840,00
Positive control	13,00	27,13	910,67
Sappan wood	13,43	15,57	798,00
Red ginger	14,63	19,17	855,67
F1 (1:1)	13,30	16,57	928,67
F2 (2:1)	13,00	21,83	1059,00
F3 (1:2)	13,37	18,50	746,33

Conclusion

Based on the outcomes of the anti-arthritis experiment utilizing the adjuvant-induced arthritis technique, it can be deduced that the oral administration of a combination of sappan wood and red ginger ethanol extract in a 0.5% carboxymethyl cellulose (CMC) solution, specifically F2 (consisting of sappan wood extract at a dosage of 60 mg per 200 g body weight and red ginger extract at a dosage of 30 mg per 200 g body weight), exhibited the most notable anti-arthritis effect. This effect was evidenced by an 86.72% reduction in rat leg edoema and a statistically significant anti-arthritis impact ($p<0.05$) when compared to the negative control, as indicated by the decrease in edoema volume. A statistical analysis using one-way ANOVA was conducted on the haemoglobin, white blood cell (WBC), and platelet (PLT) data in blood haematology. The results indicated that there was no statistically significant difference observed in the haematological tests of rats across the various treatment groups ($p>0.05$).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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