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RESEARCH ARTICLE

Immunomodulator Activity and Antirheumatoid Arthritis Extract of Ethyl Acetate Ginseng Bugis *Talinum Paniculatum* (jacq.) Gaertn)

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Abstract

The Immunomodulator is one of the compounds that can improve immune system. Chemical compounds work through the immune system to prevent and treat disease. Rheumatoid arthritis is an autoimmune disease that is chronic-systemic inflammation. The purpose of this study was to determine the effects of imonomodulator and antirheumatoid arthritis of ethyl acetate extracts of ginseng bugis leaves (Talinum Paniculatum (Jacq.) Gaertn). This study was divided into 2 groups, namely the immunomodulatory testing using sheep red blood cells and CFA-induced antirhematoid. This study used wistar rats which divided into 6 groups for immunomodulatory activity and 5 groups for antirematoid arthritis. The dose of ethyl acetate extract used was 0.05 g/KgBW; 0.1 g/KgBW, and 0.15 g/KgBW. Statistical test results for immunomodulatory activity showed significantly different results between the extract group doses of 0.05 g/KgBW; 0.1 g/KgBW, and 0.15 g/KgBW ($p < 0.05$) with a negative control group. Rheumatoid arthritis testing showed ($p < 0.05$) to the foot volume and arthritis index. The results showed that ethyl acetate extract of ginseng bugis leaves have immunomodulatory and antirheumatoid arthritis activity.

Keywords: *Immunomodulator, Anti Rheumatoid arthritis, Ethyl acetate, Talinum Paniculatum (Jacq.) Gaertn.*

Introduction

Indonesia is a country which has abundant herbal medicinal plants. Herbal medicine is advantageous and diverse. One type of herbal medicinal plants commonly found in South Sulawesi is ginseng bugis (*Talinum paniculatum (Jacq.) Gaertn*). Gingseng bugis leaf (*Talinum paniculatum* (*Jacq.*) *Gaertn)* contains chemical compounds such as saponins, flavonoids, and tannins.

Flavonoid and tannin compounds are some compounds that can modulate the immune system [1, 2]. Ethanol extract of *Talinum paniculatum* (*Jacq.*) *Gaertn* leaves at a weight-based dose of 1.25 g/KgBW, 2.5 g/KgBW, 3.75 g/KgBW has an anti-inflammatory effect in rat [3]. Its leaf (*Talinum paniculatum* (*Jacq.*) *Gaertn.)* also exerts an immunomodulatory activity effect at a weight-based dose of 7.5 g/KgBW [4]. Rheumatoid arthritis (RA) is an example of complex immune diseases.

The disease, immunoglobulin is formed in the form of IgM (called rheumatoid factor, RF) which is specific to the Fc fraction of the IgG molecule. Rheumatoid arthritis (RA) is an autoimmune disease that is chronic-systemic inflammation. Chronic inflammation causes hypertrophy, thickening of the membrane of the synovium, obstruction of blood blow, and cell necrosis.

One of the experimental models in developing rheumatoid arthritis studies is to use Complete Freund's Adjuvant (CFA). CFA contains Mycobacterium tuberculosis which, when induced, will cause an autoimmune and chronic-systemic inflammatory response [5]. This study exploited ethyl acetate extract of

gingseng bugis leaf (*Talinum paniculatum* (*Jacq.*) *Gaertn)* to investigate the CFA induced immunomodulatory and antirheumatic activities.

Materials and Methods

The materials used were gingseng bugis leves obtained in South Sulawesi Indonesia, sheep red blood cells, PBS, CFA, plethysmometer, Wistar rats.

Sample Extraction

The gingseng bugis (*Talinum paniculatum (*Jacq.) Gaertn) simplicia powder weighed 1 kg was put in maceration container. Ethyl acetate solvent was added as much as 2 (two) liters to the maceration container. Thus, the simplicia powder was wetted and submerged. The sample was left 1 x 24 hours. After that, it was macerated by filtering. Then, macerated was conducted with the same solvent. Maceration results were put in a rotary evaporator and froze drying to get the dry extract.

Making Test Extracts

Ethyl acetate extract of gingseng bugis (*Talinum paniculatum (*Jacq.) Gaertn) that had been obtained was made in a weight-based dose of 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW. Ethyl acetate extract of gingseng bugis at a dose of 0.05g/KgBW was made by weighing the extract of 0.05 g which was dissolved in 10 mL Na-CMC 1% b/v. Ethyl acetate extract of gingseng bugis at a weight-based dose of 0.1 g/KgBW and 0.15 g/KgBW were made by respectively weighting as much as 0.1 g and 0.15 g which then dissolved in 10 mL Na-CMC 1% b/v. Ethyl acetate extract of gingseng bugis at a weight-based dose of 0.1 g/KgBW and 0.15 g/KgBW were made by respectively weighing as much as 0.1 g and 0.15 g which then dissolved in 10 mL Na-CMC 1% b/v.

Creating Sheep RBC Suspension (SHRBC) 10% v/v

Sheep red blood cells were pipetted as much as 1mL and put into a clean and dried Eppendorf tube containing 1 mg of EDTA as an anticoagulant. Then it was centrifuged at 1500 rpm. SHRBC was washed with *Phosphate Buffered Saline (PBS*) solution. After centrifuge, the PBS solution was separated until 100% SHRBC was left. After that, PBS solution was added again with the same amount until 50% SHRBC was obtained and pipetted as much as 2 mL. 8 mL PBS solution was then added to obtain antigen suspension with 10% v/v SHRBC concentration.

Selection and Preparation of Test Animals

The selection and preparation of test animals that were used in the study were Wistar rats weighing 150-200g which had been adapted for ± 1 week in the cages of the Pharmacology Laboratory of the Faculty of Pharmacy at Universitas Muslim Indonesia. The adaptation was conducted to make the Wistar rats adapted to the new environment. Accordingly, they were fed and drink in moderation (Ethics: N0:076/A.1/KEPK-UMI/III/2019).

Delayed Type Hypersensitivity Test

Prepared test animals were divided into 5 groups which the Group I (only induced SHRBC without extracting) is the control group. The Group II (SHRBC + *Phyllanthus niruri* L), the Group III, IV, and V are the treatment group (induced by SHRBC and ethyl acetate extract at a weight-based dose of 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW). Administration of test extracts was carried out for seven days orally according to the volume of administration. On the third day, all the five groups were induced with a 10% v/v SHRBC antigen of 1 mL intraperitoneally. Rat foot volume was measured on the seventh day after the induction of 10% v/v SHRBC antigen at 0, 4, 24, and 48 hours using a plethysmometer [6].

Anti rheumatoid Arthritis Test

Test animals that had been adapted were induced with CFA on day 1 and let them be until the 16th day. On the 17th day, they were randomized into 5 groups. The group I is the control group (only CFA-induced without extracting), the group II (CFA + Methylprednisolone), and the groups III, IV, and V are the groups of CFA-induced groups and extracts were given in successive weight-based doses of 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW. The extracts was administered orally for 14 days with a frequecy once a day. Foot volume measurements were performed on the 1st day before CFA induced, and 17th, 21st, 23rd, 26th, 29th, and 31st days [7]. Arthritis index observations were made on the 17th and 31st days [8].

Foot Volume Measurement

The effect of arthritis drugs was assessed from the percentage of inhibition of swelling Foot volume:

caused by *Complete Freund's Adjuvant* (CFA) which was calculated using the following formula [9].

$$\% \text{ inhibition average} = \left(\frac{a-b}{a}\right) \times 100\%$$

Description:

a = Average volume of rat's feet before therapeutics (17th day)

b = Average volume of rat's feet after therapeutics (31st day)

The Arthritis Index Observation

Arthritis index observations were made on days 17th and 31st. Arthritis index observations were made according to the Table 1.

Table 1: Observation parameters of the arthritis index [10]

No	Symptoms of arthritis occurred	score
1.	There is no symtoms of arthritis	0
2.	Changes in 1 joint	1
3.	Changes in 2 joints	2
4.	Changes in more than 2 joints	3
5.	Changes in more than 2 joints are characterized by polyarthritis and ankylosis	4
6.	Severe polyarthritis and ankylosis	5

Furthermore, each rat from each group was observed to the scale index of the occurrence of rheumatoid arthritis [8]. The rats were declared as rheumatoid arthritis if they had an arthritis index > 2

Results and Discussion

This study was conducted to determine the immunomodulatory effects of ethyl acetate extract of gingseng bugis (*Talinum Paniculatum* (Jacq.) Gaertn) with the Delayed-Type Hypersensitivity and antiremathoid methods. The weight-based doses used were 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW.

Delayed-type Hypersensitivity Testing

Hypersensitivity reaction parameters of the slow type were also known as Delayed-Type hypersensitivity (DTH). The response to type IV hypersensitivity reaction appears 24 hours after the body is exposed to the antigen for the second time. This study involved 5 groups namely the control group or Group I (only induced SHRBC without administration of extracts), Group II (SHRBC+ *Phyllanthus niruri* L), and Group III, IV, and V induced SHRBC and given ethyl acetate extract with weight-based doses of 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW consecutively. Data from the research findings of immunomodulatory effects of ethyl acetate extract of gingseng bugis leaves (*Talinum Paniculatum* (Jacq.) Gaertn) using the Delayed-Type Hypersensitivity parameters were served in Table 2.

Table 2: Data on the measurement results of the average change in the rat feet volume in the treatment group after antigen administration

Group	± Average of Rat Feet Volume			
	$T_0 \pm SD$	$T_4 \pm SD$	$T_{24} \pm SD$	$T_{48} \pm SD$
I.Control (SHRBC)	0.37 ± 0.01	0.56 ± 0.02	0.52 ± 0.06	0.46 ± 0.03
II. SHRBC + Phyllanthus niruri L	0.40 ± 0.03	0.56 ± 0.02	0.36 ± 0.02	0.32 ± 0.01
III.SHRBC + 0.1 g/KgBW extract	0.44 ± 0.04	0.60 ± 0.12	0.36 ± 0.02	0.31 ± 0.02
IV.SHRBC + 0.05g/KgBW extract	0.39 ± 0.01	0.58 ± 0.06	0.42 ± 0.01	0.35 ± 0.02
V. SHRBC + 0.15 g/KgBW extract	0.39 ± 0.01	0.59 ± 0.05	0.42 ± 0.01	0.36 ± 0.03

Changes in foot volume at the 4th hour presented in Table 2. The data indicate that the extract group with a dose of 0.5 g/KgBW had an increase in foot volume higher than T_0. The increase in foot volume was caused by the activation of macrophages. Antigen (SHRBC) containing lipopolysaccharide-stimulated macrophages to secrete TNF (Tumor Necrosing Factor) and IL-1 (Interleukin-1) which were inflammatory mediators. The inflammatory process run until the antigen was eliminated [11].

Changes in foot volume at the 24th hour indicate there has been a decrease in foot volume which was lower compared to the 4th hour. Decreased foot volume at T24 shows a repair effect in the body. Based on a review of literature, a macrophage will secrete IL-12

(*Interleukin-12*) which will simulate the production of IFN-γ (*Interferon* γ) through T $CD4^+$ (*Cluster of Differentiation*) which differentiate into Th1 (*T helper)* cell. IL-12 will also enhance the cytolytic function of NK (Natural Killer) cell and T cell of $CD8^+$.

Thus, the secretion of IFN- γ can be stimulated, where the IFN-γ activate macrophages to eliminate antigens [11]. However, the T24 showed extract group had a decrease in edema volume lower than the control group. This means the extract was successful in stimulating the action of the immune system. The change in volume at the 48th hour shows the greatest decrease in foot volume. This can be inferred that the immune system had succeeded in eliminating antigens in test animals. The IL-12 cytokines which stimulated IFN-γ encouraged a decrease in foot volume at the 48th hour more rapidly [11, 12].

This shows that ginseng Bugis leaf extract can modulate the immune system of test animals. Statistical analysis of ANOVA group of *ginseng bugis* leaf extract with a weight-based dose of 0.05 g/KgBW and 0.1 g/KgBW showed the insignificantly different results ($p>0.05$). Analysis of foot volume at T-48 compared to the control group showed significantly different results ($p<0.05$). It means that the ethyl acetate extract of ginseng Bugis *leaves* was with various doses used has immunomodulatory activity with DTH parameters compared to those in the control group.

Antirhematoid Arthritis Testing

Test animals that had been adapted were then induced with CFA on day 1st and left until the 16th day. On the 17th day, they were distributed into 5 randomized groups namely control group I (only CFA-induced without extracting), group II (CFA + Methylprednisolone), and groups III, IV, and V (CFA-induced groups and extracts were given in successive weight-based dose of 0.05 g/KgBW, 0.1 g/KgBW, 0.15 g/KgBW. Provision of the test preparation was given orally for 14 days with a frequency of administration once a day.

Foot volume measurements were performed on day 1 before CFA induction. The measurements were also conducted on days 17th, 21st, 23rd, 26th, 29th, and 31st. Arthiritis index observations were made on the 17th and 31st days [8]. The provisions of various doses of extract for 14 days showed a change in the volume of rat feet compared to the group without the administration of the extract (negative control). The average score was served in Table 3.

Table 3: Initial average feet volume (mL) after induction and extraction

Group		The Average Feet Volume (mL)					
	Initial	Day-17	Day-21	Day -23	Day -26	Day -29	Day -31
I. Control (CFA)	0.27	0.55	0.55	0.54	0.52	0.57	0.68
II. CFA + Methylprednisolone	0.29	0.73	0.46	0.31	0.30	0.32	0.30
III. CFA+ extract of 0.05 g/KgBW)	0.31	0.72	0.51	0.30	0.30	0.30	0.29
IV. CFA+ extract of 0.1 g/KgBW	0.30	0.73	0.58	0.35	0.32	0.31	0.27
V.CFA+ extract of 0.15 g/KgBW	0.29	0.77	0.52	0.27	0.27	0.26	0.29

Table 3 proves that group I did not notice a decrease in leg volume in the control group (induced CFA without administration of extracts). They also showed an increase in foot volume. This means that CFA has succeeded in inducing edema in mice feet. Group II induced by CFA and given methylprednisolone decreased foot volume compared to the control group. This is because methylprednisolone is a pharmacological therapeutic drug for rheumatoid arthritis which binds to glucocorticoid receptors that affect genes involved in the inflammatory response [2].

The groups given extracts (group III, IV, and V) showed a decrease in the volume of mouse feet compared to the control group. This indicates the extract can reduce rat foot edema. The findings of rat foot decrease were presented in Table 4.

Table 4: Percentage of foot volume reduction of induction-therapeutic from the anti rheumatoid arthritis test of extract

Group	Percentage of foot volume reduction (%)
Group I Negative Control (Na.CMC 1%)	-10.55
Group II Positive Control (Methylprednisolone)	57.75
Group III Ethyl acetate extract of 0.05 g/kgBW	59.22
Group IV Ethyl acetate extract of 0.1 g/kgBW	62
Group V Ethyl acetate extract of 0.15 g/kgBW	61.98

The group that experienced the highest percentage of foot volume reduction was in the group with a weight-based dose of 0.1 g/kgBW which was 62%. It was followed by the group with a weight-based dose of 0.15 g/kgBW that was equal to 61.98%. Then, a dose of 0.05 g/kgBW followed with 59.22%. The smallest reduction was found in the positive control group (Methylprednisolone) which was 57.75%

Percentage reduction in foot volume was analyzed statistically using the One Way Anova test. The results of the One Way Anova test showed that group I (control group) was significantly different ($p<0.05$) to other groups (group II, III, IV, and V). In the group given Methylprednisolone and the groups were given extract with a dose of 0.05 g/kgBW, 0.1 g/kgBW, and 0.15 g/kgBW, there was no significant difference ($p> 0.05$).

This means that the group given methylprednisolone statistically had the same effect in reducing foot volume. The findings in the group with a dose of 0.05 g/kgBW, in the group with a dose of 0.1 g/kgBW, and in the group with a dose of 0.15 g/kgBW showed that there was no significant difference ($p>0.05$). Similarly, there was no significant difference ($p>0.05$) between the group with a dose of 0.1 g/kgBW and the group with 0.15 g/kgBW. Thus, from these findings, it can be seen that statistically, the doses of 0.05 g/kgBW, 0.1g/kgBW, and 0.15g/kgBW have the same effect in reducing the volume of CFA-induced rat feet. The average index of arthritis of rat induced and after administration of the extract can be seen in Table 5.

The results of the statistical analysis of the Mann-Whitney test on the measurement of the arthritis index indicate changes in the arthritis index after administration of the extract for 14 days. The control group was significantly different from all groups, namely Methylprednisolone group, the dose group of 0.05 g/kg BW, 0.1 g/kg BW, and 0.15 g/kg BW. This means that the control group did not have the same effect with a dose of 0.05 g/kg BW, 0.1 g/kgBW, 0.15 g/kgBW, and Methylprednisolone in reducing the arthritis index.

Table 5: Inductive arthritis index of rat (*Rattus norvegicus*) induction and after administration of the extract

Group	The average index of arthritis ± SD	
	Induction	Therapeutics
Group I: control	2.7 ± 0.57	3.7 ± 0.57
Group II: Methylprednisolone	3.7 ± 0.57	1.33 ± 0.57
Group III Extract of 0.05 g/kg BW	3.7 ± 0.57	0.66 ± 0.57
Group IV Extract of 0.1 g/kgBW	3.3 ± 0.57	0.33 ± 0.57
Group V Extract 0.15 g/kgBW)	3 ± 1	0.33 ± 0.57

There was no significant difference ($p>0.05$) between the control group with Methylprednisolone and other groups with extracts at a dose of 0.05 g/kg BW, 0.1 g/kgBW, and 0.15 g/kgBW. This means that Methylprednisolone gives the same effect with all dosage groups of 0.05 g/kgBW, 0.1 g/kgBW, and 0.15 g/kgBW

Additionally, from the results of statistical analyses that had been carried out on the arthritis index, it can be concluded that the ethyl acetate extract of ginseng Bugis leaves has an anti rheumatoid arthritis effect. This shows that the three variations of the extract dose of 0.05 g/kgBW, 0.1 g/kgBW, and 0.15 g/kgBW were effective in reducing the arthritis index.

The decreasing of foot volume and arthritis index after administration of ethyl acetate extract was considered to be due to the presence of saponins, flavonoids, and tannins contained in the leaves of *Talinum paniculatum* (*Jacq*.) *Gaertn*. Those compounds were able to modulate the immune system [1, 2] which played a role as anti rheumatoid arthritis. From the findings

of this study, it can be concluded that a decrease in edema of CFA-induced rat foot volume was associated with the presence of immunomodulatory activity from the administration of various weight-based doses of ethyl acetate extract (0.05 g/kgBW, 0.1 g/kgBW, and 0.15 g/kgBW).

Conclusion

Based on the results of ethyl acetate extract dose of 0.05 g/KgBW, 0.1 g/KgBW, and 0.15 g/KgBW conclude that ethyl acetate extract of ginseng bugis leaves has immunomodulatory and antirhematoid arthritis activity.

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