The test anti-inflammatory activity of infusing Bryophyllum pinnatum (Kalanchoe pinnata) leaves (Kalanchoe pinnata) on edema in mice leg thigh male swiss webster

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Abstract. Research to know the effect of anti-inflammatory of infuse bryophyllum pinnatum (Kalanchoe pinnata) against edema on leg thigh Swiss Webster male mice and to see relationships increased concentrations with increased anti-inflammatory effect. This research was conducted in the Science Laboratory, Faculty of Fisheries, Agriculture, and Biology of the Bangka Belitung University. The method: The animals test that used were 25 Swiss Webster male mice were divided into 5 groups, namely group I: negative control (aquadest), group II: positive control (aspirin), group III, IV, V given infuse bryophyllum pinnatum (Kalanchoe pinnata) with concentrations 20%, 40%, and 80%. This research uses a method by injecting acetic acid in mice thigh. The measured data is a murine thigh volume for 3 hours using a caliper. Data analysis using the Kruskal-Wallis, the results showed that there were no significant differences as a value greater than 0.05 is 0.533. This suggests that increasing the concentration did not give a considerable influence on the volume of edema thigh male mice Swiss Webster. From the Mann Whitney test between the concentration of 20%, 40%, 80%, and negative controls showed that the difference in significance because of the value of significantly less than 0.05. This shows that there is a potential infusion of leaves bryophyllum pinnatum (Kalanchoe pinnata) towards healing leg edema in the thigh swiss webster male mice.

1. Introduction

Inflammation is a protective response of the body against injury or lesion, this situation is not a disease but a manifestation of the disease. This reaction is the body's defense efforts to eliminate the cause of injury [1]. Inflammation caused by various adverse stimuli caused by various harmful agents such as infection or physical injury [2]. Signs of inflammation of a general nature are redness (rubor), heat (calor), swelling (tumor), pain (dolor), and impaired function (fungisiolesa)

Anti-inflammatory drugs have been widely used, especially from the group of non-steroidal (NSAID) and a small portion of this NSAID group which have adverse side effects such as peptic ulcer body [3]. Therefore the use of medicinal herbs with anti-inflammatory properties needs to be done to find alternative treatment with relatively small side effects.

Bryophyllum pinnatum (Kalanchoe pinnata) Infuse is a traditional crop that has many properties that are as anti-tumor, anti-inflammatory, to stop bleeding, reduce swelling and accelerate the healing of wounds, bryophyllum pinnatum (Kalanchoe pinnata) in traditional medicine is used to treat infections, inflammation, hypertension, kidney stones, arthritis [4]. Bryophyllum pinnatum plant
chemical constituents that appelzuur, resins, agent lenders, magnesium malate, calcium oxalate, formic acid, tannins, saponins, flavonoid, quercetin-3-glucoside, polyphenols, lemon acid, apple acid, vitamin C, bryophyllin, glucose [5].

Flavonoid compounds from plants have been reported to have anti-inflammatory by inhibiting the ability of edema. Edema is a bubble of fluid from multiple organ or tissue that is the accumulation of excess lymph fluid, without increasing the number of cells in a tissue effect. Edema can be collected at several locations on the body but is usually found on the foot and ankle [6]. Edema occurs due to increased volume and extravascular extracellular fluid (fluid interstitium) is accompanied by an abnormal accumulation of fluid in the sidelines of the network and serous cavities (loose connective tissue and body cavities).

Edema can be local (local) and public (general). Localized edema occurs only in the abdominal cavity, chest cavity, under the skin, pericardium of the heart or in the lungs. While edema which is characterized by the occurrence of edema fluid collection in many places is called generalized edema (general edema) [7]. The cause of edema is reduced plasma protein concentration, increased permeability of the capillaries, increasing venous pressure, blockage of the lymph vessels [6].

Based on the study, flavonoids contained in the infusion of bitter herbs (Andrographis paniculata) showed response inhibition of edema at a dose of 30 mg/kg and 50 mg/kg in male mice in vivo [8]. Flavonoids work by inhibiting the cyclooxygenase enzyme that inhibits the formation of prostaglandins [9].

Based on the above data that bryophyllum pinnatum (Kalanchoe pinnata) Infuse then the plant is expected to have efficacy as an anti-inflammatory by inhibiting edema. Therefore this study aimed to determine the anti-inflammatory effects through inhibition of edema. Based on the description of the background above, the problem in this research are about infusion bryophyllum pinnatum (Kalanchoe pinnata) Infuse can cure mice thigh edema in male Swiss Webster and how much influence each leaves infusion concentration bryophyllum pinnatum (Kalanchoe pinnata) on edema in mice thigh male Swiss Webster. Knowing the potential bryophyllum pinnatum (Kalanchoe pinata) to cure mice thigh edema in male Swiss Webster.

2. Methods
2.1 Types of research
This study is an experimental study that is research done by testing the effect of infusion of leaves bryophyllum pinnatum (Kalanchoe pinnata) on edema that occurs in the thigh legs of mice by injection of acetic acid 1%, then see how the influence of anti-infuse the leaves bryophyllum pinnatum (Kalanchoe pinnata) against edema in the thigh of mice, and thigh edema volume measurements of mice for 3 hours every 30 minutes using a caliper. In this experiment, three concentrations of 20%, 40%, 80% v/v.

2.2 Time and place of research
This research was conducted in April-June 2015 and was carried out in the Laboratory of Mathematics, Faculty of Agriculture, Fisheries and Biology, University of Bangka Belitung.

2.3 Object of research
bryophyllum pinnatum (Kalanchoe pinnata) that has been dried in the form of bulbs weighing 100 grams, was obtained in the Belinyu, Bangka, then mashed and made infuse.

2.4 Tools and materials
The tools used in this study, an analytical balance, hot plate, knife, mortar and stamper, test tubes and shelves, a pipette, filter paper, stir bar, beaker glass, pans infuse water bath, a syringe injection of 1 cc, Erlenmeyer, jar, blender, oven, filter paper, animal rearing cage, gloves, drinking water and food animals, calipers, stopwatch, spatula. Materials used in this study are simplistic bryophyllum pinnatum (Kalanchoe pinata), aspirin 500 mg, distilled water, 1% acetic acid.
2.5. Research procedure

2.5.1. Determination of test plants. The first phase of this study is determination bryophyllum pinnatum (Kalanchoe pinnata) relating to the characteristics of the macroscopic bryophyllum pinnatum (Kalanchoe pinnata) by matching the morphological traits in plants. Determination conducted at the Laboratory of Mathematics, Faculty of Agriculture, Fisheries and Biology University of Bangka Belitung.

1. Making the leaves powder bryophyllum pinnatum (Kalanchoe pinnata). Bryophyllum pinnatum (Kalanchoe pinnata) Simplicia obtained in the Belinyu area, Bangka. Furthermore, Simplicia bryophyllum pinnatum (Kalanchoe pinnata) leaves crushed using a mortar and Stemper or blended so that the leaves powder will form bryophyllum pinnatum (Kalanchoe pinnata).

2. Preparation of Aspirin solution. 500 mg aspirin tablets as much as 2 grains of the finely ground until a homogeneous, then diluted with distilled water 10 ml. Dose aspirin= 500 mg. Dose mice were 20 grams = 500 mg x 0,0026= 1.3 mg / 20 gram BB= 0.065 mg / g BW= 65 mg / Kg BW.

3. Testing Procedure Anti-inflammatory. Swiss Webster male mice which amount to 25 animals were divided into 5 groups that each group consisted of 5 mice. All mice have fasted for 12-18 hours with the aim that the same conditions of test animals. The treatment is done orally using an oral needle.

a. Group I: Distilled water as a negative control
b. Group II: Aspirin solution as a positive control
c. Group III: Infuse leaves of bryophyllum pinnatum (Kalanchoe pinnata) 20% v/v
d. Group IV: Infuse leaves of bryophyllum pinnatum (Kalanchoe pinnata) 40% v/v
e. Group V: Infuse leaves of bryophyllum pinnatum (Kalanchoe pinnata) 80% v/v

Thirty minutes after administration of distilled water in group I, group II aspirin solution, and infuse the leaves bryophyllum pinnatum (Kalanchoe pinnata) in group III, IV and V, each mouse has injected 1% solution of acetic acid in the thigh 1ml mice. The volume of edema thigh of mice was measured for 3 hours every 30 minutes using a caliper.

3. Result and discussion

3.1. Test anti-inflammatory infuse leaves of bryophyllum pinnatum (kalanchoepinnata) against male swiss webster mice

Test anti-inflammatory bryophyllum pinnatum (Kalanchoe pinnata) infuse is done by looking at the ability of bryophyllum pinnatum (Kalanchoe pinnata) leaves in test animals reduce leg swelling due to the injection of 1% acetic acid solution. Acetic acid is an organic acid chemical compound which has the empirical formula C2H4O2. This formula is often written in the form of CH3COOH, CH3COOH, or CH3CO2H. Acetic acid is a colorless hygroscopic liquid, and has a freezing point of 16.7 °C. Volatile acetic acid in the open air, flammable, and can cause corrosion of the metal. Acetic acid is soluble in water with a temperature of 20°C, ethanol (9.5%) acid, and concentrated glycerol. Diluted acetic acid if still reacting acid [10]. Statement acid selected to test the anti-inflammatory drug for non-antigenic and do not have a systemic effect [11].

The observation of the leaves infuse anti-inflammatory test in male Swiss Webster acquired data such as attachments 8. Edema measurement data used to calculate the percentage increase in the volume of edema (% KVU). To obtain the data shown below:
Table 1. Percentage of anti-inflammatory power of bryophyllum pinnatum (kalanchoe pinnata) leaves infusion in male webster swiss mice injection with acetic acid 1%

| Group          | t (minute) | %KVU  |
|----------------|------------|-------|
|                | 30         | 60    | 90    | 120   | 150   | 180   |
| control (-)    | 53.65      | 75.45 | 76.79 | 75.97 | 69.35 | 69.23 |
| control (+)    | 55.88      | 38.81 | 30.36 | 25.20 | 15.64 | 7.84  |
| Infusa 20%     | 64.43      | 76.09 | 22.47 | 30.64 | 21.09 | 7.52  |
| Infusa 40%     | 68.65      | 63.59 | 29.66 | 34.54 | 19.38 | 14.93 |
| Infusa 80%     | 52.68      | 42.01 | 29.56 | 19.32 | 16.21 | 5.80  |

The data in table 1 shows that the negative control group (distilled water) had the greatest percentage of edema volume compared with the other groups. This is because the negative control group was given to mice did not contain active substances that affect an anti-inflammatory. On the positive control group (aspirin), the percentage of edema volume decreased. This is because aspirin has efficacy as an anti-inflammatory. In the group of bryophyllum pinnatum (Kalanchoe pinnata) leaves infusion concentration of 20%, 40%, and 80% the percentage of edema volume decreased, this is because the infusion of bryophyllum pinnatum (Kalanchoe pinnata) given to the mice contain active ingredients as an anti-inflammatory effect.

Acetic acid is a foreign substance that, when entered into the body stimulates the release of inflammatory mediators such as prostaglandins that cause inflammation due to antibodies the body reacts to these foreign substances to fight against its influence.

On the negative control group, edema increased rapidly in the minutes to-30 at % KVU (Percentage Increase in Volume Edema) of 53.65%, and continued to rise until the 90 minutes with % KVU amounted to 76.79%. Then declined in the 120 minutes with % KVU amounted to 75.97% and continued to decline until the 180 minutes with % KVU amounted to 69.23%. But the decline of edema is not too large compared to the positive control group.

On the positive control group, edema increased in the 30 minutes with % KVU amounted to 55.88% and gradually declined in the 60 minutes with % KVU amounted to 38.81% and continued to decline until the 180 minutes with % KVU by 7.84%. The percentage of the group treated with aspirin inflammation smaller when compared to the negative control. NSAIDs such as aspirin works by me prostaglandin enzyme synthesis endopiroksid and inhibit COX enzymes work permanently. Aspirin relatively selectively inhibits COX-1 and COX-2 bit [2].

Percentage edema bryophyllum pinnatum (Kalanchoe pinnata) leaves infusion group concentration of 20% less when compared to the negative control. Edema percentage increased in the 30 minutes with % KVU amounted to 52.68 % and gradually down in the 60 minutes with %, KVU amounted to 42.01% and continued to decline until the 180 minutes with %KVU amounted to 5.80%. Percentage edema bryophyllum pinnatum (Kalanchoe pinnata) leaves infusion group concentration of 40% increased until the 60 minutes with % KVU amounted to 76.09%, and gradually fall in the 90 minutes with % KVU amounted to 22.47% and continued to decline until to-minute 180 with % KVU by 7.52%. Percentage of edema in the group of bryophyllum pinnatum (Kalanchoe pinnata), concentration bryophyllum pinnatum (Kalanchoe pinnata) infuse 80% smaller compared to the negative control and increased edema in the 30 minutes with % KVU amounted to 68.65%, and then declined in the 60 minutes with % KVU amounted to 63.59%, and continued to decline until the 180 minutes with % KVU amounted to 14.93%.
Table 2. Percentage anti-inflammation of southwestern Bryophyllum pinnatum (Kalanchoe pinnata) leaves to infuse in mice injected male swiss webster acetic acid 1%

| Treatment group | AUC   | % Power anti-inflammatory |
|-----------------|-------|--------------------------|
| Control (-) (Aquadest) | 44063.31 | 0 |
| Control (+) (Aspirin) | 15139.62 | 61.076 |
| Bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse 20% | 14428.47 | 66.274 |
| Bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse 40% | 19228.38 | 49.704 |
| Bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse 80% | 20208.456 | 54.348 |

The % increase in the volume of edema was made relationship % KVU with time, AUC0-6 then calculated using the formula:

\[
\text{AUC0-6 + C0} = \frac{(\text{C0} \times \text{t0.5} + \text{t0} + \text{C1} + \text{C0} \times \text{t1.5} + \text{t0.5} + \ldots + \text{C6} + \text{C0} \times \text{t5.5})}{2}
\]

C0 - C6 is the percent increase in the volume of edema (% KVU) on the clock to 0.5 to 6 in each group. AUC 0-6 % KVU is the area under the curve % increase of individual mice of the data AUC 0-6% calculated % KVU anti-inflammatory power of each individual by the formula:

The ability of a material to reduce inflammation in the leg test animals due to injection of acetic acid 1% expressed as anti-inflammatory power. Anti-inflammatory value is obtained by comparing the area under the curve edema volume infusion bryophyllum pinnatum (Kalanchoe pinnata) leaves and positive control to the area under the curve of the negative control.

\[
\text{anti-inflammatory power : } 100\% \times \left( \frac{\text{Auck} - \text{AUCp}}{\text{Auck}} \right)
\]

Auck is the area under the curve of the average in the negative control group and AUCp is the area under the curve of each individual in each treatment group. Statistical analysis of the data AUC0-6 and anti-inflammatory % power with Kruskal-Wallis and Mann Whitney test.

The area of the curve provides information about the potential of bryophyllum pinnatum (Kalanchoe pinnata) leaves to reduce edema when compared to the negative control. The larger the area under the curve, the greater the volume of edema caused. Based on table 3, the area of the curve in the treatment group bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse smaller than the negative control and the treatment group infuse bryophyllum pinnatum leaves (Kalanchoe pinnata) at a concentration of 20%, 40%, and 80% is almost the same when compared to the positive control. Bryophyllum pinnatum (Kalanchoe pinnata) leaves have the potential to reduce inflammation and nearly as effective when compared to the positive control.

Table 4. Test results in kruskalwallis

| The percentage of anti-inflammatory power |
|------------------------------------------|
| Chi-Square | 1.26 |
| df         | 2    |
| Asymp. Sig. | .533 |

Test Statistics,
a. Kruskal Wallis Test
b. Grouping Variable: concentration

Levene test of the anti-inflammatory power of concentration and% indicates homogeneous data results, while the Shapiro-Wilk test showed that the data were not normally distributed then performed the analysis of non-parametric statistical tests to test Kruskalwallis. Kruskal-Wallis test is a nonparametric test were used to compare three or more groups of data samples. Kruskal-Wallis test is used when the assumptions Way Anova has not met, besides as an extension of the Mann Whitney test [12].

The test showed that no significant difference was not significantly different or because of the significant value of more than 0.05 in 0.533 (Table 4). This suggests that increasing the concentration did not give a great influence on the decline in the volume of edema thigh webster male Swiss mice.

In the treatment group and the bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse positive control than the negative control, statistically by the Mann Whitney test. Mann Whitney test was conducted to determine if there are potential bryophyllum pinnate leaves infuse duck (Kalanchoepinnata) to cure mice thigh edema in male Swiss Webster. Mann Whitney test showed no significant differences significant or meaningful because the significance value <0.05 is 0.00. It is clear that infuse bryophyllum pinnatum (Kalanchoe pinnata) leaves effective in reducing the volume of edema in the thigh leg male Swiss Webster mice.

Bryophyllum pinnatum (Kalanchoe pinnata) leaves to infuse and aspirin has a way of working together, wherein the test solution has undergone the absorption, distribution, metabolism, and elimination in the body of mice that causes a decrease in the volume of edema in mice. The decline in the volume of edema in the infusion group bryophyllum pinnatum (Kalanchoe pinnata) leaves suspected to be caused by the flavonoids in leaves bryophyllum pinnatum (Kalanchoe pinnata). The mechanism of action of flavonoids in reducing the volume of edema which inhibit the cyclooxygenase enzyme that inhibits the formation of prostaglandin. The selectivity of cyclooxygenase inhibition of COX-1 which will prevent the formation of thromboxane. While the selectivity of the inhibition of COX-2 would prevent the formation of prostaglandins, which is a mediator of inflammation, and products of arachidonic acid metabolism thereby inhibiting the formation of exudation thus inhibiting the formation of edema [13].

4. Conclusion
Based on the results of this study concluded: There is the potential of bryophyllum pinnatum (Kalanchoe pinnata) leaves infuse to cure mice thigh edema in male Swiss Webster. Increasing the concentration of infusion bryophyllum pinnatum leaves (Kalanchoe pinnata) does not give a great influence on the volume of edema thigh Swiss Webster mice.

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