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SHORT COMMUNICATION

Effect extract of *Ipomoea pes-caprae* leaf as anti-inflammatory non-immunological in rat *Rattus norvegicus*

Safrida Safrida*, Hasanuddin Hasanuddin, Nurul Asri Agusdinianti

Department of Biology Education, Faculty of Teacher Training and Education, Universitas Syiah Kuala, Darussalam Banda Aceh, Indonesia. 23111. *Corresponding author's email: saf_rida@unsyiah.ac.id

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ABSTRACT

Ipomoea pes-caprae is one of the plants in Indonesia that has the potential as a traditional herbal medicine to treat inflammation. Hence, the objective of this study was to evaluate the effect of I. *pes-caprae* leaf extract as an anti-inflammatory in edema rats induced by 5% egg white solution. The experimental design used was a Completely Randomized Design (CRD) consisted of 5 treatments; each consisted of 5 rats. The treatment consisted of KN = negative control, KP = positive control, giving of 25 mg diclofenac sodium, K 100 = giving 100 mg kg^{-1} body weight (BW) of I. *pes-caprae* leaf extract , K300 = 300 mg kg^{-1} BW of *I. pes-caprae* leaf extract, and K500 = 500 mg kg^{-1} BW of *I. pes-caprae* leaf extract. The anti-inflammatory data obtained were tested statistically with a one-way ANOVA test at 99% confidence level and followed by Duncan's test. The ANOVA test results showed that the giving of *I. pes-caprae* leaf extract had a very significant effect ($p < 0.01$) on the percentage of inflammatory, obtained Fcount > Ftable, 21.46> 4.79 at the level of $\alpha = 0.01$. The treatment of *I. pes-caprae* leaf extract of 500 mg kg^{-1} BW had the effect of decreasing the volume of edema with the positive control variable using diclofenac sodium 25 mg. It is concluded that *I. pes-caprae* leaf extract can be used as an anti-inflammatory by decreasing rat feet edema volume. I. *pes-caprae* leaf extract has a prospect for non-immunological inflammatory natural drug candidates.

Keywords: Anti-inflammatory, Rat, Edema, *Ipomea Pes-Caprae*

INTRODUCTION

Indonesia is a tropical country with many potential plants that still use traditional medicines in their daily lives (Ministry of Trade Republic of Indonesia, 2014). Most Indonesian conventional medication has not been studied scientifically for their efficacy. Proving the benefits of traditional medicine through clinical trials supported by immunological research, both through qualitative and quantitative evaluations, need to be encouraged (Subijanto and Diding, 2008).

One of the plants in Indonesia that overcomes traditional herbal remedies to deal with inflammation is horse tread *Ipomoea pes-caprae* L. *Ipomoea pes-caprae* leaves are used in traditional medicine in the world to prevent jellyfish stings (da Silva Barth *et al.*, 2017) *I. pes-caprae* is a medicinal plant that is used for many treatments for several diseases, including inflammatory and algesic processes. The initial phytochemical analysis shows the presence of steroids, terpenoids, alkaloids, and flavonoids (de Souza *et al.*, 2000). The *I. pes-caprae* plant contains active components such as naphthalenone, mellein, eugenol, 4-vinyl-guaiacol. lipophilic glycosides, 2-methylpropanoic, (2S)-methylbutyric, n-hexanoic, n-decanoic, and n-dodecanoic acid (Manigaunha *et al.*, 2011), also contain alkaloids, flavonoids, tannins, steroids, acetic acid, melissic acid, myristic acid (Muthalib *et al.*, 2013).

Flavonoids are derivatives of flavones (2-phenyl-benzo-y-piron) and some (called isoflavonoids) from isoflavones (3-phenyl-benzo-y-piron) which contain anti-inflammatory, anti-oxidant, anti-bacterial, anticoagulant, anti-hepatotoxic and are spasmolytic. Flavonoids work by reducing the permeability and fragility of capillaries (Supriyatna *et al*., 2014) to relieve pain (analgesics), relax muscles and inhibit prostaglandin synthesis (Ling *et al*., 2009). Besides flavonoids, eugenol is also efficacious in treating inflammation. Eugenol is a common substance as an inhibitor of prostaglandin synthesis and also has anti-inflammatory activity. This substance also supports being able to prevent leukocyte chemotaxis and prevent the production of oxygen free radicals by leukocytes (Maria *et al*., 2011).

Ipomoea pes-caprae leaf extract can be obtained by maceration technique using ethanol solvent. Maceration (softening) is an extraction technique carried out at room temperature which allows for solvents to determine cellular structure in plants and dissolve the active components contained in these plants (Supriyatna *et al*., 2014). Macerate is chosen because it is the easiest method with high extraction yields and not many physical disturbances (Saifudin, 2014). Maceration using ethanol solvents is often used in laboratories because it has relatively high solubility and is inert, so it is not compatible with other components (Susanti, 2012).

Therefore, the researchers were interested in conducting further research to study extracts of anti-inflammatory *I. pes-caprae* in the form of 95% ethanol extract of Ipomoea pes-caprae leaves using maceration method. The objective of this study was to evaluate the effect of I. *pes-caprae* leaf extract as an anti-inflammatory in edema rats induced by 5% egg white solution.

MATERIALS AND METHODS

Site and Time

The leaves of a young *I. pes-caprae* were taken at Ulee Lheu, Meuraxa District, Banda Aceh City in December 2018. The criteria for the leaves taken are dark green leaves but with normal conditions without any damage to the color and shape of the leaf surface. Chemical analysis has been carried out at the Physiology Laboratory, Faculty of Veterinary and biology laboratory, FKIP, Universitas Syiah Kuala.

The technique of Making Ethanol Extract of Leaves of *Ipomoea pes-caprae*

Ipomoea pes-caprae leaves are finished then dried air until dry, then blended until smooth to powder, and finally sifted. Making ethanol extract of Ipomoea pes-caprae leaves is done by maceration. 200 gram Ipomoea pes-caprae leaf powder was extracted using 95% ethanol as much as 2000 mL. The maceration process is carried out in a black container that is tightly closed for 5 x 24 hours while occasionally stirring for 30 minutes per day. Macerate obtained with filter paper (filtrate 1) and received was extracted again with 95% ethanol as much as 600 mL for 2 x 24 hours then filtered (filtrate 2). Filtrate 1 and filtrate two were collected and then evaporated using an evaporator at 50 ^{o}C. The extract obtained is evaporated with water to get a thick extract (Ali *et al*., 2015).

Testing for Anti-inflammatory Effects in Animals

The stages of testing the anti-inflammatory effect of ethanol extract of Ipomoea pes-caprae leaves in rats *Rattus norvegicus* were as many as 25 male rats *R. norvegicus* selected to be divided into 5 treatments. Rats fasted to eat for about 18 hours. Then on the testing day, the test animals were weighed and grouped randomly, namely negative control, positive control, and test treatment. Each

test needs to consist of 5 rats, each rat is marked as limited to the ankles. Before starting the procedure, the volume of the rat's feet was measured to determine the initial volume (Vo) using a measuring cup by dipping the feet of the mouse to the limit made. Then the rats were treated orally using 2 ml of oral test solution in each rat. Then 2 ml of distilled water is given to treatment I, and 2 ml of diclofenac sodium in treatment II, then 2 ml of ethanol extract of Ipomoea pes-caprae leaves (previously diluted first according to the desired dose) in treatment III, IV and V. Thirty minutes later, each one of them was induced by a 5% egg white solution of 0.4 ml on the rat's feet. The volume of rat foot edema is then measured again using a measuring cup immediately after induction and every 1 hour for 7 hours after induced egg white solution.

Data Collection

Data obtained from measurements of the volume of rat feet were tabulated at all times in all treatments. Furthermore, a curve is made between the relationship between the average amount of edema and time (t) to form the Under Curve Area (AUC) using a formula (Anggraini, 2008).

$$AUC^{tn}_{tn-1} = \frac{Vt_{n-1} + Vt_n}{2} (t_n - t_{n-1})$$

Where, Vtn-1 = average volume edema(ml) on tn-1, Vtn = average volume edema(ml) on tn

The amount of anti-inflammatory power is expressed by Percent Anti-Inflammatory Power (% DAI), with a formula (Anggraini, 2008):

$$\%\ DAI = \frac{AUC_k - AUC_p}{AUC_k} X\ 100\%$$

Where, AUCk = AUC the volume edema (ml) curve averages over time for negative controls, AUCp = AUC the volume edema(ml) curve averages for time for the treatment group for individual.

Data Analysis

The anti-inflammatory data obtained were subjected to one-way Analysis of Variant (ANOVA) test with a 99% confidence level, and further testing was done using Duncan's test.

RESULTS

Tests carried out on the feet of rat which induced a solution of 5% egg white as much as 0.4 ml, this compound will cause cell edema with the release of intra-cell fluid into the interstitial fluid which initiates inflammation. Test materials can be said to have anti-inflammatory effects if they can reduce the volume of edema after induction. In this experiment using the AUC (Area Under the Curve) parameter, namely the area under the curve, this AUC shows the effect because in the graph there are areas that show the magnitude of anti-inflammatory values, the higher the area under the curve. Maximum and if the area under the curve gets smaller, the test material is minimal in inhibiting edema volume. The results of the study which showed an increase in edema and research variables in preventing inflammation in the wistar male rats' legs *Rattus norvegicus*. The average enlargement of rat feet edema at each hour given treatment of *I. pes-caprae* leaf extract in various treatments can be seen in Table 1.

Table 1. The average volume of edema of rat feet before and after treatment in various treatments

Treatments	Volume Edema (ml) at hour to (t=n)								
	t=0	**t=1**	**t=2**	**t=3**	**t=4**	**t=5**	**t=6**	**t=7**	**Mean**
KN = negative control	0.34	0.39	0.42	0.46	0.49	0.52	0.52	0.53	0.46[c]
KP = positive control, giving of 25 mg diclofenac sodium,	0.34	0.35	0.34	0.33	0.33	0.32	0.31	0.30	0.32[a]
Giving 100 mg kg^{-1} BW of *I.Pes-Caprae* leaf extract	0.34	0.35	0.35	0.34	0.33	0.31	0.31	0.30	0.38[b]
300 mg kg^{-1} BW of *I. Pes-Caprae* leaf extract	0.34	0.36	0.36	0.37	0.37	0.37	0.36	0.36	0.36[ab]
500 mg kg^{-1} BW of *I. Pes-Caprae* leaf extract	0.34	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.32[a]

*The letters of different superscripts in the same column are significantly different at $p<0.01$

The ANOVA test results showed that the giving of *I. Pes-caprae* Leaf Extract gave the significant effect ($p < 0.01$) on the percentage of inflammatory, obtained Fcount > Ftable, 21.46> 4.79 at the level of $\alpha = 0.01$. Based on the Duncan test the results showed that the highest AUC data on the volume of rat leg edema occurred in the treatment of *I. Pes-Caprae* leaf extract dose of 500 mg kg^{-1} BW when compared with negative controls, and extract of 100 mg kg^{-1} BW. In the extract treatment the dose of 500 mg kg^{-1} BW showed the same results as the positive control and the extract treatment dose of 300 mg kg^{-1} BW, but the treatment value of the extract dose of 500 mg kg^{-1} BW was very close to the positive control value This means that the best dose for treatment of decreased volume of edema is a dose of 500 mg kg^{-1} BW.

Figure 1 showed that in the positive control there was an increase in the volume of edema at the first hour by 0.35 ml and a gradual decrease in the volume of edema at 2 hour from 0.34 ml to 7 hour the edema volume was 0.30 ml on *I. Pes-Caprae* leaf extract 500 mg kg^{-1} BW there was an increase until the second hour of 0.35 ml and there was a decrease in the 3rd hour from 0.34 ml until the 7th hour the edema volume was 0.30 ml. Leaves of *I. Pes-Caprae* leaves at a dose of 500 mg kg^{-1} BW caused the largest decrease in volume of edema when compared with *I. Pes-Caprae* leaf extract dose of 300 mg kg^{-1} BW which only decreased edema volume at 6 to 7 hours to 0.36 ml. *I. Pes-Caprae* leaf extract of 100 mg kg^{-1} BW cannot reduce edema volume but can suppress an increase in edema volume of only 0.42 ml compared to the negative control an increase in edema volume found 0.53 ml. The treatment of *I. Pes-Caprae* leaf extract dose of 500 mg kg^{-1} BW has the effect of decreasing amount of edema, which is almost the same as the positive control variable using diclofenac sodium 25 mg. The anti-inflammatory effect of *I. pes-caprae* leaf extract can be determined by comparing AUC treatment of *I. pes-caprae* leaf extract with negative controls, the higher the volume of edema, the higher the price of the AUC (Table 2). The average percentages inflammatory illustrates that in the treatment, there is a decrease in the volume of edema, so it is possible that the leaves of *I. pes-caprae* extract have an anti-inflammatory effect.

Table 2. AUC Data (Area Under Curve) Volume of Edema Against Time and percentages of Inflammatory Power at Various Treatments

Treatments	Data Area Under Curve (ml h^{-1})	Inflammatory Power (%)
KN = negative control	3.22	-
KP = positive control, giving of 25 mg diclofenac sodium,	2.29	28.97
Giving 100 mg kg^{-1} BW of *Ipomea Pes-Caprae* leaf extract	2.29	28.85
300 mg kg^{-1} BW of *Ipomea Pes-Caprae* leaf extract	2.55	20.97
500 mg kg^{-1} BW of *Ipomea Pes-Caprae* leaf extract	2.67	17.09

DISCUSSIONS

The mechanism of the anti-inflammatory effect of ethanol extract of *I. pes-caprae* leaves by decreasing rat feet edema volume. It caused be bioactive compound contained in the ethanol extract of *I. pes-caprae* leaves. The amount of anti-inflammatory on leaves is better than the infusion of Ipomoea pes-caprae leaves because 70% ethanol is the right solvent. According to Supriyatna *et al.* (2014) the content of flavonoids and eugenol is a derivative of flavone (2-phenyl-benzo-y-piron) and from isoflavones (3-phenyl-benzo-y-piron) which have activity anti-inflammatory. Flavonoids work by reducing permeability and capillary fragility.

Treatment with bioactive compound 3-hydroxy, 2-methoxy-sodium butanoate reduced the paw edema induced by carrageenan and Freund complete adjuvant (FCA) dose-dependently. The 3-hydroxy,2-methoxy sodium butanoate isolated from plant leaves displays considerable potency in anti-inflammatory action (Prakash *et al.,* 2014). The 2-methyl-3-(4-(5-(4-(trifluoromethyl)phenyl) isoxazol-3-yl)phenyl)quinazolin-4(3H)-one 5e was found to be the most active compound as anti-inflammatory (Saravanan *et al.,* 2013). The 40% ethanolic extracts of the three plants were almost non-toxic at the dose of 5g kg^{-1} and all of them showed significant anti-inflammatory effects in the tests of xylene-induced ear edema and formalin-induced inflammation (Chen *et al.,* 2013). The edema formation 0.1 ml carrageenan at a dose of 1% w/v was injected into paw of left hind. It showed a fall of edemas 37.50%, 48.34%, and 55.83% while used stem bark of *Oroxylum indicum* (*O. indicum*) doses were 100, 200, and 400 mg/kg BW (p.o.) individually (Begum *et al.,* 2019)

The methanol extracts (200 and 400 mg/kg) and the ethyl acetate extract (400 mg kg^{-1}) exhibited significant ($P < 0.05$) anti-inflammatory activity when compared with that of their control groups (saline and vegetable oil respectively), with an onset of 150 min and a duration of 2.5 hours. The methanol extract (200 mg/kg) exhibited significant ($P < 0.05$) analgesic activity, with an onset of 60 min and a duration of 2 hours. The present study provided scientific justification that the extracts of *Smilax ornata* Lem. possess significant anti-inflammatory (Khan *et al.,* 2019).

CONCLUSIONS

Giving *I. pes-caprae* leaf extract can be used as an anti-inflammatory by decreasing rat feet edema volume. *I. pes-caprae* leaf extract has a prospect for non-immunological inflammatory natural drug candidates.

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