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ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF MARBOSI-BOSI LEAVES (*Tarennapolycarpa* (Miq.) Koord.ExValeton) ON WHITE RAT IN CARRAGEENAN INDUCED PAW INFLAMMATION.

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ABSTRACT

Traditionally, (Tarenna polycarpa (Miq.) Koord Ex Valeton as known as marbosi-bosi which commonly found in Sibolga, North Sumatera, Indonesia has been used as antidiabetes, cholesterol, anti-inflammatory and antibacterial. Tarenna species has been found its activities as antimicrobial, anti-oxidant and anti-inflammatory activity. The aim of this study was to investigate the anti inflammatory effect ethanol extract of marbosi-bosi leaves (Tarenna polycarpa (Miq.) Koord Ex Valeton in terms of decreased edema volume of male white rat in 1% carrageenan-induced and also to determine the effective dose of extract to decrease the volume of rat paw edema Ethanol extract of marbosi-bosi (Tarenna polycarpa (Miq.) Koord Ex Valeton was obtained by maceration. The antiinflammatory activity test was divided into 5 groups. The Group I (negative control) was given CMC 0.5%, Group II (positive control) was given diclofenac sodium 2,25 mg / kg BW, while Group III, IV and V were given marbosi-bosi leaf extract at a dose of 50, 100 and 200 mg/kgBW respectively. Each rat was induced by 1% carrageenan subplantar injection. Examination of antiinflammatory effect was measured by using digital plethysmometer *at minute of 30 to minute of 360. The data were analyzed statistically using ANOVA (analysis of variance).The results showed that negative control did not show anti-inflammatory effect had significant differences with other treatment groups. In conclusion, ethanol extract marbosi-bosi (Tarenna polycarpa (Miq.) Koord Ex Valeton has an effective anti-inflammatory activity at a dose of 100 mg / kgBW.*

Keyword: *Antiinflammation, Marbosi-Bosi, Rat Paw, Edema, Carrageenan*

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INTRODUCTION

Traditionally, medicinal plants of Rubiaceae family that is (*Tarenna polycarpa* (Miq.) Koord Ex Valeton as known as marbosi-bosi which commonly found in Sibolga, North Sumatera, Indonesia has been used as antidiabetes, cholesterol, anti-inflammatory and antibacterial [1].The previous study stated that the Rubiaceae family (*Tarenna asiatica*) contains of flavonoids that used as antimicrobials and antioxidants [2]. Its isolation contain flavon and terpenoid compounds [3]. Rubiaceae family also mentioned that it has antiinflammation effect [4]. Tarenna polycarpa (Miq.) Koord. Ex Valeton) also has antibacterial activity antioxidant and antidiabetic activity [5].

The content of secondary metabolite compounds such as flavanoid, saponin and triterpenoid / steroid has an

effect as anti-inflammatory. Flavonoid compounds are known to play an important role in inhibiting the biosynthesis of prostaglandins (PGE) and lipooksigenase (LOX) [6]. The mechanism of flavonoids in inhibiting the process of inflammation through two ways, namely by inhibiting capillary permeability and inhibit the metabolism of arachidonic acid and lysosomal enzyme secretion of neutrophil cells and endothelial cells [7]. Flavonoid type compounds such as hesperidin, apigenin, luteolin, and quercetin have anti-inflammatory and analgesic effects [8]. Quercetin, apigenin and luteolin, reduce the expression of cytokines. In this case, flavonoids may have therapeutic potential in the treatment of inflammatory-related diseases as cytokine modulator [9].

Currently, there are various medications used to treat inflammation. Antiinflammatory classes of steroids and non steroids are harmful when it used improperly, long-term use may cause severe side effects such as gastric ulcers, osteoporosis, susceptible to infection and muscle weakness [10].

Because of that, it is necessary to conduct research on marbosi-bosi leaf (*Tarenna polycarpa* (Miq.) Koord Ex Valeton) to know its anti-inflammation effect against carrageenan-induced rat paw edema.

MATERIALS AND METHODS

Plant Material

Marbosi-bosi leaf (*Tarenna polycarpa* (Miq.) Koord Ex Valeton) sample was collected from Sibolga , Northern Sumatera, Indonesia.

Experimental Animals

Male wistar rat (150-200g) were used in the entire study. The animals were fed with standard laboratory diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Fig. 1: Male wistar rat

Extraction of Marbosi-bosi leaves

An amount of 1000 g dried Marbosi-bosi leaves were crushed in a blender, then macerated in ethanol 80 % for 5 days thereafter countinue to remecerated for 2 days. The solvent was evaporated at low pressure with a temperature of not more than 40 ° C using a Rotary evaporator, then dried using freeze dryer.

Preparation suspension of ethanol extract marbosi-bosi leaves

Suspension of the extract was prepared using 0.5% CMC-Na with certain concentration

Preparation carrageenan solution

Weighed as much as 100 mg lambda-carrageenan, was dissolved using 0.9% NaCl solution ad libitum 10 ml, and incubated at 37 ° C for 24 hours.

Experimental Design

The Animals were injected 0.05ml of 1% carrageenan into the sub plantar surface of rat hind paw. It shown in Figure 2

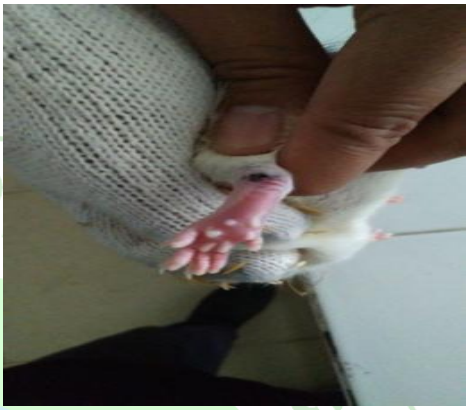

Fig. 2: The sub plantar surface of rat hind paw

Group I **:** Served as negative control (Na-CMC 0,5 %)

Group II **:** Served as positive control. Rats were received Diclofenac Sodium 2.25 mg/kg BW

Group III **:** Rats were received ethanol extract of marbosi-bosi leaves at a dose of 50 mg/kg BW

Group IV **:** Rats were received ethanol extract of marbosi-bosi leaves at a dose of 100 mg/kg BW

Group V **:** Rats were received ethanol extract of marbosi-bosi leaves at a dose of 200 mg/kg BW

Treatment was given orally before carrageenan induced. In 30 minute after oral administration, the animals were injected 0.05 ml 1% carrageenan solution. Furthermore, rats paw volume was measured every 30 minute in minute of 30 to 360. Data of edema volume was determined using plethysmometer. Percentage of edema inhibition is calculated with the following formula: [11-12]

$$\%\ edema\ Inhibition = \frac{a-b}{a}\ x\ 100\%$$

Where,

a-represents the mean inflammation percentage negative control group

b- represents the mean inflammation percentage treatment group

STATISTICAL ANALYSIS

Analysis of all results was performed using Anova followed by tukey test

RESULTS AND DISSCUSION

Percentage of edema inhibition was illustrated the effectiveness of ethanol extract of marbosi-bosi leaf to inhibit inflammation that caused by carrageenan induction. The percentage inflammatory inhibition showed in table 1 and table 2.

Table 1. Percentage of edema inhibition

No	Group	Percentage of edema inhibition (minute of)					
		30	60	90	120	150	180
1	Negative control	0#	0#	0#	0#	0#	0#
2	Positive control	19.90+ 2,31*	24.92+1,00*	32.72+1,71*	38.76+ 5,54*	33.22+1,46*	34.74+ 0,80*
3	Extract 50 mg/kgbw	9.27+ 3,34*#	13.92+0,30*#	17.75+2,03*#	18.43+3,23*#	21.98+ 3,88*#	25.94+ 3,34*#
4	Extract 100 mg/kgbw	16.84+ 2,79*	21.19+0,71*	26.69+2,16*	27.36+ 0,91*	30.59+1,37*	32.13+0,89*
5	Extract 200 mg/kgbw	14.10+ 2,27*	20.81+1,42*	28.25+1,20*	32.46+ 2,31*	36.80+2,17*	37.60+ 2,39*

Where: * (Significantly different to negative control) # (Significantly different to positive control)

Table 2. Percentage of edema inhibition

No	Group	Percentage of edema inhibition (minute of)					
		210	240	270	300	330	360
1	Negative control	0#	0#	0#	0#	0#	0#
2	Positive control	36.87+0,79*	41.14+ 0,35*	48.15+ 1,27*	49.86+3,91*	54.13+2,34*	61.05+1,76*
3	Extract 50 mg/kgbw	26.74+3,00*#	30.78+2,97*#	37.74+ 1,35*#	34.21+2,79*#	37.14+2,83*#	38.86+2,75*#
4	Extract 100mg/kgbw	33.36+0,91*	38.08+ 0,51*	44.96+ 0,93*	43.16+ 2,43*	48.68+1,27*	51.44+1,64*
5	Extract 200mg/kgbw	37.87+2,59*	42.62+ 1,70*	48.09+1,03*	49.46+ 0,94*	48.77+3,44*	50.13+3,67*

Where: * (Significantly different to negative control), # (Significantly different to positive control)

The negative control group which given 0,5% Na CMC suspension in the absence of active compounds, there was a significant increase in edema volume with a much larger volume than the other treatment groups. This negative group becomes the reference in comparing the results that achieved by other groups. The test group with significantly different results to the negative control group showed the anti-inflammatory effect of the extract or the drug material given to the test animals.

The positive control group was administered suspension of Diclofenac Sodium with a dose of 2.25 mg / kgBW. The results obtained that significantly different to the negative control in each measurement time in the minute of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360.

The treatment group which given ethanol extract marbosi-bosi leaf in dose of 50 mg/kgbw, 100 mg/kgbw and 200 mg/kgbw had different results.

Based on Table 1 and Table 2, the percentage of edema inhibition of diclofenac sodium 2,25 mg/kgbw as a positive control and became standard references in looking fot the potential drug compounds in suppressing inflammation animal tested that induced by carrageenan.

The statistic result test showed that Group III which received an ethanol extract of marbosi-bosi leaves at a dose of 50 mg/kg BW, in minute 360 edema inhibition percentage value 38,87%. Group IV which given extract as much as 100 mg / kg bb BW has value 51,44% and Group V which given extract as much as 200 mg / kg BW has value 50,14 %. All extract treatment showed an activity in suppressing rat paw edema, but Group IV which given extract at a dose of 100 mg / kg BW showed effective results, this group was able to approach the value of 2.25 mg / kg BW of diclofenac sodium which had inflammatory inhibition percentage of 61.06%.

The presence of anti-inflammatory effects based on the experimental test due to secondary metabolite activity in the ethanol extract of marbosi-bosi leaf, namely flavonoids, steroids / triterpenoids, saponins and tannins. This is supported by the results of phytochemical screening tests that indicating the presence of those metabolites [5].

The anti-inflammatory mechanisms of flavonoids through multiple pathways. Flavonoid Inhibit Cyclooxygenase and lipoxygenase pathways directly also leads to inhibition of eicosanoid and leukotriene biosynthesis, which is the end product of COX and lipoxygenase pathways that lead to induce inflammation.

Inhibition of the cyclooxygenase pathway may have a wider effect because the cyclooxygenase reaction is the first step on the path leading to the eicosanoid hormone such as prostaglandins and thromboxane [13].

Flavonoid also Inhibit leukocyte accumulation, thereby decreasing the adhesion of leukocytes to the endothelium and resulting in decreased inflammatory response of the body. [6].

Terpenoid and Saponin also play a role in as anti-inflammatory [14] . Saponins interact with many lipid membranes, such as phospholipids that are the precursors of prostaglandins and other inflammatory mediators. Previous research proved that salient extracts

which containing saponins have anti-inflammatory effects on carrageen-induced rats [7].

CONCLUSION

Ethanol extract of marbosi-bosi leaf at the dose of 50,100 and 200 mg /kgBW have anti-inflammatory effects on carrageen-induced rat paw edema. Treatment dose which has effective activity is 100 mg/kgBW.

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