The Analgesic Effect of Ethanol Extract Soursop (*Annona muricata L.*) Leaves in Wistar Rats

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Abstract

Background: Pain is an emotional and sensory experience that is unpleasant and related to tissues damage. In the past, Soursop (*Annona muricata L.*) leaves have been believed to be able to relieve pain. This study aimed to explore the analgesic effect of soursop leaves and its effective dose in an animal model.
Methods: Wistar rats (n=25) had been used in this experimental study, divided into 5 groups; consisting of a negative control group, experiment groups using extract soursop leaves with doses of 200mg/kgbw, 400 mg/kgbw and 600 mg/kgbw, and natrium diclofenac as a positive control. One hour after treatment, all groups of rats were induced by carrageenan-lambda in the feet. The basal retraction of rats' legs was measured in 47°C water and repeated at two, four, and six hours. The data were analyzed using analysis of variance and Tukey's test.
Result: The dose of 200 mg/kgbw had no analgesic effect ($p>0.05$), while the dose of 600 mg/kgbw had the highest analgesic effect at 7.72 seconds on the 4th hour of induction. On the 6th hour, the dose of 400 mg/kgbw had the highest analgesic effect at 3.58 seconds.
Conclusions: Extract soursop (*Annona muricata L.*) leaves in this study have been proven to have an analgesic effect.

Keywords: Analgesic. *Annona muricata L.*, soursop leaves

Introduction

Annona muricata L. (Annonaceae), known as soursop plants, are commonly found in Central America to South America, including the North, Northeast and Southeast regions of Brazil.[1,2] In Indonesia, especially West Java province, many soursop plants are cultivated in the area of Pelabuhan Ratu Sukabumi and Rajamandala Bandung. Traditionally, the soursop leaves can be used for headaches, insomnia, cystitis, liver problems, diabetes mellitus, sedative, hypotensive activity, analgesic, anti-inflammatory, and antidysenteric.[2,3] Moreover, the leaves have parasiticidal, anti-rheumatic and anti-neuralgic effects.[1,2] Bioactive compounds of soursop leaves have been reported to be found such as acetogenins, alkaloids (isoquinoline, aporphine, protoberberine), tannin, coumarins, flavonoids, polyphenolic components, phytosterol (β-sitosterol, stigmasterol), vitamins B, C, and E.[4–6] Flavonoid is a strong inhibitor to lipid peroxidation, as a captor of reactive oxygen or nitrogen and also able to inhibit lipoxygenase and cyclooxygenase enzymes and as such, this compound can be used to reduce pain and thus serve as analgetic agent.[7–9]

Studies regarding soursop leaves as anti-inflammation, anti-rheumatic, and anti-neuralgia supported by pharmacological and clinical validation are still scarce[1], especially soursop leaves cultivated in West Java. This study aimed to explore the analgesic effect of the ethanol extract of soursop leaves from West Java, using an animal model.

Methods

This was an experimental study, conducted from May 2012 to January 2013, using Wistar rats, to explore whether soursop leaves extract had an analgesic effect. The

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Table 1 Leg Retraction Time After Administration of Soursop Leaves (*Annona muricata L.*) Ethanol Extract

Group	2 hours			4 hours			6 hours		
	$\overline{X}$	±	SD	$\overline{X}$	±	SD	$\overline{X}$	±	SD
Negative control	1.35	±	0.27	1.61	±	0.48	1.30	±	0.27
Dose 200mg/kgbw	1.91	±	0.50	3.72	±	1.65	1.49	±	0.24
Dose 400mg/kgbw	2.39	±	0.61	4.86	±	1.65	3.58	±	1.49
Dose 600mg/kgbw	3.36	±	1.66	7.72	±	2.04	3.36	±	0.85
Positive control	3.08	±	1.06	3.73	±	1.03	2.02	±	0.57

Note: $\overline{X}$ = average of leg retraction time in seconds; SD= standard deviation. Natrium diclofenac was given for positive control.

hyperalgesia test applied was according to a modified Randall-Sellito.[10–12] This study was performed in the Pharmacology Laboratory, Faculty of Medicine, Universitas Padjadjaran, and after ethical clearance, granted by the Health Research Ethic Committee Faculty of Medicine Universitas Padjadjaran. Inclusion criteria in this study were white male Wistar rats weighed at 180–220 grams, aged 2–3 months in healthy conditions and normal activity. Experimental rats were randomly assigned to groups 1 to 5. Group 1 was given 2 mL carboxymethyl cellulose (CMC) per oral and served as a negative control group. Group 2 to 4 were given ethanol extract of soursop leaves per oral, with doses of 200, 400, and 600 mg/kgbw, respectively. Group 5 as a positive control group was given natrium diclofenac per oral with a dose of 4.5 mg/kgbw. One hour after giving the experimental dose, carrageenan-lambda was given to the left foot to induce inflammation and pain. After two, four, and six hours of induction, respectively, measurement of left leg retraction time was examined in each group, by plunging their left leg to a water bath filled with 47°C water. The ankles of the rats were marked with waterproof tint as the marker of plunging. The length of the leg retraction time was limited to 15 seconds to avoid any thermal injuries.

Statistical analyses were conducted to compare the analgesic effect of different doses given and leg retraction time at two, four, and six hours after of induction, by using analysis of variance (F test), followed by Tukey test.

Results

The leg retraction time from various doses of soursop leaves ethanol extract treatment

Table 2 Tukey Test on Leg Retraction Time 2 hours After Administration of Soursop Leaves (*Annona muricata L.*) Ethanol Extract

Group (I)	Group (J)	Mean Difference (I-J)	p
Control negative	Dose 200mg/kgbw	-0.564	0.073
	Dose 400mg/kgbw	-1.046*	0.003*
	Dose 600mg/kgbw	-2.016*	0.000*
	natrium diclofenac	-1.736*	0.000*
Dose 200mg/kgbw	Dose 400mg/kgbw	-0.482	0.623
	Dose 600mg/kgbw	-1.452	0.104
	natrium diclofenac	-1.172	0.134
Dose 400mg/kgbw	Dose 600mg/kgbw	-0.970	0.751
	natrium diclofenac	-0.690	0.822
Dose 600mg/kgbw	natrium diclofenac	0.280	1.000

Note: *significancy was set on p<0.05

Table 3 Tukey Test on Leg Retraction Time 4 hours After Administration of Soursop Leaves (*Annona muricata L.*) Ethanol Extract

Group (I)	Group (J)	Mean Difference (I-J)	p
Control negative	Dose 200mg/kgbw	-2.110	0.199
	Dose 400mg/kgbw	-3.252	0.018*
	Dose 600mg/kgbw	-6.112	0.000*
	natrium diclofenac	-2.128	0.193
Dose 200mg/kgbw	Dose 400mg/kgbw	-1.142	0.739
	Dose 600mg/kgbw	-4.002	0.003*
	natrium diclofenac	-0.018	1.000
Dose 400mg/kgbw	Dose 600mg/kgbw	-2.860	0.043*
	natrium diclofenac	1.124	0.749
Dose 600mg/kgbw	natrium diclofenac	3.984*	0.003

Note: *significancy was set on p<0.05

per oral to Wistar Rats after 2 hours of carrageenan-lambda induction were as followed; there was a significant increase of leg retraction time with doses of 200, 400, and 600 mg/kgbw compared to negative control. This study indicated that the experiment doses shown an analgesic effect on the increase of leg retraction time. The soursop leaves ethanol extract dose of 600 mg/kgbw had an average analgesic effect of the highest at 7.72 seconds on the 4th hour of induction. The dose of 600 mg/kgbw had reached the maximum time equivalent to the treatment induced by natrium diclofenac, as a positive control. The result of leg retraction time after 2, 4, 6 hours of carrageenan-lambda induction with various doses of soursop leaves was shown in Table 1.

In summary, the leg retraction time from various doses of soursop leaves ethanol extract treatment per oral to Wistar Rats after 4 and 6 hours of carrageenan-lambda induction were as followed; there was a significant increase of leg retraction time with doses of 200, 400, and 600 mg/kgbw compared to negative control (Table 2, 3, 4). As for the induction of carrageenan-lambda after 4 hours, there was a maximum leg retraction time comparable to positive control already with the dose of 200

Table 4 Tukey Test on Leg Retraction Time 6 hours After Administration of Soursop Leaves (*Annona muricata L.*) Ethanol Extract

Group (I)	Group (J)	Mean Difference (I-J)	p
Control negative	Dose 200mg/kgbw	-0.192	0.844
	Dose 400mg/kgbw	-2.276	0.000*
	Dose 600mg/kgbw	-2.064	0.000*
	natrium diclofenac	-0.716	0.101
Dose 200mg/kgbw	Dose 400mg/kgbw	-2.084	0.003*
	Dose 600mg/kgbw	-1.872	0.003*
	natrium diclofenac	-0.524	0.502
Dose 400mg/kgbw	Dose 600mg/kgbw	0.212	1.000
	natrium diclofenac	1.560	0.091
Dose 600mg/kgbw	natrium diclofenac	1.348	0.091

Note: *significancy was set on p<0.05

mg/kgbw that was further increased with the dose of 400, and 600 mg/kgbw; whereas for induction of carrageenan-lambda after 6 hours, the dose of 400 and 600 mg/kgbw showed comparable leg retraction time (3.58s and 3.36s, respectively) and showed no significant difference to positive controls (2.02s).

Discussion

Annona muricata produced antinociception action of activity in both neurogenic and inflammatory phases. Metabolites of arachidonic acid (called eicosanoids) are involved in the inflammation process.[1] In this study, Carrageenan-lambda has been used. Carrageenan-lambda is a red sea seaweed extract that is often used to induce pain in the laboratory setting for experimental study.[2,13] This study shown that soursop leaf extract given an hour before administration of carrageenan-lambda induction has a comparable analgetic effect with natrium diclofenac. Natrium diclofenac has been proven to have an analgesic effect and in this study, it is used as a positive control. The metabolites are produced via cyclooxygenase and lipoxygenase when the cell is activated by mechanical trauma, cytokines, growth factors or other stimuli. It has been proposed that the mechanism of antinociception may be caused by inhibition of cyclooxygenase (COX) and lipoxygenases (LOX) and other inflammatory mediators such as flavonoids, present in the plant extract.[1,2] Flavonoids contained in soursop leaves are strong inhibitors to lipid peroxidation, and serve as a captor of reactive oxygen or nitrogen and also able to inhibit cyclooxygenase enzymes thereby reducing the biosynthesis of prostaglandins which are pain mediators and as such this compound can be used to reduce pain (analgesic).[1,14] Diclofenac is a phenyl acetic derivate that is relatively nonselective as a COX inhibitor. This drug has analgesic, antipyretic and anti-inflammatory effects by inhibiting prostaglandin synthesis.

The inhibition of the synthesis of pro-inflammatory prostaglandins is one of such therapeutic targets to which some of the potent analgesic and anti-inflammatory agents.[15] The analgesic effect can be seen by lengthened time of foot withdrawal. Hyperalgesia by the inflammatory process because of an increased sensitivity to pain receptors and a decrease in the pain threshold. Our study has shown that soursop leaf extract has an analgesic effect compared to the negative control group, and comparable to the positive control group Diclofenac. Interestingly, the length of induction with carrageenan-lambda has shown that there is an optimum effect of the inflammatory effect of carrageenan-lambda (i.e. 4 hours) and soursop leaves extract might be useful in lower doses. In this study, the strongest analgesic effect occurred at the 4th hour of induction probably the inhibitory effect on pain mediators was the most optimal, while at the 6th hour the inhibitory effect was reduced and caused no different dosage of 400 mg/kgbw and 600 mg/kgbw soursop leaf extract.

This study has hampered some limitations. The result of leg retraction time varies greatly with a high standard deviation. Since this experiment used an animal model, a minimal number of rats had been used. Further study is needed to confirm the true effect of soursop leaves as an analgesic agent.

Soursop (*Annona muricata L.*) leaves ethanol extract has been proven to have an analgesic effect on Wistar rats induced by carrageenan-lambda. Soursop (*Annona muricata L.*) extract may be useful in human, therefore, further in vivo study is needed.

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