

ORIGINAL ARTICLE

Analgesic Effect Study of Young Coconut Water (*Cocos nucifera L.*) on Mice (*Mus musculus*) Induced with Pain using Acetic Acid

Dini Indah Berlianti¹, Danti Nur Indiasuti^{2*}, Gondo Mastutik³, Shaohong Lai⁴

¹Faculty of Medicine, Universitas Muhammadiyah Surabaya, Surabaya, Indonesia

²Department of Anatomy, Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

³Department of Anatomical Pathology, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

⁴Faculty of Medicine, Graduate School of Medicine, Oita University University, Yufu, Japan

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* Corresponding author:

danti-n-i@fk.unair.ac.id

ABSTRACT

Introduction: Pain signals tissue damage that is capable of reducing the quality of life. Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are known as effective analgesic drugs which have various side effects, therefore natural minerals are used as an alternative medicine for pain and inflammation, one of which is known to be coconut water. Hence this research was conducted to find out the differences of the analgesic effect between young coconut water (*Cocos nucifera L.*) with non-selective and COX-2 selective NSAID on mice induced with pain from acetic acid 0.6% 1 ml/100gBW of mice.

Methods: True experimental, conducted at the Pharmacology Laboratory in Faculty of Medicine of Airlangga University involving the sample of 48 mice (6 groups). The recorded data was tested using the oneway ANOVA methodology before then continued with the posthoc test of LSD.

Results: The addition of young coconut water (*Cocos nucifera L.*) with the dosage of 3 ml/100gBW, 4 ml/100gBW, and 4.5 ml/100gBW of mice doesn't give any significant analgesic effect even though the analgesic protection percentage increases accordingly to its dosage (12.32%, 18.72%, 26.88%), but non-selective and COX-2 selective NSAID give significant analgesic effect ($p < 0.05$) on mice induced with pain from acetic acid 0.6% 1 ml/100gBW of mice.

Conclusion: There are differences in the analgesic effect of young coconut water (*C. nucifera L.*) with non-selective and COX-2 selective NSAID on mice induced with pain from acetic acid 0.6% 1 ml/100gBW of mice.

Introduction

Pain signals tissue damage that is capable of reducing the quality of life and becoming the primary cause of disability.¹ Tissue damage will cause arachidonic acid to be formed, and then the cyclooxygenase enzyme (COX) is converting it to be the pain mediator, called the prostaglandin, which will stimulate nociceptive to deliver pain to be perceived by the thalamus.²

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are the drugs used to tend to pain and inflammation that have high effectiveness.^{3,4} NSAID works on the inhibition of cyclooxygenase enzyme.⁵ NSAID is classified into non-selective (e.g. aspirin and ibuprofen), and COX-2 selective (e.g. celecoxib and meloxicam). Aspirin and celecoxib are capable to work on peripheral pain

stimulation that's delivered by nerve fiber C, for example on the chemical pain stimulation from acetic acid as an irritant.⁶ However, NSAID has harmful side effects, which generally affect the digestive and cardiovascular systems.⁵ Therefore, we need alternative medicine which has minimalistic side effects compared to NSAID.

Humans have been using herbs and natural minerals as an alternative medicine to tend to pain. One of them is the coconut, *Cocos nucifera L.* (Arecaceae).⁷ According to studies, the extract of coconut coir is capable of inhibiting the writhing reflex on mice.⁸ Matured coconut water (*C. nucifera L.*) that is consumed with the dosage of 4 ml/100 g BW of mice for 6 weeks can give an analgesic effect on mice.⁹ Coconut water also has an anti-inflammation effect, and it has been proved that young coconut water is



more potent than matured coconut water.¹⁰ The analgesic and antiinflammation effects inside the coconut water are caused by its content, namely the flavonoid and salicylic acid which will inhibit the forming process of prostaglandin.¹⁰ Salicylic acid (2-hydroxybenzoic acid) acts as the fitohormon on coconut that has the analgesic effect (Inhibition of enzyme COX-1&2), antipyretic, and antiinflammation.^{11,12} Young coconut water possesses much more salicylic acid concentration compared to matured coconut water.¹⁰

Hence why, the plan for research in order to learn the differences of the analgesic effect between young coconut water (*C. nucifera L.*) with non-selective and COX-2 selective NSAID on mice induced with pain from acetic acid 0.6% 1 ml/100gBW of mice with writhing reflex methodology was arranged.

Methods

Ethical Clearance

This research has been approved by the Ethical Committee of Faculty of Medicine Universitas Airlangga.

Research Design

This research is using a true experimental design where the total sample was divided into six treatment groups. Group 1 (P1) was given aquadest as K(-), group 2 (P2) aspirin (non-selective NSAID) with the dosage 6.5 mg/100gBW of mice as K(+), group 3 (P3) was given celecoxib (COX-2 selective NSAID) with the dosage 2.6 mg/100gBW as K(+), group 4 (P4) was given young coconut water with the dosage 3 ml/100gBW, group 5 (P5) was given young coconut water with the dosage 4 ml/100gBW, and group 6 (P6) was given young coconut water with the dosage 4.5 ml/100gBW of mice. Each treatment was given 30 minutes before the intraperitoneal injection of acetic acid 0.6% 1ml/100g BW of mice as pain induction. This was based on how long the drug's onset of work until it evokes the desired effect, for instance on aspirin whose onset starts at the 20-minute mark.¹³ The young coconut water (*C. nucifera L.*) is around 5-6 months old with the consistency of fruit flesh that is still soft and has a white with a little clear color. Male *Mus musculus* has the body weight of 26-35 gr and lives in a group inside a husk cage and was fed Charoen Pokphand 593 with the same water, which was acclimatized first for 14 days. The dosage of young coconut water was determined based on the previous research and the volume capacity of the mice's stomach, which is 1ml for 20g BW (body weight) of mice.¹⁴ The dosage of aspirin and celecoxib were determined by calculating the human-to-animal dosage conversion based on the conversion table by Laurence and Bachrach with the maintenance dosage of aspirin of 500 mg and celecoxib of 200 mg.¹⁵ The result of aspirin conversion was found to be 6.5 mg/100g BW of mice and celecoxib to be 2.6 mg/100g BW of mice. Both medicines were given to the mice with suspension preparations, thus they first needed to be dissolved with CMC Na on water. Suspension and aquadest given to mice were corresponding to the result of calculation for average mice bodyweight that is 30 g which were then calculated with the average volume of coconut water given, thus obtaining the given volume of 1.15ml/30gBW for treatment K(+), K(+), dan K(-). Given suspension

volume was calculated for each mouse according to their BW. Trial and error were done to see if the dosage volume that was given and the injected acetic acid gave the desired reaction.

Settings

The research was conducted at the Pharmacology Laboratory in the Faculty of Medicine of Airlangga University.

Time Frame

The research was conducted since March 2020 until October 2020.

Variables

The independent variables in this research are the giving of young coconut water (*C. nucifera L.*) with the dosage 3 ml/100g, 4 ml/100g, and 4.5 ml/100gBW of mice, aspirin suspension, celecoxib suspension, and aquadest according to the dosage calculated on the mice with the given volume 1.15 ml/30g BW. The dependent variables in this research are the amount of abdomen writhing based on the writhing reflex methodology. The control variables in this research are the male mice (*Mus musculus*) which received the same treatment and induced with acetic acid 0.6% 1 ml/100 g BW of mice.

Population, Samples, and Sampling

The population used for this research is the male mice (*Mus musculus*). The sample needed for this research was determined through the simple random sampling technique with calculation using Federer formula, and another sample was also added as an anticipation measure for sample error. The sample needed was 48 male mice *Mus musculus*.

Instruments

The research instruments used during the acclimatization process of mice were the body weight scale, cage, and food. During the process of treatment given, the instruments used were young coconut water (*C. nucifera L.*) with the dosage 3 ml/100g, 4 ml/100g, and 4.5 ml/100gBW of mice, aspirin 6.5 mg/100gBW of mice, celecoxib 2.6 mg/100gBW of mice, aquadest 1.15 ml, and a pipette. The instruments used during writhing reflex test and its data collecting process were acetic acid 0.6 % 1 ml/100g BW of mice, stopwatch, stationery, camera, tripod, and the writhing result table on writhing reflex.

Data Analysis

The data acquired was the amount of writhing reflex (stretching or extension movement of its back leg, contraction of abdominal muscle, or its back arch). The amount of writhing from each group was recorded every 5 minutes for as long as 60 minutes since the time they were given the treatment, before then calculating the average of each group. The data was calculated and analyzed using the comparison test using the one way ANOVA (Analysis of Variance) method; so that it was necessary to first conduct the normality test and homogeneity test. If the test result using the one way ANOVA method provides a result with a rather significant difference, then it will be followed by a posthoc test of Least Significant Difference (LSD). All tests were conducted using the IBM SPSS Statistics Subscription application version 25.

Results

The average writhing calculation was acquired as shown in table 1.

Table 1. Writhing average

Treatment	Average at the Minute-											
	5'	10'	15'	20'	25'	30'	35'	40'	45'	50'	55'	60'
P1	11.50	10.38	8.25	8.25	7.38	7.50	6.25	6.00	4.88	3.63	2.63	1.50
P2	7.63	7.00	5.13	2.75	3.13	2.38	1.63	2.13	1.50	0.75	1.00	0.63
P3	8.13	7.88	6.00	5.25	4.63	3.25	2.63	1.38	1.25	1.00	0.63	0.25
P4	11.75	11.88	8.75	6.25	5.88	5.75	4.13	4.38	3.63	2.38	1.63	2.13
P5	10.13	8.75	7.88	7.25	6.38	6.13	4.50	3.63	2.88	2.63	2.00	1.38
P6	9.88	9.00	7.13	5.50	4.88	4.63	4.13	3.13	2.00	2.88	2.50	1.50

Normality and homogeneity tests were conducted on the acquired data, from which we obtain the significance (sig.) value each of ($p > 0.05$) which indicates that the data was distributed normal and homogeneous. The data was calculated using the one way ANOVA method whose results are shown in Table 2.

Based on the result of the correlation test shown above, the significance value $p = 0.03$ ($p < 0.05$) was obtained. This shows that there is a significant difference between each group. And then, it was followed by further correlation test using the Posthoc method of LSD to see the significant difference between groups.

The interpretation of the analgesic effect can be seen through protection percentage, which is a calculation to see the inhibition of the writhing amount in percentages (%) on each treatment if compared to the writhing amount in control negative group K(-). The following formula was used:

$$\% \text{ Protection Percentage} = 100\% - (P/K \times 100)\%$$

P: Cumulative writhing amount for each treatment

K: Cumulative writhing amount for K(-) treatment

Table 2. Correlation Test Result with ANOVA One Way Method

Treatment	Average	ANOVA (p)
P1	78.13±25.21	0.03
P2	35.63±16.88	
P3	42.25±19.95	
P4	68.5±24	
P5	63.5±27.42	
P6	57.13±26.38	

Table 3. Analgesic Protection Percentage

Treatment	% Protection Percentage
P1	0%
P2	54.4%
P3	45.92%
P4	12.32%
P5	18.72%
P6	26.88%

Discussion

Based on Table 1, the result of average mice writhing from group P2, P3, P4, P5, and P6 have less value compared to the result of mice writhing from group P1. Based on the results of Table 2, there are significant differences in the research with significance value ($p < 0.05$) between each group. The test results using the posthoc method show the significant differences in the test results between P2 (K(+1)) and P3 (K(+2)) towards group P1 (K(-)). Based on Table 3, the result of analgesic protection in percentage (%) shows that the biggest inhibition value is found on group P2 (K(+1)), followed by group P3 (K(+2)) and group P6 (young coconut water with the highest dosage) with the values respectively being 54.4%, 45.92%, and 26.88%.

The analgesic test results of aspirin and celecoxib are parallel with the researches that have been conducted, wherein aspirin possesses analgesic effects and is capable to give a relatively high percentage of inhibition for mice writhing.^{16,17,18} Aspirin is an acetylsalicylate acid compound that is capable of inactivating the work of cyclooxygenase enzyme irreversibly and inhibits the expression of factor NFκB so that the transcription process of inflammatory mediator gene decreases.^{15,19} Celecoxib as COX-2 selective NSAID also possesses analgesic effects that are just as effective as those of non-selective.²⁰ Enzyme COX-2 plays the main role in producing inflammatory prostanoid mediators.¹⁵

The result of giving young coconut water doesn't statistically show any significant analgesic effect, but there is an increase of protection percentage along with the increase of the amount of dosage given. The biggest protection percentage was found at the given dosage of 4.5 ml/100gW, with the mice's stomach filling percentage of 90%, which if implemented on human then the consumption of young coconut water with the same capability to give an equal protection percentage is as much as 810 ml. This refers to the capacity of human's stomach which is established to be 900 ml.^{21,22} The result of this research isn't parallel with the result of previous ones conducted. According to studies, coconut water is capable of giving significant analgesic effects when given for six weeks at maximum with the dosage 4 ml/100gBW through the hot plate, tail flick, formalin-induced paw licking, and writhing reflex methods with the dependent variable that is the duration of the giving of coconut water, which are set to be two, four, and six weeks.⁹ Young coconut water has

an antiinflammatory effect on inflammation and edema in the subplantar tissue of a rat's leg using plethysmometer, it's because young coconut water has flavonoid content that can inhibit the synthesis of prostaglandin as inflammatory mediator, which is also the mediator for pain.^{10,23}

This difference may be caused by the influence from the duration of the coconut water giving to the final condition of the test animals, and is related to one of the contents in the coconut water, which is salicylic acid, whose long-term effect can affect the metabolism of connective tissue through the changes of composition, biosynthesis, or the metabolism of mucopolysaccharides in connective tissue on ground substance that is capable of suppressing the spread of inflammatory process, so that when given in longer duration it will give better end results.¹⁵ Aside from the water in the coconut, previous researches also explains that the aqueous extract and hydromethane extract from *C. nucifera L.* also possess analgesic effects that are effective when experimented on animals.^{8,24,25} Coconut water is safe for consumption, and the only general symptoms we may experience when consuming coconut water is the sensation of the stomach being full which resulted from consuming too much in a relatively short period time, other than that, there are no recorded side effects.^{26,27}

Conclusion

Young coconut water (*C. nucifera L.*) with non-selective and COX-2 selective NSAID given on mice that were induced with acetic acid 0.6% 1 ml/100g BW of mice have differences because the young coconut water given in this research didn't show an analgesic effect as significant as aspirin and celecoxib did, despite the writhing protection increasing accordingly to each addition of dosage given. More research regarding the analgesic effect of young coconut water will be needed using various variables.

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Conflict of Interest

The author stated there is no conflict of interest

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