The Effect Of Papaya Leaf Infusa (*Carica Papaya L.*) On Analgesic Effects In Male Swiss Mice

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Abstract. Pain is an unpleasant sensory and emotional experience resulting from tissue damage, either actual or potential or described as damage. One of the traditional plants that can be used as an anti-pain agent is papaya leaves (Carica papaya L.). The compounds in papaya leaves are flavonoids, alkaloids, saponins, and tannins. The mechanism of this compound as an anti-analgesic is by inhibiting the action of the cyclooxygenase enzyme. This will reduce the production of prostaglandins by arachidonic acid thereby reducing pain. This research is experimental with a Post-test-only Control Group Design. The test subjects used 25 male Swiss strain mice and were divided into 4 treatment groups, namely negative control, positive control, and papaya leaf infusion with concentrations of 25% and 75%. Before treatment, mice were fasted and induced with a hot plate. Observations were made by observing the activity and number of jumps and licks of mice for 15 minutes in 1 hour. Numerical data was then processed using SPSS with the One Way ANOVA test. The results showed that papaya leaf infusion was given with concentrations of 25% and 75%. Which has an analgesic effect on mice with an effective concentration of 25%.

Key words: Papaya leaves (Carica papaya L.), Analgesic, male mice

INTRODUCTION

Pain is an unpleasant sensory and emotional experience resulting from tissue damage, either actual or potential, or described in terms of such damage. These phenomena can vary in intensity (mild, moderate, severe), quality (dull, burning, sharp), duration (transient, intermittent, persistent), and spread (superficial or deep, localized or diffuse). Although pain is a sensation, it has cognitive and emotional components, which are described in the form of suffering. Pain is also related to avoidance reflexes and changes in autonomic output and pain is a subjective experience, just like when someone smells fragrant or rotten smells, tastes sweet or salty, all of which are perceptions of the five senses and are felt by humans from birth. However, pain is different from sensory stimuli, because the pain stimulus is something that originates from tissue damage or has the potential to cause tissue damage (Meliala, 2004).

The mechanism for the emergence of pain is based on multiple processes, namely nociception, peripheral sensitization, phenotypic changes, central sensitization, ectopic excitability, structural reorganization, and decreased inhibition (Bahrudin, 2017). Pain can be relieved by analgesics, which is a class of drugs most often used for the treatment of pain. Although it is generally safe to use, if it is used incorrectly, toxicity can occur. Analgesics are divided into two groups, namely the non-steroidal anti-inflammatory group (NSAID) and the opioid group, which works peripherally or centrally (Soenarjo, 2010).

One of the traditional medicines uses the papaya plant or papaya leaves. Papaya leaves (Carica papaya L.) Chemical compounds contained in papaya leaves (Carica papaya L.) Flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. One of the ingredients that have analgesic properties is flavonoids which inhibit the expression of isoforms of induced NOS (nitrite oxide synthase), COX (cyclooxygenase), LOX (lipooxygenase) which play a role in the formation of inflammatory mediators (Kumar & Pandhey, 2013).

In research, papaya leaf infusion (Carica papaya L.) in white mice contains flavonoid active substances that function as an analgesic by inhibiting the cyclooxygenase enzyme in the brain in prostalglandin biosynthesis. According to research by Lasarus, Najoan & Wuisan (2013) stated that papaya leaf extract (Carica papaya L.) has an analgesic effect at a dose of 100mg/50kgBB. Based on the description above, researchers are interested in conducting further research on the analgesic effect of papaya leaves (Carica papaya L.). This research was carried out by testing the analgesic effect of papaya leaves (Carica papaya L.) in the form of an infusion preparation. This dosage form was chosen because it is the simplest way to make herbal preparations such as leaves, stems and flowers.

METHODS

Research Design

This type of research is experimental using test animals: male Swiss mice. The research design used was Post-test Only Control Group Design. This research has received an ethical clearance test. Approval from the Bioethics Commission for Medical Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang. Ethical clearance No. 115/IV/2020/Bioethics Commission.

The research design can be seen in Figure 1.

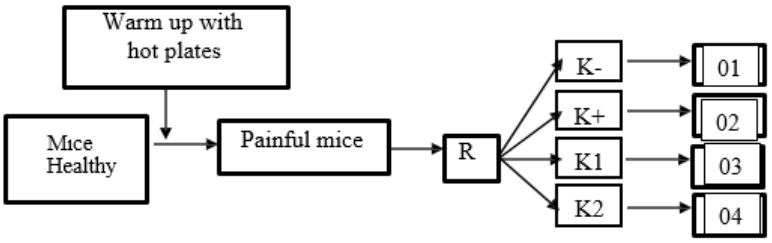

Figure 1. Research design

Information:
MS: Healthy Mice
R: Random
K-: Negative Control Group
K+: Positive Control Group
K1: 25% Concentration Group
K2: 75% Concentration Group
O1–O4: Observation of male Swiss strain mice licking or jumping

The tools used in this research were analytical scales, infusion pans, Erlemeyers, spatulas, glass stirrers, filter paper, funnels, stopwatches, beakers, measuring cups, pipettes, 1cc injection splits, markers, test animal cages, mouse probes, flannel cloth, stemper, mortar, test tube, test tube rack, test tube clamp, Bunsen lamp, water bath, water bath, drop plate, water bath. Materials used in the research were papaya leaves, distilled water, hot plate as a pain inducer, distilled ibuprofen, CMC Na powder, Mg powder, concentrated Hcl, chloroform, ammonia, 2N sulfuric acid, Mayer's reagent, Dragendroff's reagent, Wagner's reagent, HCl and solution $FeCl_3$ 1%.

Making papaya leaf infusion

Papaya leaves obtained from Kedungwinong village, Sukolilo District, Pati Regency. Sorted to separate from other plants. Then wash the papaya leaves thoroughly with running water, drain, and let air dry. Papaya leaves are dried by heating them in the sun and covered with black cloth. After the simplicia is dry, the simplicia is then blended and sieved with a 40 mesh sieve. Then make 100 ml of papaya (Carica papaya L.) leaf infusion with each papaya (Carica papaya L.) leaf weighed according to its concentration. 25 grams, and 75 grams. After that, each Simplicia is put inside an infusion pot, and 100ml water (+2x the weight of Simplicia). Each infusion pot is heated over a water bath for 15 minutes starting from the solvent temperature reaching 90°C, then filtered using a flannel cloth until the infusion water reaches 100 mL (Indonesian Pharmacopoeia Edition III 1979).

Phytochemical Screening

The phytochemical examination carried out was an examination of flavonoids, alkaloids, tannins, and saponins.

a. Test flavonoid phytochemicals using 4 gram samples of papaya leaves Add hot water and boil for 15 minutes at temperature 90°C and filtered. The filtrate is added with a little mg powder and concentrated I mL HCl then shaken. Positive results are red, yellow, or orange
b. Alkaloid phytochemical test by infusion of papaya leaves dissolved in 5 mL of HCl 2N. The solution obtained was then divided into 3 tubes. The first tube was blank, the second tube was added with 3 drops of Dragendroff's reagent and the third tube was added with Mayer's reagent. 3 drops formed orange in the second tube and a white precipitate in the third tube.

c. Tannin phytochemical test was done by infusion of papaya leaves with 3 drops of 1% $FeCl_3$. a blackish-green color is formed.
d. Saponin phytochemical test by adding papaya infusion The HCl is then shaken. Forms stable foam.

Grouping and test animals

The test animals used in the research were Swiss strain mice aged 2-3 months with a body weight of 20 -30 grams. All mice are maintained including food, water, cage, and bedding. Before treatment, they were fasted for 11 hours while still being given water. This aims to reduce the influence of food on test results. The mice used were 25 mice, which were randomly assigned to 4 groups**.**

1. Group 1 negative control cmc na 0.1% 1ml in each mouse
2. Group 2 positive control ibuprofen dose of 41 mg/kg BW was given 1ml to each mouse
3. Group 3 papaya leaf infusion 25% concentration
4. Group 4 papaya leaf infusion 75% concentration

Each was given I ml/gram BW to the test animals orally after an interval of 15 minutes within 1 hour of hot plate chemical stimulation and then the results of the number of jumps and licks were observed. The research results were analyzed using One-way ANOVA. If there is a significant difference followed by the LSD (Least Significant Different) test. To determine whether there are differences between groups.

RESULTS AND DISCUSSION

Research on the analgesic test of papaya leaf infusion (Carica payaya L.) by observing the number of jumps and licks with male Swiss mice as test animals. Because the test animal chosen has the advantage of being small in size, economical and the results are expected to be accurate and easier to control (Safitri, 2013). This research uses hotplate induction. The results of the phytochemical screening contained flavonoids, saponins, tannins, and alkaloids as analgesics. Flavonoids as analgesics work to inhibit the oxygen-binding enzyme cyclooxygenase catalyst in the formation of prostaglandins.

Table 1. Phytochemical screening results

Content	Preaction	Results	Information
Flavonoids	Mg + concentrated HCl	+	Qred or Jingga
AlkMayer's aloid	Chloroform+ammonia + sulfuric acid	+	A precipitate is formed in white
Alkaloid Wagner	Chloroform+ammonia + Concentrated HCl	+	A precipitate is formed brownish red
Saponin	Mg + water + HCl	+	Formed table foam
tannin	Ethanol + $FeCl_3$ 1%	+	Forms dark blue or greenish black

Source: Primary Data 2020

Analgesic Effect of Papaya Leaf Infusion (Carica papaya L.)

Number of jumps and licks in mice

In testing the analgesic effect of papaya leaf infusion (Carica papaya L.) is obtained by counting the number of jumps and licks on mice. The results are the average number of jumps and licks of mice for each group by testing the analgesic effect of papaya leaves (Carica papaya L.) can be seen in Table 2.

Table 2. Results - Average number of jumps and licks of mice per group

	Time					
Group	**Before treatment**	**15'**	**30'**	**45'**	**60'**	**SD means**
	L J	L J	L J	L J	L J	L J
Control (-)	30	26	27	24	20	25.4 ± 3.7 #
Control (+)	9	8	10	11	8	9.2 ± 1.30 *
Infusion 25%	10	9	11	13	11	10.8 ± 1.48*
Infusion 75%	12	11	8	14	10	11 ± 2.23 *

Source: Primary Data 2020

Information :
L : Jump
A : Lick
(*) : Indicates a significant difference from the negative control group (P<0.05)
(#) : Indicates a significant difference from the positive control group (P>0.05)

The results show the average number of jumps and licks of mice there was a decrease in the number of jumps and licks in the positive control group and papaya leaf infusion group with concentrations of 25%, and 75% compared with the negative control group. Observation result The number of jumps and licks of mice that have been obtained is then continued with data normality test, homogeneity test, One Way Anova test and test LSD with SPSS 22.0

The results of the normality test show that the data is normally distributed with (P>0.05). The results of the normality analysis can be seen in Table 3.

Table 3. Data Normality Test

Variable	Group			
	Negative	Postitive	Infusiona 25%	Infusiona 75%
p-value	0,955	0,421	0,777	1,000

Source: Primary Data 2020

Next, a homogeneity test was carried out. The data homogeneity results show that P=0.083 (P>0.05) which means the data is homogeneous. After the data was homogeneous, it was continued with the One Way ANOVA test. The results obtained were significant at P= 0.000 (P<0.05), which means that the data contained differences between groups. So it was continued with the Post Hoc LSD (Least Significance Different) test. In this test, there was a significant difference between the negative control and the positive control, 25% infusion, and 75% infusion, whereas there was no significant difference between the positive control with 25% infusion and 75% infusion. %.

In the LSD (Least Significance Different) test, the results showed that there was a significant difference between the negative control and the positive control, 25% infusion, 50% infusion, and 75% infusion. This is because, in the negative control, induction was carried out with a hot plate, which gave rise to a jumping and licking effect. But no medication was given, only CMC-Na. Where CMC-Na does not have an analgesic effect because it is only a solvent. CMC-Na is a derivative of cellulose and is often used in the food industry, or used in food ingredients to prevent retrogradation. CMC-Na belongs to the class of emulsifiers, thickeners, and stabilizers (Fennema *et al*., 1996).

Then the results obtained were that there was no significant difference between the positive control with 25% infusion, 50% infusion, and 75% infusion. This is because this group was given medication or given anti-analgesics. The positive control here is given ibuprofen as an analgesic drug. The mechanism of ibuprofen as an anti-analgesic is by inhibiting the cyclooxygenase enzyme in the formation of prostaglandins so that it can reduce the number of jumps and licks in mice. Papaya leaf infusion with a concentration of 25%, 50% concentration, and 75% concentration contains flavonoids, alkaloids, saponins, and tannins so it has an analgesic effect (Safitri, 2013).

The mechanism of flavonoids as anti-analgesics is by inhibiting the action of the cyclooxygenase enzyme. Thus, it will reduce the production of prostaglandins by arachidonic acid thereby reducing pain (Gunawan, 2008). The mechanism of alkaloids as anti-analgesics is by inhibiting the enzyme cyclooxygenasediota in prostaglandin biosynthesis (Sulaiman *et al*., 2008). The mechanism of saponin as an anti-analgesic is by inhibiting the formation of exudates and increasing vascular permeability. The mechanism of tannin as an anti-analgesic is by inhibiting the cyclooxygenase enzyme from prostaglandin so that tannin has an analgesic effect (Hasan *et al*., 2014).

CONCLUSION

Papaya leaf infusion (Carica papaya L.) has analgesic activity against male Swiss strain mice induced with a hot plate. The optimal concentration of papaya leaf infusion has an analgesic effect against male Swiss strain mice induced with a hot plate is a concentration of 25%.

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