Journal of Health Management and Pharmacy Exploration
https://shmpublisher.com/index.php/johmpe
p-ISSN: 2985-4814
e-ISSN: 2985-5543

Antipyretic activity test of papaya leaf extract (*Carica papaya L.*) against peptone-induced male white mice

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Article Info

Article history:

Received July 26, 2024
Revised August 06, 2024
Accepted August 08, 2024

Keywords:

Antipyretic
Carica papaya L.
Peptone

ABSTRACT

Fever increases body temperature above normal (98.6°F/ 37°C). To treat complaints of fever, antipyretic drugs are given. Papaya leaves are believed to have antipyretic effects. The purpose of this study was to test the antipyretic activity and effective dose of ethanol extract of papaya leaves (*Carica papaya* L.) against peptone-induced mice. The test animals were grouped into 5 groups consisting of group I (negative control) CMC Na 0.5%, group II (positive control) paracetamol drug 65 mg/KgBB mice, group III papaya leaf ethanol extract 140 mg/KgBW mice, group IV papaya leaf ethanol extract 280 mg/KgBW mice, and group V papaya leaf ethanol extract 560 mg/KgBW mice, with peptone fever inducer 10 mL/kgBW subcutaneously. The body temperature of the test animals was observed every 30 minutes for 240 minutes after peroral administration of the preparation, then obtained data on T0, Tdemam and body temperature measurements at each time. The data was then used to calculate the AUC and the average AUC calculation data was analyzed by Shapiro wilk test and One way Anova test. The results of the study of ethanol extract of papaya leaves have antipyretic effects, namely due to the presence of flavonoids, alkaloids, tannins, steroids, and saponins. Papaya leaf ethanol extract has the most effective antipyretic activity, namely a dose of 560 mg/kg BW, which is comparable to the positive control of paracetamol.

1. Introduction

Fever is a disorder of the center responsible for regulating body temperature, which is located in a region of the brain known as the hypothalamus [1]. Fever is not a disease but rather a sign of several conditions, including colds, coughs, teething in children, fever after

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DOI: https://doi.org/10.52465/johmpe.v2i2.437

vaccination, and fever due to bacterial and viral infections. Prolonged fever is related to higher nutritional needs, which can be dangerous and result in weakness [2].

This biodiversity can be used for treatment, such as raw materials for making modern or traditional medicines [3]. Indonesians have long known natural ingredients as medicines for various diseases. Many types of Indonesian medicinal plants have been used as raw materials in the manufacture of medicines. Even these plants have been clinically tested for their phytochemical content, efficacy and safety of use. Traditional medicine has been used as a complementary therapy, even before official health facilities began using contemporary medicine. Traditional medicine continues to serve as the vanguard of pharmacotherapy for many people in different parts of the world [4].

One of the plants used for medicine by the community is the papaya plant (*Carica papaya* L.). This plant is used by the community to overcome fever, vaginal discharge, acne, increase appetite, increase breast milk, and treat toothache [5]. People use papaya leaves as a fever reducer by boiling the leaves. Previous research has researched the antipyretic activity of papaya leaves, which have antipyretic activity with 3 test groups, namely 50 g/kgBW, 100 g/kgBW and 200 g/kgBW. The dose had a pyrative effect of 200 g/kgBW, but the antipyretic effect was lower than that of the positive control paracetamol. Papaya leaves are reported to contain several significant compounds, such as flavonoids, tannin alkaloids, saponins, and steroids. Flavonoids have a wide variety of bioactivities. The bioactivity shown includes antipyretic, anti-inflammatory and analgesic effects [6]. Flavonoids work as cyclooxygenase (COX) inhibitors. Cyclooxygenase (COX) functions to trigger the formation of prostaglandins. Prostaglandins play a role in inflammatory processes and increased body temperature. If prostaglandins are not inhibited, there will be an increase in body temperature, which will result in fever. Saponins can inhibit the COX-2 enzyme so that the production of prostaglandins will be inhibited, and then the levels of prostaglandins in the hypothalamus will decrease, causing fever to decrease. Tannins can be efficacious as antipyretics by inhibiting arachidonic acid in prostaglandin biosynthesis [7]. The mechanism of alkaloids as antipyretics is suspected to be by inhibiting the biosynthesis of prostaglandins so that the level of prostaglandins in the hypothalamus decreases and the body temperature will drop [7]. Steroids can be used as antipyretics by inhibiting the COX-2 enzyme so that the prostaglandins formed during fever can be reduced [8]. Based on research by [9] regarding antipyretic activity using the 5% Pepton induction method. Pepton induction usually uses mouse test animals, and after the temperature rises, measurements can be made of the antipyretic activity of the test compound.

Pepton is a hydrolyzed protein that is a strong fever inducer without harmful effects [10]. Excessive protein administration in mice can be toxic (pyrogen), so that it affects the balance of body temperature. Protein is a type of pyrogen that can be detected by the body as a foreign body so that it can cause a stimulus effect on the center of body temperature regulation. Peptones work by stimulating the hypothalamus to increase prostaglandins, which can then lead to an increase in body temperature [11]. Based on the description above, the author is interested in conducting a study titled antipyretic activity test of Papaya leaf ethanol extract (*Carica Papaya* L.) against peptone-induced male white mice.

2. Method

The harvested papaya leaves are cleaned of dirt or other foreign materials from the leaves with running water and then dried in the oven at 50°C until they become dry. The dried simplicia is ground and finally sifted with the number 40 sieve to obtain simplicia powder.

Preparation of ethanol extract of Papaya leaves simplicia Powder as much as 250 grams is extracted by maceration method using 96% ethanol solvent with an ingredient ratio of 1 : 10 parts, stored at room temperature and avoided from direct sunlight and soaked for 5 days, filtered. The results of the filtration are concentrated using an evaporator, and a viscous extract is obtained, and then the yield is calculated [12].

Antipyretic activity test

The test animals used for the study were 25 animals. The day before the experiment was carried out, the animals were fasted for 8 hours before the treatment by not being fed but still given a drink. The animals were weighed and grouped into 5 groups, each group consisted of 5 mice. Group I is the negative control group given a 1% Na CMC solution. Group II is the positive control group that is given paracetamol suspension. Group III is the control group that tests ethanol extract of papaya leaves 140 mg/kg BW mice. Group IV is the control group for testing ethanol extract of papaya leaves at a dose of 280 mg/kg BW in mice. Group V is the control group for testing ethanol extract of papaya leaves at a dose of 560 mg/kg BW in mice. 33 Before the observation, the normal temperature of each mouse was measured using a digital thermometer before being given peptone induction and after peptone induction as much as 0.2 mL subcutaneously and 30 minutes after induction the temperature of the mice was measured again using a digital thermometer to determine the degree of increase in body temperature after peptone injection. The average temperature (normal temperature of mice) is determined. The mice were given a test preparation after the mice's body temperature reached fever temperature (>37°C). Temperature measurements of mice were carried out at minutes 30, 60, 90, 120, 150, 210 and 240 and then the temperature decrease from fever temperature to temperature at each measurement was calculated.

3. Results and Discussion

The thick extract obtained was 31 grams with a yield value of 12.4%. This study conducted antipyretic tests on mouse test animals. The antipyretic activity test was divided into 5 (five) groups consisting of 5 (five) groups of CMC Na 0.5% treatment used as a negative control because it had no pharmacological effect on the drug body, paracetamol as a positive control, and using papaya leaves containing flavonoid compounds that are antipyretic.

Table 6. Average rectal temperature of mice (°C)

Ex.	Average rectal temperature (°C)									
	T0	TD	T30	T60	T90	T120	T150	T180	T210	T240
I	36.52 ±0.29	37.52 ±0.33	37.6 ±0.35	37.76 ±0.21	37.8 ±0.16	38.06 ±0.17	38 ±0.12	37.92 ±0.11	37.78 ±0.08	37.72 ±0.13
II	36.54 ±0.36	37.62 ±0.30	37.26 ±0.21	37.12 ±0.16	37 ±0.19	36.84 ±0.29	36.62 ±0.26	36.46 ±0.17	36.4 ±0.10	36.4 ±0.19
III	36.64 ±0.25	37.92 ±0.24	38.06 ±0.21	37.94 ±0.23	37.94 ±0.15	37.68 ±0.21	37.58 ±0.08	37.48 ±0.15	37.48 ±0.17	37.16 ±0.35
IV	36.68	37.84	37.7	37.66	37.58	37.46	37.36	37.06	36.86	36.7

	±0.24	±0.25	±0.34	±0.19	±0.18	±0.23	±0.23	±0.15	±0.27	±0.34
V	36.52	37.78	37.46	37.22	37.1	36.96	36.68	36.7	36.52	36.5
	±0.11	±0.18	±0.15	±0.19	±0.19	±0.21	±0.26	±0.29	±0.23	±0.19

Information:
I = Negative control of CMC Na 0.5 %
II = Positive control of paracetamol 65 mg/Kg BW
III = Ethanol extract of papaya leaves 140 mg/Kg BW
IV = Papaya leaf ethanol extract 280 mg/Kg BW
V = Ethanol extract of papaya leaves 560 mg/Kg BW
T0 = Initial minutes of mouse temperature
TD = Temperature after 1 hour of peptone administration
T30 = Temperature after treatment every 30 minutes

The manufacture of papaya leaf extract uses the maceration method with 96% ethanol because it can attract beneficial substances. The preparation of Papaya Leaf Ethanol Extract (EEDP) is made in 3 different doses, namely 140 mg/kgBB, 280 mg/kgBB and 560 mg/kgBB. The peptone antipyretic research method is used to induce fever/heat. Pepton is a polymer of amino acids with many peptide bonds. However, the number is still less than proteins, so its mass is relatively large enough to be able to activate the immune system. In contrast, by the body peptone is considered an exogenous pyrogen that stimulates phagocytes to form endogenous pyrogen, which initiates an increase in prostaglandin synthesis and regulates higher temperature threshold values. After setting the threshold value at a higher temperature level, the normal body temperature works in a cold state. This causes vasoconstriction of the skin vessels, trembling due to subjective cold. Before the mice were induced peptone, the mice's body weight was weighed first to determine the volume of preparation that was appropriate to the mice's body weight, then the body temperature in the mice was measured at their normal temperature to determine whether there was an increase in temperature after peptone injection. Peptones are injected subcutaneously into the skin of the nape of the neck. Subcutaneous administration of peptones aims to be absorbed for a long time so that it prolongs the performance of peptones and causes fever conditions in induced mice to persist for a long time. Mice that have been induced with fever are left for 1 hour to give a period of time for peptones to be active in the mice's body, then the mice's body temperature is measured again to find out whether the mice have fever or not. If the mice have a fever, they are then given oral preparations with the volume of preparation according to body weight and the temperature is measured after 30, 60, 90, 150, 180, 210 and 240 minutes. The thermometer used to measure body temperature in mice is calibrated first before being inserted into the mice's rectum. Calibration of the thermometer is carried out by immersing the thermometer tip in an aquades whose temperature is monitored by another thermometer, this aims to reduce the measurement inaccuracy in thermometers that are very sensitive to temperature changes of 0.1°C. Before being inserted into the rectum, the thermometer is inserted into the oil liquid to reduce the risk of abrasions on the rectum of mice and facilitate the entry of the thermometer.

All test animals that have an increase in body temperature of more than 0.6 ºC from the initial temperature can be categorized as having a fever. The results of this study were obtained in that the test animals experienced a temperature increase of more than 0.6 ºC after 1 hour of peptone injection. Judging from the rectal temperature data of mice under

normal circumstances, it has a range of 36.52°C – 36.68°C, while the temperature of mouse fever has a range of 37.52°C – 37.92°C. Based on the results of the above research data, it can be seen that in the negative control group where this group used CMC Na 0.5% showed a continuous increase in temperature after 1 hour of fever induction, this is because CMC Na has no effect on the inhibition of the cyclooxygenase enzyme in the body so that the fever temperature does not decrease. The positive control group with paracetamol 65 mg/kg BB showed a continuous decrease in temperature from minute 30 to minute 42 to 180, while at minute 210 to minute 240 the mice's body temperature returned to normal. The decrease in temperature occurred because the highest concentration of paracetamol in plasma was achieved within 30 minutes with a plasma half-life of between 1-3 hours. Paracetamol works by inhibiting the cyclooxygenase enzyme in peripheral tissues so that it produces analgesic and antipyretic effects. The antipyretic properties of paracetamol have a direct effect on the center of heat regulation in the hypothalamus resulting in peripheral vasodilation, sweating, and the presence of excessive heat dissipation aimed at normalizing body temperature again. The results of the group given papaya leaf ethanol extract at a dose of 140 mg/KgBW were comparable to the negative control, at the 60th minute there was a decrease in body temperature, in the positive control the temperature decrease started at the 30th minute until the last minute. This is suspected because the effects of peptone pyrogen are still working dominantly, and papaya leaf extract at a dose of 140 mg/kg BW has been eliminated in the blood. The results of the group that were given papaya leaf ethanol extract 280 mg/kg BW and papaya leaf ethanol extract 560 mg/kg BW, there was a constant temperature drop from the 30th minute to the 120th minute like a positive control (paracetamol). This is suspected to be due to the larger the dose of papaya leaf ethanol extract, the greater the ability to lower body temperature in mice.

The results obtained from the rectal temperature measurement data of mice were then calculated Area Under Curva (AUC). The Under Curva Area (AUC) value in this study was used to identify the effectiveness of the test animal temperature reduction test. The smaller the AUC value, the better the antipyretic activity in the treatment group. The body temperature of the mice was observed for 240 minutes.

Table 7. Results of the calculation of the average AUC

Treatment Groups	Average AUC ± Elementary School
Negative Control (CMC Na)	1134.525B ± 6.250
Positive Control (Paracetamol)	1105.163A ± 12.041
EEDP 140 mg/KgBW	1131.375B ± 8.954
EEDP 280 mg/KgBW	1121.063AB ± 11.829
EEDP 560 mg/KgBW	1109.175A ± 12.280

Information:
A : Different meaning with negative control
B: Meaningful difference with positive control

Based on the AUC results, it can be seen that EEDP 560 mg/KgBW has a smaller AUC value compared to the EEDP dose group of 140 mg/KgBW and EEDP 280 mg/KgBW. The paracetamol-positive control group has a smaller AUC value than other groups, this is because paracetamol has better antipyretic activity compared to other treatments. The CMC Na negative control group had the highest AUC value compared to other groups, this

is because CMC Na does not have antipyretic activity, so that the rectal temperature of mice does not decrease.

After the AUC calculation is carried out, the data is then analyzed statistically. The initial stage in this statistical analysis is the normality test where this normality test is carried out mainly to see whether the data produced is normally distributed or not. The results of the research from the normality test obtained a value of 0.267, which is greater than 0.05, which is the standard of normality, namely P>0.05, so it can be concluded that the data in this study is normally distributed. Furthermore, a parametric analysis of one way ANOVA statistics was carried out, the result of this one way ANOVA analysis was 0.000 where this value was small from 0.05, which is the standard measurement of one way ANOVA P<0.05 this marked a significant difference from the group of variation in the dose of papaya leaf ethanol extract to the decrease in the average rectal temperature of mice, so it can be said that papaya leaf ethanol extract has antipyretic activity. After the analysis using one-way ANOVA, the post hoc test was continued using the Tukey HSD multiple comparison test method to determine the differences between each treatment group. The results of this test were significantly different between groups. The negative control group was comparable to an EEDP of 140 mg/KgBW. This showed that an EEDP of 140 mg/KgBW was equivalent to a negative control. The EEDP 280 mg/KgBW treatment group was significantly different from all positive control treatment groups, negative controls, EEDP doses of 140 mg/KgBW and 560 mg/KgBW. This showed that EEDP doses of 280 mg/KgBW showed antipyretic activity but were not comparable to positive controls. The EEDP treatment group of 560 mg/KgBW was comparable to the positive control. This shows that EEDP 560 mg/KgBB has antipyretic activity equivalent to positive control.

The results of tests that have been carried out on papaya leaf ethanol extract prove that there is antipyretic activity, this is due to the presence of flavonoid compounds, saponins, tannins, alkaloids and steroids in papaya leaves. Flavonoid compounds have pharmacological activity as an antipyretic, namely from the flavonols, flavones and isoflavones group that have the potential to inhibit the enzyme cyclooxygenase so that the synthesis of prostaglandins is inhibited. The mechanism of flavonoids as antipyretics is by suppressing TNF-α or related compounds and inhibiting arachidonic acid, which reduces prostaglandin levels thereby reducing the occurrence of fever [11]. Saponins can inhibit the COX-2 enzyme so that the production of prostaglandins will be inhibited, then the levels of prostaglandins in the hypothalamus will decrease so that fever will decrease. Tannins can be efficacious as antipyretics by inhibiting arachidonic acid in prostaglandin biosynthesis [4]. The mechanism of alkaloids as 45 antipyretics is thought to be by inhibiting the biosynthesis of prostaglandins so that the level of prostaglandins in the hypothalamus decreases and the body temperature will drop [5]. Steroids can be used as antipyretics by inhibiting the COX-2 enzyme so that the prostaglandins formed during fever can be reduced [6].

4. Conclusion

Ethanol extract of papaya leaves (*Carica papaya* L.) has antipyretic activity against peptone-induced mice. The effective dose of papaya leaf ethanol extract (*Carica papaya* L.) had antipyretic activity against peptone-induced mice was a dose of 560 mg/KgBW papaya leaf ethanol extract in mice that was comparable to the positive control of paracetamol.

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