Antipyretic Effect of Combination of Moringa Leaf Extract and Andrographis paniculata Ness on Mice Induced by 5% Peptone

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Abstract. Fever is a physiological response of the body to infection or inflammation, characterized by an increase in body temperature above 37.5°C. Antipyretic drugs such as paracetamol are commonly used to reduce fever through the mechanism of prostaglandin inhibition in the central nervous system. The purpose of this study was to determine the antipyretic effectiveness of Moringa oleifera leaf extract and Andrographis paniculata leaf extract in mice induced with 5% peptone, and to compare it with the positive control (paracetamol). The study was conducted in vivo with a pre and post test group design using 30 mice divided into five groups, each consisting of 6 mice. The negative control group (K−) was given 0.5% CMC Na, the positive control (K+) was given 1.3 mg paracetamol, the K1 group was given 400 mg/kg BW of Andrographis paniculata extract, the K2 group was given 400 mg/kg BW of moringa extract, and the K3 group was given a combination of Andrographis paniculata and moringa extracts of 200 mg/kg BW each. Fever induction was carried out by administering 5% peptone, and temperature measurements were taken at 30, 60, 90, 120, 150, and 180 minutes. The results of the analysis showed that the combination of extracts in the K3 group was able to significantly reduce the body temperature of mice, and had comparable effectiveness to the positive control group (paracetamol), with a p value = 0.561 (p> 0.05). Thus, the combination of Andrographis paniculata and moringa leaf extracts has the potential as an alternative natural antipyretic.

Key words: antipyretic, Andrographis paniculata leaves extract, Moringa leaves extract,peptone 5%, body themperature

INTRODUCTION

Fever is a body temperature that exceeds the normal limit where the body temperature is above 37°C. Fever can be caused by liposaccharide toxins (pyrogens) released by bacterial cell membranes, causing an increase in the set-point of the hypothalamic thermostat (Guyton & Hall, 2011). The prevalence of fever is currently increasing due to the increase in infectious diseases such as viral, bacterial, and parasitic infections. The 2018 National Basic Health Research (Riskesdas) report stated that diseases or conditions with symptoms of fever in the Indonesian community were ARI 9.3%; pneumonia 4.0%; hepatitis 0.39%; malaria 0.37%; pregnancy 2.4%; postpartum 1.5%; and KIPI 37.5%. The prevalence of fever according to the World Health Organization (WHO), is estimated that fever is experienced by children aged 6 months to 5 years as much as 2-5% in developed countries and more than 21.65 million children in the world (Paudel *et al*., 2018). Treatment of fever, antipyretics or fever-reducing drugs can be given, antipyretics can help restore abnormal hot temperatures by inhibiting the synthesis and release of prostaglandin E2, which is stimulated by endogenous pyrogens in the hypothalamus (Sweetman, 2019).

The antipyretic drug that is often used today is paracetamol, because it has been proven effective, with a mechanism that stimulates the heat center in the hypothalamus which causes peripheral vasodilation in the skin characterized by sweating and decreased body temperature, but long-term use of paracetamol has hepatotoxic side effects (Tjay & Rahardja, 2015), therefore a breakthrough in traditional medicine is needed which is considered safer than the use of modern drugs, because the side effects are relatively smaller (Kemenkes of the Republic of Indonesia, 2021). Medicinal plants that can be used as antipyretics include Moringa oleifera L. and Andrographis paniculata Nees. because they contain secondary metabolites that have pharmacological benefits. The results of research by Bhattacharya *et al*. (2014) stated that moringa oleifera leaf extract is effective as an antipyretic with a dose of 400 mg/kgBW, while the results of research by Nety *et al*., (2019), on the antipyretic activity of Andrographis paniculata Nees leaf extract showed that a dose of 400 mg/kgBW was proven to be effective in lowering body temperature.

METHODS

This research was conducted experimentally using the Pre- and Post-Control Group Design method. Laboratory experiments using mice were conducted in the pharmacology laboratory of the Institut Teknologi Kesehatan Cendekia Utama Kudus. The test animals were healthy, active male Swiss Webster mice with no anatomical abnormalities. This research has obtained ethical clearance with registration number KEPK/UMP/41/III/2024.

Tools and materials

The equipment needed in the study is a moisture balance (OHAUS), digital thermometer, stopwatch, mortar and stamper, glassware, oral sonde, 1 mL injection syringe.

Test animals Swiss webster mice, Andrographis paniculata Nees (PT Industri Jamu Borobudur), Moringa oleifera L from (PT Industri Jamu Borobudur), generic Paracetamol tablets (Kimia Farma), 5% peptone (Merck KgaA), CMC Na 0.5%, distilled water, aqua pro injection, sterile NaCl solution 0.9%.

a. Water Content Testing.
   Weigh 1 gram of extract powder and put it into a prepared moisture balance at a temperature of 105°C for 10 minutes and record the content listed on the moisture balance. Water content testing was replicated 3 times on each extract.
b. Preparation of 5% Peptone Solution
   5% peptone inducer solution is made from 5 grams of peptone dissolved in 100 mL of aqua pro injection.
c. Preparation of 0.5% CMC Na Suspension.
   Weigh 0.5 grams of CMC Na and put it into a mortar containing 10 mL of hot aquadest, leave it to expand, and stir it until it forms a mucilage. Transfer it into a measuring flask and dilute it with aquadest until the total volume of the suspension is 100 mL.
d. Preparation of Paracetamol Tablet Suspension
   Paracetamol suspension is made from 32.5 mg of paracetamol powder suspended in 0.5% CMC Na given orally with a volume of 1 mL.
e. Preparation of Andrographis paniculata Ness Leaf Extract Suspension
   The Andrographis paniculata Ness leaf extract suspension is made from 200 mg of dry Andrographis paniculata Ness leaf extract suspended in 0.5% CMC Na, given orally with a volume of 1 mL.
f. Preparation of Moringa oleifera L. Leaf Extract Suspension
   The Moringa oleifera L. leaf extract suspension was made from 200 mg of dry moringa leaf extract suspended in 0.5% CMC Na, given orally with a dose volume of 1 mL.
g. Preparation a Combination Suspension of Andrographis paniculata Leaf Extract and Moringa Leaf Extract
   Combination suspension of Andrographis paniculataf leaf extract and moringa leaf extract. Made from 100 mg of dry Andrographis paniculata Ness leaf extract and 100 mg of dry moringa leaf extract suspended in 0.5% CMC Na, given orally with a dose volume of 1 mL.
h. Antipyretic Effect Examination
   A total of 30 Swiss Webster mice were adapted for 7 days and fasted for 6 hours in the cage, grouped into 5 groups (K1, K2, K3, K4, K5) each group consisting of 6 mice selected randomly. Weigh the weight of each mouse. The initial rectal temperature of the mice was measured with a digital thermometer. 1 mL of 5% peptone was induced per mouse subcutaneously (on the nape of the mouse). The rectal temperature of the mice was measured after 1 hour after administration of 5% peptone. Rectal temperature was measured again at 30, 60, 90, 120, 150, 180 minutes using a digital thermometer after injection for each treatment group, then compared with negative and positive controls to see the activity of the test preparation.

   The data obtained was then calculated as % antipyretic power:

$$\% \text{ antipyretic power} = \frac{t0 - tn}{t0} \times 100\%$$

Description:

t0 = Temperature after being induced by 5% peptone
tn = Temperature at minutes 30, 60, 90, 120, 150, 180

RESULTS AND DISCUSSION

a. Water Content Test Results

Determination of the water content of dry extracts of Moringa leaves (Moringa oleifera L.) and Andrographis paniculata Nees.) was carried out using a Moisture balance tool and replicated 3 times to obtain accurate results. The results of the water content test can be seen in table 1.

Table 1. Water Content Test Results

Replication	Water Content of Dry Extract of Andrographis Leaves	Water Content of Dry Extract of Moringa Leaves
I	1,75%	1,07%
II	1,93%	1,48%
III	2,15%	1,44%
Rata-rata	1,94%	1,33%

Examination of dry extracts of Moringa oleifera L. and Andrographis paniculata Nees. was carried out using organoleptic tests by describing the shape, smell, taste and color. The results obtained on the dry extract of Moringa oleifera L. were 1.33% and Andrographis paniculata Nees. were 1.94%. The results of the Certificate of Analysis (COA) from PT. Industri Jamu Borobudur showed that the water content test of the dry extract of Moringa oleifera L. was 1.33% and Andrographis paniculata Nees. was 2.76%, so it can be concluded that the water content test conducted by researchers with the water content test conducted by the industry did not have a specific difference. The dry extract of Moringa leaves (Moringa oleifera L.) and Andrographis paniculata Nees.) was declared in accordance with the requirements for water content of simplicia, which is <10%. Based on the Kemenkes, in Republic Indonesian (2015), the water content of the sample should not be >10% because it can cause the growth of fungi or microbes which results in a decrease in the stability of the extract or sample.

b. Antipyretic Test Results

Mice were measured for initial temperature (normal temperature) before being induced with 5% peptone using a digital thermometer via the rectum or anus, after which the mice were induced with 1 mL of 5% peptone subcutaneously. Research by Oktifanny & Trisna, in (2022) explained that peptone compounds are proteins that are used as fever induction in test animals such as mice. Excess protein in the body of mice will cause a stimulating effect on the temperature regulation center (hypothalamus) thus causing an increase in body temperature. Fever can be caused by the presence of toxic substances that affect the hypothalamus. The temperature measurement of mice was carried out after 1 hour of 5% peptone induction to determine the increase in temperature (fever temperature), after the mice had fever, randomization treatment was carried out. Mice were divided into 5 treatment groups, where the first group was a negative control given 0.5% CMC Na, the negative control was used as a comparison with the positive control group and the group given the extract suspension. The second group was a positive control given paracetamol which is an antipyretic drug, the dose given to mice was 1.3 mg / kg of mouse body weight. The third group was given dry extract of Andrographis paniculata leaves with a dose of 400 mg / kg of mouse body weight. The fourth group was given dry extract of moringa oleifera leaves with a conversion dose of 400 mg / kg of mouse body weight. The fifth group was given a combination of dry extract of moringa oleifera L. leaves and Andrographis paniculata Nees.leaves with a dose of each extract of 200 mg / kg of mouse body weight. The data results can be seen in table 2.

Table 2. Average Result Rectal Themperature (°C) Minute to- Minute

Groups	Average Result Rectal Themperature (°C) Minute to-Minute							
	T0	**T1**	**30**	**60**	**90**	**120**	**150**	**180**
K 1	36,23	37,43	37,56	37,30	37,96	37,93	37,90	38,00
K 2	36,23	37,90	37,63	37,40	37,06	36,80	36,73	36,36
K 3	36,36	38,00	37,80	37,73	37,63	37,40	37,16	36,90

K 4	36,43	37,83	37,60	37,60	37,46	37,40	37,03	36,73
K 5	36,80	38,16	37,83	37,73	37,30	37,13	36,73	36,53

The results of the study showed that the rectal temperature of mice in the positive control group (K2) given paracetamol at a dose of 1.3mg and the combination group of dry extracts of Andrographis paniculata leaves and Moringa oleifera leaves was the temperature that reduced fever the fastest compared to other groups. The positive control group of the induction temperature of 37.90°C decreased by 36.36°C at 180 minutes, and the combination group of the induction temperature of 38.16°C experienced a decrease in temperature of 36.53°C.

The average graph of the decrease in rectal temperature at minutes 30, 60, 90, 120, 150, and 180 is shown in Figure 1

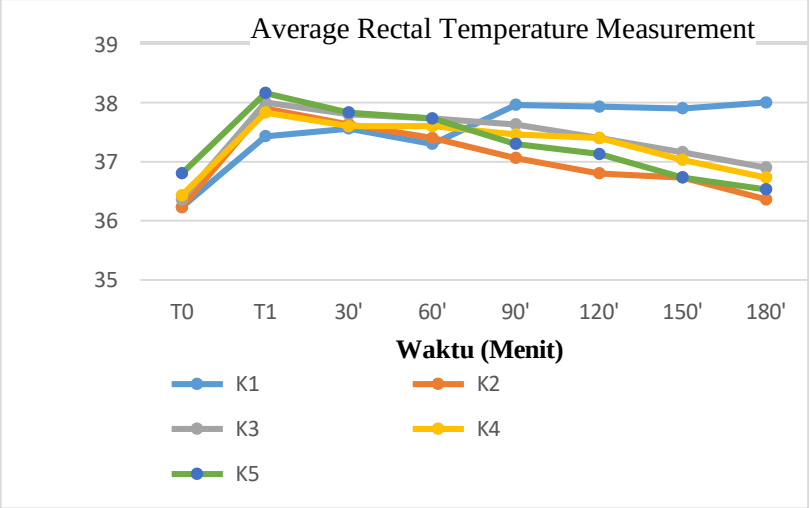


Figure 1. The average graph of the decrease in rectal temperature

The positive control group of paracetamol dose 1.3mg average induction temperature of 37.90°C experienced a decrease in temperature from minute 30 to minute 180 to 36.36°C. The combination group of dry extract of Andrographis paniculata leaves and moringa oleifera leaves average induction temperature of 38.16°C experienced a decrease in temperature from minute 30 to minute 180 to 36.53°C. The positive control group of paracetamol and the control group of combination of dry extract of Andrographis paniculata leaves and moringa oleifera leaves tended to experience a decrease in temperature from minute 30 to minute 180. Based on research by Nurfadhila *et al*. (2023), it is explained that paracetamol has been known to reduce fever by inhibiting the cyclooxygenase enzyme mechanism, causing the set-point in the hypothalamus to decrease and heat dissipation does not occur. The induction temperature of the dry extract group of Andrographis paniculata leaves averaged 38.00°C, with a decrease in temperature from minute 30 to minute 180 to 36.90°C. The results of research by Azizah *et al*. (2022) stated that Andrographis paniculata leaf extract has antipyretic activity because it contains flavonoids that can block the cyclooxygenase pathway so that it can inhibit the process of fever. The average induction temperature of the dry extract group of moringa oleifera leaves was 37.83°C, with a decrease in temperature from minute 30 to minute 180 to 36.73°C. The results of Silalahi's research in (2020) showed that moringa oleifera leaf extract contains flavonoids so that it is effective for use as a fever reducer.

Average Results of Antipyretic Power Percentage

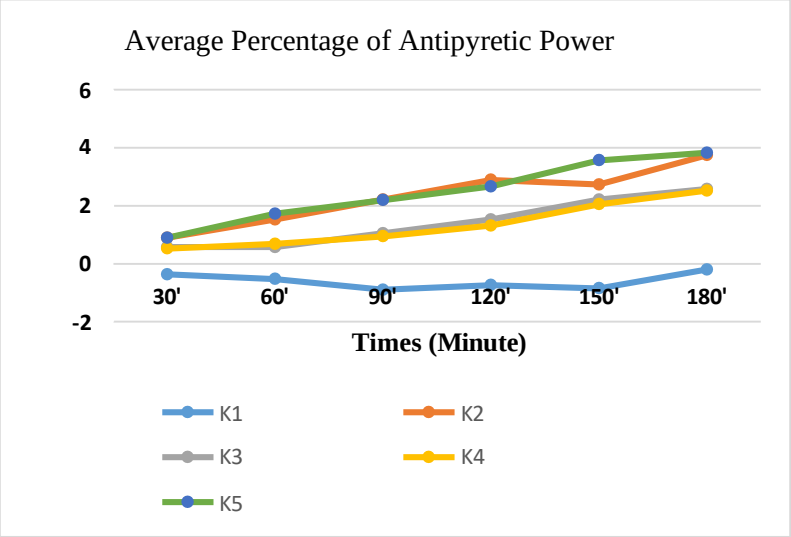
The percentage of antipyretic power is calculated using the formula % Antipyretic Power : t0 – tn x 100%/ t0. The calculation of the percentage of antipyretic power was carried out after 5 groups of mice were treated from 30, 60, 90, 120, 150 and 180 minutes. The percentage of antipyretic power in this study for all treatment groups is presented in table 3.

Table 3. Average Results of Percentage of Antipyretic Power

Groups	**30**	**60**	**90**	**120**	**150**	**180**
K 1	-0.36%	-0.71%	-1.42%	-1.33%	-2.24%	-0.08%

K 2	0.70%	1.31%	2.19%	2.90%	3.07%	4.04%
K 3	0.52%	0.69%	0.96%	1.57%	2.19%	2.88%
K 4	0.61%	0.61%	0.96%	1.14%	2.10%	2.97%
K 5	0.87%	1.74%	2.26%	2.70%	3.75%	4.27%

The results of the average percentage of antipyretic power showed that the combination group of 200 mg of dry extract of moringa oleifera leaves and 200 mg of Andrographis paniculata leaves obtained the highest percentage of antipyretic power, with an average result of 4.27% at the 180th minute which was equivalent to the percentage result of the positive control of paracetamol 1.3 mg.



The percentage of positive control results obtained results of 4.04% at the 180th minute. The percentage of antipyretic power of the 400mg Andrographis paniculata leaf dry extract group at the 180th minute obtained results of 2.88% while the 400mg moringa oleifera leaf dry extract group obtained results of 2.97%. The negative control group (K1) of CMC Na 0.5% did not show any decrease in temperature. Puspitaningrum's research, in (2015) stated that CMC Na is only a suspending agent or carrier that does not have antipyretic activity.

CONCLUSION

1) The combination of Moringa oleifera L. and Andrographis paniculata Nees. leaf extract has antipyretic activity in mice induced by 5% peptone.
2) The highest percentage of antipyretic power of the combination of Moringa oleifera L. and Andrographis paniculata Nees. leaf extract was obtained at the 180th minute with an average of 3.82%.
3) The combination of Moringa oleifera L. leaf extract at a dose of 200 mg and Andrographis paniculata Nees. leaf at a dose of 200 mg was effective in reducing rectal temperature in mice. Statistically not significantly different from the positive control group p = 0.561 (> 0.05)

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