Antipyretic properties of carica (*Vasconcellea pubescens* A.DC.) fruit and seeds ethanolic extract in experimental animals

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Abstract. Fever is the condition of body temperature which more than usual caused by an increase of central control temperature in the hypothalamus. An infection or virus usually cause this condition. Some of the active substances of plant secondary metabolites had been researched lower fever; they were flavonoids, saponins, and tannins. Carica (*Vasconcellea pubescens* A.DC) or mountain papaya is one of the fruits that had the compounds. This research purposed to know the antipyretic effect of ethanolic extracts of carica fruits and seeds towards Wistar rats. The study used pre-test – post-test only controls group design. Thirty Wistar rats were divided into six groups on each extract sample. Normal group was not given any treatment, negative control was given 2.5 mL aquadest, positive control was given acetaminophen syrup with the dosage 1.5 mL/kg body weight (b.w), and the dosages of carica fruit and seeds ethanolic extracts (EECF/ EECS), there were 200, 400, and 800 mg/kg b.w. The rats were s.c induced fever with 20 mL/kg b.w 20% yeast suspension. Temperature measurement was performed every 30 minutes for 6 hours. Data processed using One Way ANOVA. The results showed that ethanolic extracts of carica fruits and seeds with a dosage of 800 mg/kg b.w were able to lower the temperature significantly (p < 0.05) towards negative control in Wistar rats.

1. Introduction
The fever is definition a sign and symptoms of an underlying condition caused by an infection or viruses [1]. Fever is a significant feature of the infective and non-infective inflammatory processes, and it is one of the commonest physical signs found in patients [2]. Fever is a very common complaint in children accounting for as many as 20% of pediatric visits to doctors. Most children aged 0–36 months who have a fever have a focus of infection, which can be identified by careful history and examination. A viral upper respiratory tract infection is the most common focus [3]. Uncontrolled fever causes seizures [4], so it can reduce using antipyretic drugs [5].

Acetaminophen or paracetamol is nonsteroidal anti-inflammatory drugs for antipyretic which is widely used in many people because it's safe and easy to get [6]. The previous study, acetaminophen had adverse effect hepatotoxicity if the drugs used in a long time and overdose [3,4]. A case report from Chinese mention a 12-month-old boy who presented with hepatotoxicity disseminated intravascular coagulation and persistent renal insufficiency four days after repeated ingestion of a supratherapeutic dosage of paracetamol [9]. This adverse effect reason many people choose natural drugs as an alternative treatment.
The carica (Vasconcellea pubescens A.DC.) is one of the plants that come from Dieng plateau, Wonosobo, Central Java. Mountain papaya has to contain flavonoid, alkaloid, tannin, and phenol. Carica had been used in pharmacology test and prove that its fruit has antioxidant activity through lipid peroxidation inhibition [10] and analgesic activity [11]. In many studies doing antioxidant, analgesic, anti-inflammatory and antipyretic activity associated with secondary metabolites in some plant [7–9]. Recently, several plant-derived secondary metabolites have been described as interfering directly with molecules and mechanisms involved in inflammatory and fever processes [15–18]. However, to the best of our knowledge, the antipyretic effect of carica has not been explored in experimental fever models. This study aims to determine the antipyretic activity of carica fruit and seeds towards Wistar rats that had been induced fever.

2. Experimental

2.1. Chemical

Acetaminophen (Sanbe Farma), yeast (Na Kok Liong), WIDA NS 0.9% NaCl® (PT Bhakti Widatra), ethanol (pharmaceutical grade).

2.2. Animal

This research used 30 white male Wistar rats (Rattus norvegicus) and its 2-3 month age (150 – 200g). All animal handling procedures have been approved by the ethics committee of the Faculty of Medicine, Universitas Sebelas Maret with the number 502 / IV/ HREC / 2018.

2.3. Extraction

The fruit of carica (Vasconcellea pubescens A.DC) used in this study was collected from the Dieng Plateau, Central Java, Indonesia. This fruit and seeds are processed with maceration extraction methods using 70% ethanol for five days.

2.4. Phytochemical screening

2.4.1. Flavonoid screening. Flavonoid screening is done with Bate-Smith Test, the extract added by HCl then boiled in a water bath during 15 minutes. The positive reaction showed red color solution [19].

2.4.2. Saponin screening. Saponin screening is done with mixing the extract with 5 mL boiled water in test tubes, then shake until showed the positive reaction that is foam [20].

2.4.3. Polyphenol and Tannin screening. Polyphenol and Tannin screening did with add the extract with 70% ethanol. Then, add 2-3 drops 1% FeCl₃ solution. The positive reaction showed a bluish black or brownish green solution [21].

2.4.4. Alkaloid screening. This screening is done with dissolving 1 g extract. Two mL solution evaporated in porcelain bowl using a water bath. The residue is dissolved with 5 mL HCl 2 N then its divided into 2 test tubes. First tubes added by three drops of HCl 2 N and second tubes attached by three drops dragendorff reagent. Formation of sediment and reaction with dragendorff reagent will show orange residues which is a positive result of this screening [19].

2.4.5. Antipyretic test of mountain papaya fruit and seeds ethanolic extracts. Antipyretic test of carica fruit was determined using Ahmadiani modification method with yeast suspension induced in animal models [22]. The temperature was measured in rectal using a digital thermometer. Measurement of temperature was done before testing to knowing pretest temperature. All rats were given 20% yeast suspension with the dose 20 mL/kg body weight (b.w) as fever induced and injected subcutaneously (at the nape of neck). Fever temperature was checked after 3 hours since injection of yeast suspension. The rats divided into six groups on each extract sample which each group consists of five rats. Group I
was given 2.5 mL aquadest as a negative control. Group II was given 1.5 mL/kg b.w acetaminophen syrup as a positive control. Group III – V was given carica fruit and seeds ethanolic extracts (EECF/EECS) 200, 400, and 800 mg/kg b.w. Group VI not given any treatment as a normal group. Rectal temperature was then measured immediately after per oral granting as 0 hours. Antipyretic activity was measured during 6 hours, and the measurement of rectal temperature was checked every 30 minutes.

2.5. Statistical analysis
The data of rectal temperature processed in Area Under Curve (AUC). AUC was then measured using the following formula 1:

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AUC = \frac{\text{First temperature} + \text{Second temperature}}{2} \times \text{interval of first and second temperature}
\] (1)

The number of AUC in each group were then statistically analyzed using One Way ANOVA and followed by Post Hoc Test using Least Significant Difference (LSD).

3. Results and Discussion
3.1. Maceration and phytochemical screening of carica fruits and seeds
The phytochemical screening of carica fruits and seeds extract showed that it contains secondary metabolites consist of flavonoid, polyphenol, tannin and saponin that can be showed in table 1.

| No | Metabolite                | Theory [19]                                      | Test         | Result | Fruits | Seeds |
|----|---------------------------|--------------------------------------------------|--------------|--------|--------|-------|
| 1. | Flavonoid                 | Red                                               | Brownish red | Positive | Positive |
| 2. | Saponin                   | Stable foam and with dragendorf reactant will form an orange precipitate | Stable foam | Positive | Positive |
| 3. | Alkaloid                  | Black precipitate formed                           | Black precipitate formed | Negative | Negative |
| 4. | Polyphehnl and Tannin     | Bluish-black or green                              | Brownish green | Positive | Positive |

have 19 phenolic compounds were identified [23]. Phytochemical analysis with the Wagner and Bladt method described that carica fruit extract showed the presence of flavonoids, tannins and phenolic [10]. The quantitative test results show that the contains total flavonoids quercetin equivalent in Dieng Plateau with value 633.35 mg / L, respectively [19].

3.2. Antipyretic Test
The antipyretic test using yeast suspension induction caused by S. cerevisiae could increase temperature by synthesis of prostaglandins [24]. The mechanism of yeast causes fever which is an increase in rat body temperature due to the presence of microorganisms in yeast that are considered as foreign objects by the immune system [25]. The S. cerevisiae and its cell wall components such as mannans induce fever by increasing plasma levels from IL-1β, interferon c, and TNF-α. This mediator works on the organum vasculosum lamina terminalis in the central nervous system. The mediator induces the formation of prostaglandins which play an important role in fever mediators [26]. The result of the antipyretic test can be seen in table 2 and 3.
Table 2. The average measurement in preliminary (t0) and fever temperature after 3 hours (ta) in the fruits extract sample

| No | Groups           | Rats Rectal Temperature (°C) |
|----|------------------|------------------------------|
|    |                  | t0   | ta   |
| 1  | Negative Control | 36.07±0.642 | 36.9±0.704 |
| 2  | Positive Control | 36.13±0.309 | 37.43±0.396 |
| 3  | EECF 200 mg/kg b.w | 35.63±0.323 | 37.13±0.532 |
| 4  | EECF 400 mg/kg b.w | 35.64±0.360 | 36.9±0.456 |
| 5  | EECF 800 mg/kg b.w | 35.92±0.271 | 37.42±0.463 |
| 6  | Normal Control   | 35.38±0.249 | 35.38±0.249 |

Table 3. The average measurement in preliminary (t0) and fever temperature after 3 hours (ta) in the seeds extract sample

| No | Groups           | Rats Rectal Temperature (°C) |
|----|------------------|------------------------------|
|    |                  | t0   | ta   |
| 1  | Negative Control | 36.07±0.642 | 36.9±0.704 |
| 2  | Positive Control | 36.13±0.309 | 37.43±0.396 |
| 3  | EECS 200 mg/kg b.w | 35.49±0.563 | 36.93±0.292 |
| 4  | EECS 400 mg/kg b.w | 35.26±0.267 | 36.71±0.363 |
| 5  | EECS 800 mg/kg b.w | 35.14±0.292 | 36.71±0.710 |
| 6  | Normal Control   | 35.38±0.249 | 35.38±0.249 |

The result from Table 2 and 3 showed that increasing the temperature 0.83°C – 1.5°C of the rat. Increasing temperature above 0.5°C can be said that rats have a fever [27]. Peroral granting was given after checked fever temperature and directly measured rectal temperature as 0 minutes. The result of rectal temperature measurements during 6 hours was showed in figure 1 and 2.

Figure 1. The graph of the decline in the average temperature of 6 groups. t0 = pre-test temperature, ta = post-test temperature, EECF = ethanolic extract of carica fruits
Figure 2. The graph of the decline in the average temperature of 6 groups. \( t_0 \) = pre-test temperature, \( t_a \) = post-test temperature, EEC\(_S\) = ethanolic extract of carica seeds

Figure 1 and 2 showed that negative control had more temperature than positive control and other groups of carica extract doses. It caused negative control as not given antipyretic agent treatment. Increased and decreased temperature in graph caused by fever induction that is still running. Yeast worked to raise the temperature during 9-18 hours \([24]\). The decrease of temperature in each group then statistically analyzed using One Way ANOVA and followed by Post Hoc LSD. Data that analyzed in statistical was AUC from all groups. AUC average in each group can be seen in Table 4.

Table 4. Data area under the curve (AUC) of carica fruit (EECF) and seeds (EECS) ethanolic extracts

| No | Groups               | AUC ± CV          | EEC | EECS ± CV          |
|----|----------------------|-------------------|-----|-------------------|
| 1  | Positive Control     | 13286.1 ± 0.96*   | EECF| 13268.1 ± 0.96*   |
| 2  | Negative Control     | 13446.9 ± 0.96    | EECF| 13446.9 ± 0.96    |
| 3  | Dose 200 mg/kg b.w   | 13427.55 ± 0.38   | EECF| 13392.15± 0.34    |
| 4  | Dose 400 mg/kg b.w   | 13303.5 ± 1.25    | EECF| 13397.55± 0.21    |
| 5  | Dose 800 mg/kg b.w   | 13277.75 ±0.83*   | EECF| 13153.66 ± 0.26*  |
| 6  | Normal Control       | 12727.65 ± 0.26*  | EECF| 12727.65 ± 0.73*  |

Description: * = significant difference between negative control groups (p<0.05)

The statistical analysis showed that normal control gave a significant difference in all groups because this control was not given any treatments. Negative and positive control gave significant differences because positive control contained acetaminophen as antipyretics agent \([28]\). Ethanolic extract of carica at the dose 800mg/kg b.w showed a significant difference (p<0.05) than other extract doses. Other carica extract had the effect of lowering temperature that can be seen in Table 4. Both AUC gave lowering temperature than negative control but still can not provide a significant difference. This result was due to the dose at 200, and 400 mg/kg b.w had not reached levels of maximum effective.

Mountain papaya fruits and seeds extract at the dose of 800 mg/kg b.w had more concentration than another dose. Furthermore, it is plausible to suggest that the antipyretic activities of plant involved, at least in part, modulation of the prostaglandin mechanism through direct inhibition of prostaglandin activity or indirect inhibition of prostaglandin synthesis, via inhibition of the enzyme cyclooxygenase \([18,29]\). Some study concludes that bioactive compounds namely flavonoids, tannins, and saponins have synergistic action, and thus confirmed the folklore of the plant for the treatment of various ailments \([29,30]\).
4. Conclusion
The results showed that carica (Vasconcellea pubescens A.DC.) fruits and seeds ethanolic extracts with the dosage of 800 mg/kg b.w was significantly (p < 0.05) able to lower the temperature towards negative control in Wistar rats. At the dose possible had more concentration of metabolite than another dose. Further research is expected to conduct a quantification test of secondary metabolites of carica fruits and seeds extract as of the correlation between the dose, and the effect can be seen more clearly.

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