

## **Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) Exocarp Extract Effectivity Against Blood Uric Acid Levels in Male Mice (*Mus musculus*)**

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### **ABSTRACT**

Uric acid is the product of the body from foods that contain high purines. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) exocarp contains active compounds such as lycopene and flavonoids, which can inhibit the formation of free radicals and inhibit xanthine oxidation. This study aims to determine the activity of watermelon exocarp extract on uric acid levels in male mice. Twenty-five mice were divided into five experimental groups, with each treatment consisting of five replicate male mice. Treatment groups consisted of a solvent control (CMC Na 0.5%), a positive control (Allopurinol 0.26 mg/20gBB), dose I (Watermelon exocarp extract 50 mg/kgBB), dose II (Watermelon exocarp extract 100 mg/kgBB), dose III (Watermelon exocarp extract 200 mg/kgBB). Then the test animals were measured for uric acid levels, and each treatment was carried out with induction of chicken liver suspension 25 mg/kgBB until it showed that the mice had hyperuricemia. Mice were treated similarly to the treatment group for 15 days until their uric acid levels became normal. Blood uric acid levels were measured on days 5, 10, and 15. The results showed that all treatment doses had activity as a reduction in uric acid levels, and dose III of watermelon exocarp extract 200 mg/kgBB with a duration of 15 days was the most effective dose as a reduction in uric acid levels with a percentage reduction of 53,16 %.

Keywords: Uric acid, watermelon exocarp extract, mice

### **Introduction**

Uric acid is the body's production of foods high in purines. Levels of uric acid in the blood could increase because of bad eating habits, such as foods containing high purines, such as meat, clam, shrimp, and chicken offal (liver, kidney, intestine, spleen, lung, brain). The average level of normal uric in men is below 7 mg/dl for men, and for women, it should be below 6 mg/dl (Misnadiarly, 2007).

Elevated uric acid level above normal value is caused by excess uric acid production and reduced uric acid excretion, which could lead to hyperuricemia. A continuous increment in uric acid levels could cause gouty arthritis (Herliani, 2019).

One of the natural ingredients that can be used to lower uric acid levels is *Citrullus lanatus* (Thunb.) Matsum. & Nakai, locally known as watermelon. According to Eltadeza's research (2018), the extract of all-part watermelon (is effective in lowering uric acid levels in white male rats of the Wistar strain (*Rattus norvegicus*). The treatment dose with the most significant effect is 1500 mg/200 g mice body weight. According to the research results of Muthia et al. (2017), watermelon exocarp extract has the potential as a diuretic with the best dosage of 100 mg per kg. That adequate amount was used in a preliminary test to see the activity of watermelon exocarp extract in reducing uric acid levels. One of the active compounds in watermelon exocarp is flavonoids. Flavonoids act to lower uric acid levels by

inhibiting the action of the enzyme xanthine oxidase, thus inhibiting or reducing the formation of uric acid. This study aimed to investigate the activity of watermelon exocarp extract on uric acid levels tested in male mice (*Mus musculus*).

### **Material and Methods**

#### *Material*

The instruments of this study consist of a sput, mice cage, drinking bottles, and feeding containers. Experimental animal scales, porcelain cup, rotary vacuum evaporator, analytical balance, stirring rod, spatula, porcelain crucible, furnace (Ney®), oven, pipette, glass beaker, dropper, chocolate bottle, aluminium foil, chemical glassware, filter paper, refrigerator and uric acid level check and test strip (easy touch) (Meilanda & Lanuari, 2023).

The materials used in this study consisted of crude drug exocarp for watermelon, male mice aged 2-3 months weighing 30-40 g, feed for mice (pellets), aquadest, 70% ethanol, chicken liver juice, allopurinol tablets, carboxy methyl cellulose 0.5%, bismuth subnitrate, acetic acid, Dragendroff's reagent, Mayer's reagent, Bouchardat reagent, gelatin, magnesium powder, 2 N hydrochloric acid, betadine solution, 1% ferric chloride.

#### *Methods*

##### *1. Plant determination*

The watermelon exocarp used in this study was collected from one of the regional markets in Bogor, West Java Province. Then it was determined at Herbarium Bogoriense for Botany, Biology Research Center, LIPI Bogor.

2. Crude drug production process

Cut the watermelon into bite-sized pieces, separate the fruit and skin, then wash the exocarp with clean water. Afterward, cut into small pieces and dried in an oven at 60°C, flavonoid compounds will be damaged if heated at temperatures over 90°C (Wahyuni, Vifta, & Erwiyani, 2018) while the levels of lycopene at a temperature of 80°C will experience a decrease in because the temperature rise causes the decomposition of these compounds. It was powdered with a blender. The finished powder is used for extraction (Annisa, 2021).

3. Extraction process

The manufacture of ethanolic extract of watermelon exocarp is by maceration method. The watermelon exocarp powder was weighed 300 g, put into a brown bottle, and added 70% ethanol solvent in a ratio of 1:10 for three days, covered and protected from light, and stirred once a day. After three days, it was filtered with a flannel cloth and then evaporated in a vacuum evaporator until a thick extract was obtained, characterized by a water content of around 5-30% (Voight, 1994).

4. Phytochemical identification

Phytochemical tests were carried out on powders and extracts, including the identification of alkaloids, flavonoids, saponins, and tannins.

5. Experimental animal induction stage

Experimental animals were induced to increase uric acid levels with chicken liver juice orally at 0.5 ml per 20 g body weight for 14 days or until uric acid levels > 1.7 mg/dl. The uric acid was measured every five days for 15 days.

6. Animal experiment treatment

Twenty-five male mice were divided into five treatment categories. Solvent control was given with 0.5% CMC-Na solution. In contrast, positive control was treated with Allopurinol suspension 0.26 mg/20g BW. In comparison, dosage I, II, and III groups were treated with watermelon exocarp extract at 50, 100, and 200 mg/kg BW, respectively. Preparation of 50 mg/kg BW watermelon exocarp extract suspension in the following way. The required extract for 20 g mice is 1 mg, based on the following calculation:

$$\frac{20g}{1000g} \times 50mg = 1mg$$

Solution stock is made as much as 25 mL, then 125 mg of the extract is required to be dissolved

with CMC-Na up to 25 ml. The calculation of the amount of extract that must be dissolved is as follows.

$$\frac{1mg}{0.2ml} \times 25ml = 125mg$$

7. Measurement of uric acid level in blood

Measurement of uric acid levels is assessed with a uric acid check tool (easy touch). Blood samples from the tails of mice were taken using a blood lancet that had been sterilized with alcohol. The first measurement of uric acid levels is after 14 days of acclimatization, which is normal uric acid levels. After that, it was induced for ten days, then the uric acid level was checked. Treatment was given for 15 days, then monitored uric acid levels every five days on days 0, 5, 10, and 15.

*Data Analysis*

Obtained data were analyzed using one way analysis of variant (ANOVA) with the SPSS version 15.0 program. Duncan's test was carried out to show the difference between treatments.

**Results and Discussion**

*Phytochemical Identification*

Based on watermelon exocarp phytochemical tests, flavonoids, alkaloids, tannins, and saponins, are found. The results obtained are in accordance with the previous research (Muthia Rahmi, 2017).

*Experimental Animal Acclimatization*

Before acclimatization, the research procedure was approved by the Animal Ethics Committee of FMIPA No. 98/KEPHP-UNPAK/04-2020. Mice were acclimatized for two weeks before uric acid induction. This acclimatization aims to adapt the mice to the environment and determine the feasibility of the mice used. Experimental mice were declared relatively homogeneous if the coefficient variant (CV) < 15% (Indriani, Zunnita, & Khairi, 2019). The CV results obtained during acclimatization were 3.42%. It could be stated that the experimental animals used were relatively homogeneous. CV was calculated by weighing the entire body weight of the mice before and after acclimatization, then the average, SD and CV were calculated before and after acclimatization.

*Increased Uric Acid Levels After Induction*

The average uric acid level of male mice in all treatment groups before induction was 3,364 mg/dL ±0,224, and Normally, uric acid levels in mice range from 1-5 mg/dL (Muhtadi, 2014).

After induction of chicken liver juice for ten days, uric acid increased to 6.3 mg/dL. The resulting mean increase in uric acid is 87.28% compared to normal pre-induction uric acid because the high purine content of chicken liver juice in the blood

stimulates the formation of uric acid by the enzyme xanthine oxidase (Suwaibah & Wijayanti, 2019).

*Decreased Uric Acid Levels After Watermelon Exocarp Extract Administration*

**Table I** shows the average uric acid levels after treatment with watermelon exocarp extract.

**Table I.** Mean  $\pm$  SD uric acid values for mice during treatment.

| Treatment Group  | Uric acid level (mg/dL) at day- |                  |                  |                  | Average                        |
|------------------|---------------------------------|------------------|------------------|------------------|--------------------------------|
|                  | 0                               | 5                | 10               | 15               |                                |
| Solvent Control  | 6,28 $\pm$ 0,141                | 6,82 $\pm$ 0,636 | 7,5 $\pm$ 0,707  | 8 $\pm$ 0,849    | 7,15 $\pm$ 0,583 <sup>b</sup>  |
| Positive Control | 6,58 $\pm$ 0,415                | 5,24 $\pm$ 0,364 | 4,02 $\pm$ 0,164 | 3,04 $\pm$ 0,207 | 4,720 $\pm$ 0,281 <sup>a</sup> |
| Dose I           | 5,94 $\pm$ 0,195                | 5,36 $\pm$ 0,434 | 4,68 $\pm$ 0,476 | 3,54 $\pm$ 0,416 | 4,66 $\pm$ 0,380 <sup>a</sup>  |
| Dose II          | 6,38 $\pm$ 0,327                | 5,44 $\pm$ 0,288 | 4,26 $\pm$ 0,230 | 3,42 $\pm$ 0,259 | 4,875 $\pm$ 0,276 <sup>a</sup> |
| Dose III         | 6,32 $\pm$ 0,5263               | 5,4 $\pm$ 0,682  | 3,98 $\pm$ 0,606 | 2,92 $\pm$ 0,207 | 4,665 $\pm$ 0,319 <sup>a</sup> |

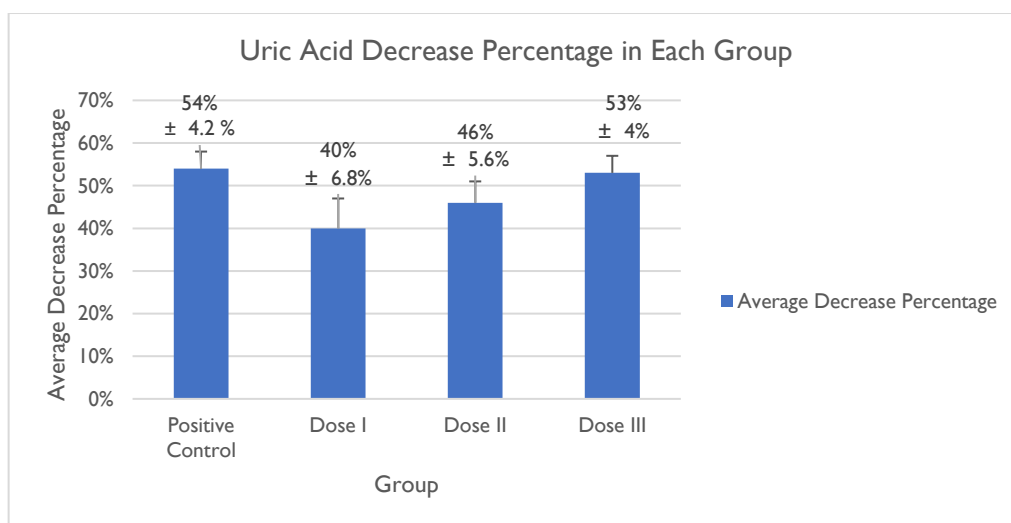
Note: The average value followed by the same superscript letter in the same row or column indicates the treatment effect is not significantly different ( $P > 0.05$ ).

The study results showed that the treatment group experienced a reduction in blood uric acid after being treated with watermelon exocarp extract. It can be seen in Table I and Figure 1. On day 0, measurements were taken after the induction of liver juice. All mice's uric acid levels indicate hyperuricemia. On day 15, each treatment group experienced a decrease in blood uric acid levels into baseline (around 3 mg/dL). On the 5th day of examination, the positive control group and watermelon rind extract treatment showed decreased uric acid levels, with the lowest average uric acid levels in the positive control group. However, until the 5th day, the uric acid level reduction did not show normal values (1-5 mg/dL). Starting on the 10th day, the four groups showed an average value of uric acid levels within the normal range. This decrease in uric acid levels continued until the 15th day of examination, even though this decrease did not cause the test animals to have uric acid values below normal.

The positive control group (allopurinol 0.26 mg/kg bw) decreased uric acid levels from day 0 to day 15 and were inversely proportional to the

solvent control (CMC-Na 0.5%). The uric acid level lowering effect of watermelon exocarp extract can be confirmed in Graph 1 by comparing the average uric acid level with the solvent control group (CMC Na 0.5% solution), which experienced an increase in uric acid levels from day 0 to day 15, this is due to the effect of giving chicken liver juice which is still given during the treatment so that uric acid accumulate (Restina, Effendi, & Wiendarlina, 2018) therefore, CMC-Na solution does not have anti-hyperuricemia activity, but it does not reduce uric acid to normal value (Jumain, Asmawati, & Karnita, 2018). This indicates that watermelon exocarp extract has a pharmacological effect on blood uric acid levels and lowers uric acid levels in mice.

All treatments showed the same effect as the positive control of allopurinol, which averaged uric acid in each group statistically not significantly different. Allopurinol has a mechanism of action by inhibiting the activity of the enzyme xanthine oxidase. It is therefore used as a uric acid-lowering drug, which plays a role in converting hypoxanthine into uric acid (Dipiro, 2017).



**Figure 2.** Decrease uric acid level percentage in mice from each group

The highest percentage decrease in uric acid levels was positive control (allopurinol) at  $54 \pm 4.2\%$ , dose III at  $53 \pm 4\%$ , dose II at  $46 \pm 5\%$  and the lowest percentage was dose I at  $40 \pm 6.8\%$ . Duncan's test results showed that the decrease in uric acid levels in the solvent control group significantly differed from that in the positive control group and all treatment doses ( $p < 0.05$ ). The Duncan test results showed that the decrease in uric acid levels in the solvent control group showed a significant difference ( $p < 0.05$ ) with the positive control group, the dose I, dose II, and dose I had relatively the same effect with the positive control group ( $p > 0.05$ ) so that it can be interpreted that watermelon exocarp extract has an anti-hyperuricemic activity which is almost the same as positive control (allopurinol).

In this study, Two way ANOVA was used to see how the effect of dose, length of time on decreasing uric acid levels, and the interaction of dose and duration of administration on reducing uric acid levels. The results indicate that the dose treatment factor and the duration of administration (days) are known to have a significant effect on lowering blood uric acid levels in mice ( $p < 0.05$ ). There is a considerable effect from the interaction of dose and duration of administration to decrease uric acid levels in mice ( $p < 0.05$ ). Therefore, further testing was performed using Duncan's test to determine the dosage and duration of watermelon exocarp extract with anti-hyperuricemia effects.

Duncan's test results for watermelon exocarp extract administration duration on days 0, 5, 10, and 15 showed a significant difference in Watermelon skin extract length to lower blood uric acid levels ( $p < 0.05$ ).

The interaction between treatment dose and duration of administration can be stated as the third dose (200 mg/KgBW) with the time of administration of watermelon exocarp extract for 15 days gave the same effect as the positive control (Allopurinol 0.26 mg/20 gBW) and significantly different from the solvent control group ( $p < 0.05$ ) so that the third dose with a duration of 15 days was the best dose duration in lowering uric acid. This shows that there are two influential variables in the study where the treatment dose and the length of time given to have a synergistic effect in helping to reduce blood uric acid levels in mice.

Watermelon exocarp, according to Muthia et al. (2017), acts as a diuretic at a dose of 100 mg/Kg BW so that it could increase urine production. Thus, it is thought to help reduce blood uric acid levels (Djohari & Paramitha, 2015) so that watermelon rind extract can be used as an anti-hyperuricemia agent.

Flavonoids in watermelon exocarp are expected to reduce blood uric acid levels in mice. Flavonoids have antioxidant properties by inhibiting the action of the enzyme xanthine oxidase, thus inhibiting or reducing the formation of uric acid and

can lower uric acid value (Eltadeza, 2018). Flavonoid compounds that have double bonds in C-2 and C-3 atoms tend to have the ability to act as inhibitors. In addition, the presence of hydroxyl groups on C-5 and C-7 and carbonyl groups on C-4 can form hydrogen bonds and play a role in the interaction of inhibitors with the active site of the xanthine oxidase enzyme (Wajdie, Kartika, & Saleh, 2018).

#### Summary

All therapeutic doses of watermelon exocarp extract had uric acid-lowering effects, and dose III (200 mg/kg body weight) administered for 15 days lowered uric acid levels with a reduced rate of  $53 \pm 4\%$ , thus being the best therapeutic dose.

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