ACTIVITIES OF RED MENIRAN ETHANOL EXTRACT (*Phyllanthus urinaria* L.) AS ANTIHYPERURICEMIA ON MICE

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Abstract

Hyperuricemia is a condition in which blood uric acid levels exceed normal value. This state may become gout disease. Therefore, a study was conducted to find a therapy which can reduce uric acid levels by utilizing Indonesian medicinal plants. Red meniran is in the same family with white meniran, which is proven effective in reducing uric acid levels, so similar efficacy is expected from red meniran. The study used six groups with each group consisted of 6 mice; they were control group, standard group, and four treatment groups. The mice were induced with hyperuricemia using potassium oxonate 250 mg/kg BW; after 30 minutes, control group was given a suspension of CMC Na 0,5%, standard group was given 10 mg/kg BW of allopurinol, and treatment groups were given ethanol extract of red meniran at doses of 25 mg/kg BW, 50 mg/kg BW, 75 mg/kg BW and 100 mg/kg BW. The effectiveness of red meniran ethanol extract was determined by measuring uric acid levels of mice in 60th, 90th, 120th, 150th, and 180th minute after treatment. The result indicated that red meniran could lower uric acid levels at an effective dose of 50 mg/kg BW. Uric acid levels reduction occurred in the 90th minute.

Key words : *red meniran, Phyllanthus urinaria L., ethanol extract, uric acid levels, peruricemia, mice*

ISBN : 978-979-18458-5-4

INTRODUCTION

Uric acid is a substance from purine final metabolism. Elevated levels of uric acid can occur due to excessive production of uric acid or disruption of the mechanism of excretion through the kidneys. Uric acid levels elevation above the normal value, > 7 mg/dL for men and > 6 mg/dL for women is called hyperuricemia. Hyperuricemia can progress into a disease called gout (Dipiro et al., 2008). Gout is not lethal, but it causes severe pain that can reduce the patient's quality of life.

Medications used for hyperuricemia can be classified into two groups, xanthine oxidase inhibitor and uricosuric. The recommended therapy for gout is allopurinol (Katzung, 2010). The use of allopurinol may lead to some side effects such as gastrointestinal disturbances, rash, vertigo, headache, hepatitis, and interstitial nephrithis (Dipiro et al., 2008). The most common side effects are gastrointestinal disturbances, rash, hepatotoxicity and skin rash. About 2-10% of patients, especially the elderly people experienced skin rash and 0.4% of patients experienced hypersensitivity reaction (Dincer et al., 2002; Kong et al., 2002).

Indonesia is a country rich in traditional plant. Traditional plants have been used for medication since long time ago. Scientific authentication that traditional plants can be used as medication needs to be developed. One of the plants which has been shown to lower uric acid levels is white meniran (Phyllanthus niruri). A study conducted by Murugaiyah and Chan (2009) stated that white meniran (Phyllanthus niruri) at doses of 50 mg / kg body weight can reduce uric acid levels in white rats. The active compounds in white meniran responsible for the anti-hyperuricemic effect are phyllanthin, hypophyllanthin, and niranthin. White meniran is also used to crush and eliminate kidney stone (Barros ME, et al, 2006); as a hepatoprotector, anti-diabetes, anti-hyperlipidemia, anti-fungal, and analgesic (Damle M.C, 2008). Besides white meniran, people are also familiar with red meniran (Phyllanthus urinaria L), which is in the same family with white meniran. In taxonomy, two plants with close genetic relationship may have similar active compounds. Therefore, a study to find out whether red meniran can reduce uric acid levels is needed.

Scientific authentication that red meniran can lower (blood) uric acid levels was carried out with experimental laboratories using potassium oxonate - induced hyperuricemic mice. After that, the mice was given ethanol extract of red meniran. The parameter observed is blood uric acid levels.

This study is expected to give information about the efficacy of red meniran as an anti-hyperuricemic agent and red meniran can be formulated into a pharmaceutical product that can be used by people, thus indirectly encourage the use of Indonesian traditional plants.

METHODS

Materials

The materials used in this study were red meniran herbs (Phyllanthus urinaria, L.) obtained from the district of Mojokerto, potassium oxonate, 96% ethanol, CMC Na, and allopurinol.

Instruments

The instruments used were the equipments for kinetic maceration process, rotary evaporator, waterbath, UA-sure meter (uric acid test kit), strips, injection spuits 1 ml, sonde (probe), beaker glass, glass stirring rods, glass funnels, graduated cylinder, Erlenmeyer flask, mortar and pestle, analytic scales, and animal scales.

Animals

The animals used were 3-4 months white male Swiss strain of Mus musculus (mice), weighing 20-35 g; visually fit with the parameters of no illness/disease; no watery consistency of feces; clean, smooth and shiny fur; clear and reddish eyes, the nose and mouth were not slimy and continuously salivate. During

the first week of environmental adaptation, mice body weight should not be reduced by 10%.

Procedure

Preparation of Ethanol Extracts of Red Meniran (Phlyllanthus urinaria L.)

Red meniran herbs were extracted by kinetic maceration process for an hour using 96% ethanol solvent, then allowed to stand for 24 hours. The bath was then filtered, the process produced filtrate and residue. The residue was re-extracted with the same process and solvent until at least three times or a clear or colorless filtrate was obtained. The filtrate obtained from the whole extraction process was collected in a container, and then the solvent was removed using rotary evaporator and followed by a waterbath at a temperature of 60° C to obtain thick extract with a constant weight (Depkes,1986). The dose of ethanol extract of red meniran used were 25 mg/kg BW, 50 mg/kg BW, 75 mg/kg BW, 100 mg/kg BW.

Administration of the treatment to the sample

In this study, 36 white male mice (Mus musculus) were used with each group consisted of 6 mice. Before the treatment, all mice were adapted to the environment for one week. During the adaptation period all mice were given food and drink with a fixed amount and frequency.

All mice were fasted for 12 hours before testing, then their initial uric acid levels were measured within normal range of 3-4 mg/dL as sample inclusion criteria. After that, 36 white male mice were given potassium oxonate at a dose of 250 mg/kg intraperitoneally (ip). Thirty minutes after the induction of potassium oxonate, treatment group was given ethanol extract of red meniran (Phyllanthus urinaria, L.) at a dose of 25 mg/kg BW, 50 mg/kg BW, 75 mg/kg BW, and 100 mg/kg BW, with volume of 25 ml/kg BW, in the suspension of CMC Na (Murugaiyah dan Chan, 2009). The control group was given 25 ml/kg BW of 0.5% CMC Na suspension orally. The standard group was given allopurinol at a dose of 10 mg/kg BW in 25 ml/kg suspension orally. Blood uric acid levels of each mice in the control group, treatment group, and standard group were measured in the 60th, 90th, 120th, 150th, and 180th minute after treatment (Zhao, et al., 2005).

The blood sample were obtained by making cuts on the tail of each mice, then the samples were processed using an uric acid measuring instrument 'UA-sure'®.

Data Analysis

Uric acid levels in the control group, treatment group, and standard group were analyzed using the Minitab.

Table I. The uric acid levels of mice in control group

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	5,7	6,5	5,3	4,8	4,4
2.	6,2	7,7	6,8	6,2	5,3
3.	5,6	6,6	5,7	4,8	3,7
4.	5,0	6,7	5,9	4,7	3,9
5.	5,5	8,3	6,9	5,8	4,3
6.	5,2	6,8	5,9	5,3	4,9
Mean ± SD	5,53±0,42	7,1±0,73	6,08±0,63	5,27±0,59	4,42±0,60

ISBN : 978-979-18458-5-4

Table II. The uric acid levels of mice in standard group.

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	3,7	4,4	3,9	3,6	3,0
2.	4,9	6,1	4,9	4,0	3,3
3.	4,9	4,3	4,2	3,8	3,5
4.	5,2	4,3	3,3	3,1	3,0
5.	4,2	3,6	3,3	3,0	3,0
6.	4,1	6,3	4,6	3,9	3,2
Mean ± SD	4,5±0,58	4,83±1,10	4,03±0,66	3,57±0,42	3,17±0,21

Table III. The uric acid levels of mice in Meniran 25 group (dose of 25 mg/kg BW)

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	5,5	10,5	8,3	5,9	4,8
2.	4,3	7,6	6,6	5,9	4,2
3.	5,1	8,1	6,2	5,5	4,3
4.	3,9	6,4	5,0	4,6	4,1
5.	4,6	6,3	5,8	5,5	4,3
6.	4,3	6,7	4,7	4,3	4,0
Mean ± SD	4,62±0,59	7,6±1,59	6,1±1,59	5,28±0,68	4,28±0,28

Table IV. The uric acid levels of mice in Meniran 50 group (dose of 50 mg/kg BW).

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	6,0	5,2	4,9	4,8	3,5
2.	5,3	4,3	4,3	4,0	3,5
3.	5,8	5,2	4,3	4,0	3,1
4.	6,4	4,8	4,1	4,0	3,3
5.	5,2	4,4	4,0	4,0	3,2
6.	5,6	4,0	3,5	3,1	3,0

ISBN : 978-979-18458-5-4

Table V. The uric acid levels of mice in Meniran 75 group (dose of 75 mg/kg BW)

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	5,5	4,3	4,0	3,2	3,1
2.	6,2	5,5	4,8	4,3	3,4
3.	5,1	6,6	4,7	4,0	3,5
4.	5,0	4,7	3,9	3,2	3,0
5.	6,5	4,7	4,6	3,8	3,0
6.	4,2	3,9	3,5	3,2	3,1
Mean ± SD	5.42±0,84	4,95±0,97	4,25±0,52	3,62±0,48	3,18±0,21

Table VI. The uric acid levels of mice in Meniran 100 group (dose of 100 mg/kg BW)

Number of mice	Uric acid levels (mg/dL)				
	Time (minute)				
	60	90	120	150	180
1.	6,5	5,8	4,7	4,0	3,5
2.	6,2	5,0	4,5	4,0	3,7
3.	5,9	5,2	4,3	3,9	3,6
4.	5,8	4,7	4,6	3,9	3,4
5.	6,5	4,8	4,0	3,5	3,3
6.	6,6	5,4	4,4	4,1	3,5
Mean ± SD	6,25±0,34	5,15±0,41	4,42±0,25	3,9±0,21	3,5±0,14

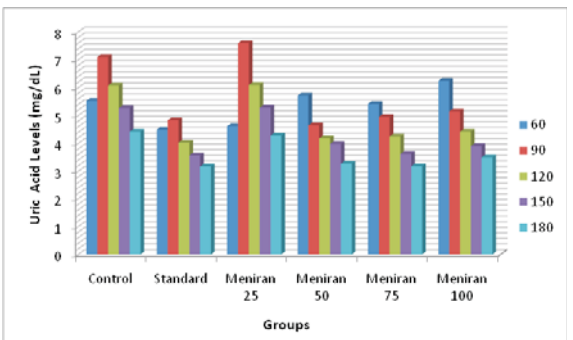

Figure 1. The diagram of the mean uric acid levels of mice in various groups and time.

RESULT

The study results of uric acid levels in various groups of mice are listed in Table I-VI, and bar charts of the mean uric acid levels in various groups and time is shown in Figure 1. The results from statistical analysis using minitab method are shown in Table VII and VIII.

There were four different doses of ethanol extract of red meniran used in this study, which were 25 mg/kg BW, 50 mg/kg BW, 75 mg/kg BW, and 100 mg/kg BW. The dose selection was based on the study conducted by Murugaiyah and Chan (2009) in which white meniran with a dose of 50 mg/kg BW can lower uric acid levels, and therefore the study was conducted to determine the effectiveness of the ethanol extract of red meniran at doses lower and higher than 50

ISBN : 978-979-18458-5-4

Table VII. Statistical Analysis Results Between the Treatment Groups

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Analysis of Variance for kadar, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS   Adj MS      F      P
treatment          5   73,8529  73,8529  14,7706  34,81  0,000
waktu              4   99,2130  99,2130  24,8032  58,45  0,000
treatment*waktu   20   46,3957  46,3957   2,3198   5,47  0,000
Error            150   63,6483  63,6483   0,4243
Total            179  283,1099

Tukey 95,0% Simultaneous Confidence Intervals
Response Variable kadar
All Pairwise Comparisons among Levels of treatment
treatment = kontrol positif  subtracted from:

treatment     Lower  Center   Upper    -+---------+---------+---------+--
meniran 100  -1,522  -1,037  -0,551*         (----*---)
meniran 25   -0,589  -0,103   0,382                   (----*----)
meniran 50   -1,805  -1,320  -0,835*      (----*----)
meniran 75   -1,882  -1,397  -0,911*     (----*----)
pembanding   -2,145  -1,660  -1,175*   (---*----)
                                  -+---------+---------+---------+--
                                -2,0      -1,0       0,0       1,0

treatment = meniran 100  subtracted from:

treatment    Lower   Center    Upper    -+---------+---------+---------+-
meniran 25   0,448   0,9333   1,4186*                          (----*-)
meniran 50  -0,769  -0,2833   0,2019               (----*----)
meniran 75  -0,845  -0,3600   0,1252               (---*----)
pembanding  -1,109  -0,6233  -0,1381*          (----*----)
                                  -+---------+---------+---------+-
                                -2,0      -1,0       0,0       1,0

treatment = meniran 25  subtracted from:

treatment    Lower  Center   Upper    -+---------+---------+---------+---
meniran 50  -1,702  -1,217  -0,731*       (----*----)
meniran 75  -1,779  -1,293  -0,808*      (----*----)
pembanding  -2,042  -1,557  -1,071*    (---*----)
                                  -+---------+---------+---------+--
                                -2,0      -1,0       0,0       1,0

treatment = meniran 50  subtracted from:

treatment     Lower   Center   Upper    -+---------+---------+---------+-
meniran 75  -0,5619  -0,0767  0,4086                (----*----)
pembanding  -0,8252  -0,3400  0,1452              (----*---)
                                  -+---------+---------+---------+-
                                -2,0      -1,0       0,0       1,0

treatment = meniran 75  subtracted from:

treatment     Lower   Center   Upper    -+---------+---------+---------+-
pembanding  -0,7486  -0,2633  0,2219              (---*----)
                                  -+---------+---------+---------+-
                                -2,0      -1,0       0,0       1,0
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Explanation *= significantly different

ISBN : 978-979-18458-5-4

Table VIII. Statistical analysis result of the interaction between control group and Meniran 50 group at various times

Group and Time Interaction					
Group - Time	Group - Time	Result			Explanation
		Lower	Center	Upper	
Control 60 min*	Meniran 50-60 min	-1,255	0,183	1,6220	Not significantly different
Control 90 min	Meniran 50-90 min	-3,889	-2,450	-1,011	Significantly different
Control 120 min	Meniran 50-120 min	-3,339	-1,900	-0,461	Significantly different
Control 150 min	Meniran 50-150 min	-2,722	-1,283	0,1554	Not significantly different
Control 180 min	Meniran 50-180 min	-2,589	-1,150	0,2887	Not significantly different

*minute

mg/kg BW. Red meniran is in the same family with white meniran, so it is expected that red meniran can also provide the same effect. The dose of allopurinol used was 10 mg/kg BW, which refered to a study conducted by Zhao, et al, 2005. The effectiveness of the ethanol extract of red meniran as an anti-hyperuricemic agent can be determined by measuring the uric acid levels of mice.

Initial uric acid levels with a normal value of 3-4 mg/dL was a sample inclusion criteria. The mice with normal uric acid levels were given potassium oxonate as an uric acid inducer intraperitoneally (ip) at a dose of 250 mg/kg BW. Based on the research conducted by Mai, et al, 2005, potassium oxonate can increase uric acid blood levels and achieve the peak levels after 2 hours of administration. Uric acid levels in mice may decline steadily even without medication because the mice have uricase, an enzyme that converts uric acid into a more polar alantoin thus is easily removed from the body through the urine. Therefore, the ethanol extract of red meniran and allopurinol were given thirty minutes after the induction process so that the materials given to the treatment and standard group could give the effect before uricase changed uric acid into alantoin.

The chart in Figure 1 showed that the uric acid levels profiles of mice in the control group, standard group, and Meniran 25 group increased from the 60th minute to the 90th minute then they were decreased; but in Meniran 50 group, Meniran 75 group, and Meniran 100 group the uric acid levels decreased after 60 minutes. This suggested that the decline in uric acid levels occurred in the 90th minute.

Based on the statistical result in Table VII, uric acid levels lowering between control group and Meniran 25 group did not differ significantly, but there were significant differences between control group with standard, Meniran 50, Meniran 75, and Meniran 100 group. Meniran 25 group differed significantly with Meniran 50, Meniran 75, Meniran 100 and standard group. Meniran 50 group did not differ significantly with Meniran 75, Meniran 100 and standard group as well as Meniran 75 group did not differ significantly with Meniran 100 and standard group. There were significant

differences between Meniran 100 and standard group. The statistical result showed that the standard group provided the most decrease in uric acid levels. Based on the descriptions it can be concluded that the ethanol extract of red meniran given to Meniran 50 and Meniran 75 group have anti-hyperuricemic effect. Given in a lower dose, the therapeutic effect of red meniran extract in Meniran 50 group did not differ significantly than in Meniran 75 group, thus the dose of 50 mg/kg BW of red meniran extract was selected as the effective dose in lowering blood uric acid levels of mice.

Based on the statistical results in Table 3.8, it was known that the uric acid levels lowering between the control and Meniran 50 group in the 60th minute was not significantly different; this could happen because ethanol extract of red meniran hadn't shown any effect, so the uric acid levels in Meniran 50 group didn't differ significantly than control group. In the 90th and 120th minute there was a significant difference between control group and Meniran 50 group, it showed that the ethanol extract of red meniran had given therapeutic effect. In the 150th and 180th minute there was not any significant difference between control group and Meniran 50 group, and that means the uric acid levels lowering in 150th minute was not because of the activity of red meniran extract, but the mice body metabolism activity to eliminate the uric acid.

CONCLUSION

Based on the study result, it can be concluded that ethanol extract of red meniran provides anti-hyperuricemic effect at a dose of 50 mg/kg BW and uric acid levels lowering occurs at minute of 90.

ACKNOWLEDGEMENTS

Research Activities and Community Service Institutions (LPPM), Kartini, Steffi and Fifi.

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