The Effect Of Kelor Leaves (Moringa oleifera) Ethanol Extract On Serum Uric Acid And Tumor Necrosis Factor-α Of Hyperuricemic White Rats (Rattus norvegicus)

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Abstract. Chronic hyperuricemia leads to various complications due to the inflammation and oxidative processes. However, the current chemical medications have caused serious side effects. Ethanol extract of Kelor (Moringa oleifera) leaves contains various active substances potential for decreasing serum uric acid and inflammatory cytokine el. The aim of this experiment is to study the effect of Kelor (Moringa oleifera) leaves ethanol extract to serum uric acid and TNF-α level. The Method used is experimental study with post test only with control group design. Twenty-five white rats were randomly assigned into 5 groups: A (healthy control), B (diseased control; hyperuricaemic), C,D,E (hyperuricemic, given ethanol extract 300,600,1200 mg/KgBW/day). Hyperuricemia induction was conducted by administration of 20 g raw cow brain/rat/day for 10 days and was continued until day 17. The result is the average of uric acid (mg/dL) in group A: 4.18±0.55, B: 15.02±0.71, C: 8.46±0.12, D: 6.55±0.14, E: 6.45±0.13. The average of TNF-α (mg/dL) in group A: 29.59±10.67, B: 122.58±40.45, C: 108.80±19.89, D: 60.54±8.40, E: 36.12±8.07. Data analysis used One Way ANOVA, followed by post hoc LSD for uric acid and Kruskall-Wallis, followed by Mann-Whitney tests for TNF-α reveal significant differences among groups. The conclusion of this study is the administration of Kelor (Moringa oleifera) leaves ethanol extract in various doses significantly decrease serum uric acid and TNF-α level of hyperuricemic model white rats.

Keywords: moringa oleifera; leaves, uric acid; TNF-α; hyperuricaemic

1. INTRODUCTION

Hyperuricemia is a condition when there is high level of serum uric acid, i.e more than 7.0 mg/dL in male and more than 5.7 mg/dL in female (Soeroso, 2011). One of the causes of hyperuricemia is daily protein intake found in meat which is high in purine. High purine intake causes an increased serum uric acid level as its end metabolic result (Pribadi & Ernawati, 2010, Manampiring, 2011). Chronic hyperuricemia may lead to the development of serious health problems such as gout arthritis and cardiovascular diseases (Liu et al., 2011). As reported in Basic Health Research 2012, the prevalence of hyperuricemia in Indonesia was 11.9 % (Depkes RI, 2013), whereas in Central Java was 24.3% in male and 11.7% in female aged 15-45 year old (Purwaningsih, 2010). Hyperuricemia prevalence is variable based on age and starts to increase from age 30 y.o. in male and 50 y.o. in female. The higher prevalence (17-28%) were reported in studies conducted in hospitals due to the underlying diseases and side effects of medications used (Liu et al., 2011).

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Uric acid is one of inflammation agents that can stimulate macrophage to releases pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (Putra, 2009). Billiet et al. (2014) reported that in male and female, serum uric acid level positively correlates with CRP, IL-6 dan TNF-α level. Inflammation process releases reactive oxygen species (ROS) that causes oxidative stress (Zhou et al., 2018).

Anti-inflammatory medications and antioxidants are potential for decreasing TNF-α level. However, the current medications may cause serious side effects (Katzung et al., 2012). Kelor plant (Moringa oleifera) has been widely known as one of vegetables and herbal medicine plants in many places in Indonesia. Rahmawati (2015) proved that daily administration of 3,75 g/kgBW kelor leaves brewed in 3,6 ml warm water for 14 days decreased serum uric acid level from 2,463 mg/dL to 1,788 mg/dL. However, the effective dose of Kelor leaves ethanol extract to decrease serum uric acid level in hyperuricemia has not been established yet.

Kelor leaves contain various active substances that are potential as antioxidant (i.e flavonoid, vitamin C and vitamin E), as hepatoprotector, immunomodulator, and as anti inflammation agent (Witt, 2013). The active substances may decrease serum uric acid and TNF-α level through inhibition of uric acid synthesis, oxidative stress and inflammation (Giancarlo et al., 2010). It was hypothesized that the administration of Kelor leaves ethanol extract decrease serum uric acid and TNF-α level of hyperuricaemic white rats (Rattus norvegicus).

2. METHOD

The experimental method used completely randomized design with post test only with control group approach. Twenty five male wistar strain white rats (Rattus norvegicus) aged 2-3 months, weighted 150-200 g, healthy, active and have no observable physical anomaly, gained from MS. Bram Mouse Farm, Purbalingga, Central Java. The rats were randomly assigned into 5 groups: A (healthy control), B (diseased control, hyperuricaemic), C,D,E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day). Hyperuricemia induction was conducted by administration of 20 g raw cow brain/rat/day for 10 days and was continued until day 17.

The independent variable was various ethanol extract dosage (300,500, 1200 mg/kgBW/day). The dependent variable was serum uric acid and TNF-α level. Kelor leaves powder was bought from Pusat Informasi dan Pengembangan Tanaman Kelor Indonesia (The Indonesian Center for Information and Development of Kelor Plant), non governmental organization: Media Peduli Lingkungan, Blora, Central Java. Masseration technique with 96% ethanol to gain ethanol extract was conducted in Faculty of Pharmacy, Jenderal Soedirman University. Uric acid level (in mg/dL) was measured using spectrophotometer from 5 mL blood sample taken from the orbital plexus. TNF-α level was measured using ELISA. The absorbance read on 492 nm wave length (Kusumastuty et al., 2015). Data analysis used One Way ANOVA, followed by post hoc LSD for uric acid and Kruskall-Wallis, followed by Mann-Whitney tests for TNF-α. Differences were considered as significant when p<0.05.

3. RESULT AND DISCUSSION

The data of uric acid and TNF-α level (presented as mean ± standard deviation) are as shown in Figure 1 and 2. As seen, the pattern of uric acid and TNF-α level result were almost similar. The least level was found in normal group (A), significantly lower from other groups. This showed the success of induction process through daily intake of cow brain which is high in purine (100-1000 mg purine /100 gram food) (Lestari et al., 2014)

Previous study by Pribadi & Ernawati (2010) reported that the administration of 20g/rat/day of cow brain for 8 days caused significant increase in serum uric acid level until reached hyperuricemic state. The result also showed that the induction of hyperuricemia have caused increase in serum TNF-α level. This was in accordance with Zhou et al.’s (2012) finding that there was an increase in TNF-α level in hyperuricemia white rats.
Figure 1. Serum Uric acid level average
Note: groups: A = 4,18±0,55 (healthy control), B = 15,02±1,59 (diseased control, hyperuricaemic), C = 8,46±0,12, D = 6,55±0,14, E = 6,45±0,13 (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).

Figure 2. Serum TNF-α level average
Note: groups: A (healthy control), B (diseased control, hyperuricaemic), C, D, E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).

| Table 1. One Way ANOVA Result of Serum Uric Acid Level |
|----------------------------------|-----------------|-----------------|
|                                  | N   | Mean±Std. Deviasi | p value |
| Group A                          | 5   | 4,18±0,55 mg/dL   |       |
| Group B                          | 5   | 15,02±0,71 mg/dL  |       |
| Group C                          | 5   | 8,46±0,12 mg/dL   |       |
| Group D                          | 5   | 6,55±0,14 mg/dL   |       |
| Group E                          | 5   | 6,45±0,13 mg/dL   |       |

Note: groups: A (healthy control), B (diseased control, hyperuricaemic), C, D, E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).
Tabel 2. Post hoc LSD Result of Serum Uric Acid Level

| Group   | Group   | P     |
|---------|---------|-------|
| Group A | Group B | .000  |
| Group C | Group D | .000  |
| Group D | Group E | .000  |
| Group B | Group A | .000  |
| Group C | Group D | .000  |
| Group D | Group E | .000  |
| Group C | Group A | .000  |
| Group B | Group E | .000  |
| Group D | Group E | .777  |

*significance at p<0,05

Note: groups: A (healthy control), B (diseased control, hyperuricaemic), C,D,E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).

Tabel 3. Kruskal-Wallis Result of Serum TNF-α Level

|      | N   | Median (min-max) | Mean±Std. Deviasi | P value |
|------|-----|-----------------|-------------------|---------|
| Group A | 5   | 30,35 (13,58-43,64) | 29,59±10,67 mg/dL |         |
| Group B | 5   | 146,35 (58,60-151,89) | 122,58±40,45 mg/dL |         |
| Group C | 5   | 115,83 (85,50-127,69) | 108,80±19,89 mg/dL | 0,001   |
| Group D | 5   | 62,21 (47,03-68,04)  | 60,54±8,40 mg/dL  |         |
| Group E | 5   | 33,43 (29,88-50,05)  | 36,12±8,07 mg/dL  |         |

Note: groups: A (healthy control), B (diseased control, hyperuricaemic), C,D,E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).
### Tabel 4 Mann-Whitney Result of Serum TNF-α Level

| Group   | P   |
|---------|-----|
| Group A | Group B | .009 |
|         | Group C | .009 |
|         | Group D | .009 |
|         | Group E | .175 |
| Group B | Group A | .009 |
|         | Group C | .347 |
|         | Group D | .076 |
|         | Group E | .009 |
| Group C | Group A | .009 |
|         | Group B | .347 |
|         | Group D | .009 |
|         | Group E | .009 |
| Group D | Group A | .009 |
|         | Group B | .076 |
|         | Group C | .009 |
|         | Group E | .016 |
| Group E | Group A | .175 |
|         | Group B | .009 |
|         | Group C | .009 |
|         | Group D | .016 |

*Significance at p<0.05

Note: groups: A (healthy control), B (diseased control, hyperuricaemic), C,D,E (hyperuricaemic, given ethanol extract 300,600,1200 mg/KgBW/day).

As shown in Figure 1, the significant decrease in group C and D was not followed by group E. The result of One Way ANOVA (Table 1), followed by Post hoc LSD (Table 2) found significant differences (p=0.000) among groups, except between group D and E (p=0.777). This could happen because dose-response of active substance is dependent on the dosage. The increase in dosage causes receptor saturation as seen as the decrease in the effect observed (Katzung, et al., 2012).

Nevertheless, as shown in Figure 2, the decrease in TNF-α level is directly proportional to the increase in ethanol extract dosage, i.e the higher the dosage of ethanol extract, the greater the decrease in TNF-α level. The same as the result of Kruskal-Wallis (Table 3), followed by Mann-Whitney test (Table 4) showed the significant difference (p=0.009) between group A and group B/C/D, except between group A and E (p=0.175).
3.1. OXIDATIVE STRESS AND INFLAMMATION IN HYPERURICEMIA

Uric acid is one of inflammatory agents. Increase in serum uric acid over 6.8 g/dL leads to the formation and deposition of uric acid crystal called monosodium urate monohydrate. The crystals then fagiosited by macrophage, thus triggers inflammation response. The stimulated macrophage releases pro-inflammatory cytokines such as interleukin 1β (IL-1β), interleukin-6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor-alpha (TNF-α) (Putra, 2009). Billiet et al. (2014) found that in male and female, serum uric acid level positively correlates with CRP, IL-6 dan TNF-α level.

TNF-α is a pleiotropic proinflammation cytokine for host defense against viral, bacterial and parasitic infection. It is released by macrophage, activated by lymphosite T, NK cell, antigen and Mast cell, thus it is frequently found in serum examination in inflammation and infection (Abbas et al., 2012). TNF-α altogether with IL-1 work after released by peripheral serum monosite and trigger the expression of endothelial E-selectin, intercellular adhesion molecule 1 (ICAM-1) dan vascular cell adhesion molecule 1 (VCAM-1). The group of immunoglobulin cause leukocyte attraction to the urate crystal deposition region (Ngestiningsih et al., 2012). The progression of inflammation process also produces reactive oxygen species (ROS) that builds up and causes oxidative stress (Zhou et al., 2018).

3.2. THE ROLE OF KELOR LEAVES

Kelor (Moringa oleifera) leaves contain various active substances that potential for reducing the level of serum uric acid through several mechanisms. Inhibited processes are the synthesis of uric acid, stress oxidative and inflammation.

3.2.1. Inhibition of Uric Acid Synthesis

Generally, flavonoid substanes (in Kelor leaves consist of quercetine and kaempherol), alcailoid, tannine and saponin decrease serum uric acid level through inhibition of xanthine oxidase (Nijveldt, et al., 2001; Anandagiri et al., 2014). According to Sutrisna et al., (2010), the 50% inhibition of xanthine oxidase causes 50% decrease in serum uric acid. One of common inhibition pathway is through n-hexane. When the n-hexane pathway is disrupted, xanthine oxidase cannot transform xanthine and hypoxanthine into uric acid. Therefore the level of serum uric acid is decreased (Yumita, et al., 2013). Moreover, quercetine inhibit xanthine oxidase by its role as competitive inhibitor that occupy active site of the enzyme because of its shape similarity with the substrate. The transformation from xanthine and hypoxanthine is not happened, therefore the level of serum uric acid is decreased (Kusmiyati, 2008; Song, et al., 2018)

3.2.2. Inhibition of Oxidative Stress Progression

Inhibition of oxidative stress progression is the result of characteristic molecular structure in flavonoid (quercetine and kaempherol) that maintain structural stability when scavanging free radicals and chelating transition metals. The free radicals scavenging protects cell from damage or inflammation process, thus prevent the increase in serum uric acid level (Hatano, et al., 1990; Tion, et al., 2013). Other uric substances in Kelor leaves with antioxidant potential are alcailoid, tannine, saponine, vitamin C and Vitamin E (Shankar, et al., 2006, Anandagiri et al., 2014)

3.2.3. Inhibition of Inflammation Reaction

Active substance of Kelor leaves with potential as antiinflammation is quercetine, saponine and vitamin C (Shankar, et al., 2006, Anandagiri et al., 2014). The inhibition of inflammation reaction conducted by transforming the expression and activity of various uric acid transporter. Verzola, et al. (2014) showed that in the regulation of uric acid transporter, URAT1 is an important transporter for uric acid to get into the renal proximal tubule cell and induce intracellular oxidative stress. Lipid peroxidation disrupts cell membrane and induces further inflammatory response by the release of pro-inflammatory cytokines such as IL-1, TNF-α, IL-6, IL-8 dan Cox-2. Damaged renal tubule cells cause decrease in uric acid excretion (Praditya et al., 2018).
Nieman (2007, in Choo et al., 2008) showed that the water extract of Kelor leaves give invitro antiinflammatory activity by decreasing TNF-α through the inhibition of Nuclear Factor Kappa B (NF-kB). Quercetine and vitamin C work by decreasing phosphorilation of Iκβ and reducing the activity of NF-κB, which later decreasing the endogenic production of TNF-α (Nair et al., 2006; Carcamo et al., 2002; Choo et al., 2008)

Statistical analysis in this research showed that serum uric acid and TNF-α level in groups given ethanol extract of Kelor leaves (group C,D,E) were significantly lower than group B (diseased control). This proves the effect of various dosage (300, 600, 1200 mg/KgBW) Kelor leaves ethanol extract administration in reducing serum uric acid and TNF-α level. The most significant decrease, nearly reach (insignificantly different from) the normal level was in group E, which was given 1200 mg/kgBW Kelor leaves ethanol extract. Therefore, the dosage was the most effective in reducing uric acid level.

Kelor leaves’ flavonoids (quercetine and kaempherol) are called nefroprotector due to their inhibitory ability to oxidative stress and inflammatory reaction process in renal tubule cells (Verzola, et al., 2014). Protection to renal function to excrete uric acid maintain the normal level of serum uric acid (Wang, et al., 2015). Moreover, Vitamin C in Kelor leaves increases the renal excretion of uric acid (Shankar, et al., 2006, Anandagiri et al., 2014)

4. CONCLUSION
The administration of Kelor (Moringa oleifera) leaves in various dosage were able to significantly decrease the serum uric acid and TNF-α in hypercaemic model white rats. The most significant decrease was found in group given dosage of 1200 mg/KgBW/day for 7 days.

5. SUGGESTION FOR FURTHER STUDY
This study can be replicated with method improvements, such as using more variable dosage and using linear regression in the data analysis. More frequent assessment of uric acid and TNF-α level should be done, i.e: after acclimatization to measure the pre-induction uric acid and TNF-α level, after induction period to make sure the quantity of pre-post test difference in uric acid and TNF-α level. It would be interesting to investigate the specific flavonoid with potential for reducing uric acid and TNF-α level. Finally, it is also needed to study the toxic dose of Kelor (Rattus oleifera) leaves.

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