Anti Hyperuricemic Activities of Some Medicinal Plants Extract from Indonesia to Decrease Serum Uric Acid White Male Mice in Balb-C Strain Induced by Potassium Antih Activity Of Salam (Syzygium Polyanthum Walp.) and Meniran (Phyllanthus niruri Linn.) Herbs Extracts in Oxonate-Induced Mice

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Abstract

The present study was carried out to evaluate in potassium oxonate (250 mg/kg b.wt) induced mice. The water extract of Salam (Syzygium polyanthum Walp) and Meniran (Phyllanthus niruri L.) administered orally to the hyperuricemic mice, produced significant decrease in the level of uric acid in blood serum. The highest of antihyperuricemia activity in uric acid levels were shown of Salam extract with doses 200 mg/kg b.w and the value of percentage reduction were 0.520 mg/dL, while the negative and positive control, was 3.100 and 0.200 mg/dL, respectively. Each extract had been analysed the standard test procedure based on the parameters of the general standardize of medicinal plant extracts suggested by The National Agency of Drug and Food Control of Indonesia. In conclusion, based on the results of standard tests common medicinal plant extracts, both materials under study had met the recommended requirements.

Keywords: antihyperuricemia activity, Syzygium polyanthum Walp, Phyllanthus niruri Linn., oxonate-induced mice

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Introduction

Uric acid is the end result of a chemical compound of purine metabolism in the body. Based on the previous reports that 90% of uric acid is the result of purine catabolism assisted by guanase and xanthine oxidase enzyme (Shamley, 2005). Uric acid is carried to the kidneys through the bloodstream to be expelled with the urine. Healthy kidneys will regulate uric acid levels in the blood to keep it under normal circumstances. However, excessive uric acid will not be accommodated and completely metabolized by the body, there will be increased levels of uric acid in the blood known as hyperuricemia. Hyperuricemia which may further develop into gout (Klippel, 2000).

Preliminary observations of the research team, proved that the water extract of Salam leaves at a dose of 1.25 g / kg body weight can reduce blood uric acid levels in white male mice effectively (Handadari, 2007) and Salam leaves infusion at a dose of 2.5 g / kg BB is able to reduce levels of uric acid with allopurinol equivalent dose of 10 mg / kg (Ariyanti, 2007). There is also research showing that the ethanol extract of the herb meniran (Phyllanthus niruri L.) can reduce levels of uric acid male Leghorn chicken made of hyperuricemia with high purine diet (Susanti, 2005). Another study mentions semi-polar fraction of methanol extract (Phyllanthus niruri L.) shows the effect of lowering uric acid levels in rats made hyperuricemic by inducing potassium oxonate (Anonymous, 2008).

In South America meniran is used to treat edema, dealing with the excess uric acid, kidney stones treatment, gallstones, flu, and fever (Anonymous, 2003). Many studies have found meniran as immunostimulator, which is needed by people with infectious diseases, as well as potentan antioxidant and antineoplastic. It is also efficacious as immunotherapy or therapeutic companion other cancer drugs (Novalina, 2003).

The use of traditional medicine in Indonesia is essentially a part of the Indonesian culture. The advantage of the use of the drug (herb) in principle is a traditional side-effects are relatively smaller than...
modern medicine. Although empirical traditional medicine can cure various diseases, but efficacy and ability has not been proven scientifically and clinically. Moreover, not much is known what chemical compounds responsible for the efficacy of traditional medicine (Wijayakusuma, 2002). Meniran that contain approximately the same chemistry with regards though there has been no research on the activity decrease uric acid levels in the blood of white mice. Therefore, it is necessary to investigate whether the extract Meniran also have the same activity in reducing blood uric acid levels of white mice and also further research to reveal the leaf extracts as Salam and Meniran standardized herbal medicine (SHM), especially in the treatment of gout, with follow the research methodology recommended by the BPOMRI. Therefore, in order to explore the competitive advantages of natural ingredients native of Indonesia, by increasing the potential and capacity of natural medicinal materials into standardized herbal medicine (SHM) phytopharmaceutical quality, with its potential to synergize with each other by Industry, Research Institutions (Universities), and full support by the Government, the research for the purpose of raising herbs into a better product is very important to be done.

Methods

Preclinical testing

Tools: injection volume of 3.0 mL syringe (Terumo), syringes for insulin injection 1.0 ml oral syringe size 15 gauge, flacone, analytical balance of mice with 2610 gram capacity scales (Lark, China) (Presica A-SCS), capillary tube (Assistent), mikrotube centrifuged (eppendorf), centrifuged (mini spin), vortex, micropipette sizes 5-40 mL and 200-1000 mL, blue tip, yellow tip, tools glass (Pyrex), FC StarDust * 15 (DyaSys) and disposable cuvette.

Materials: water extract of Salam leaves and Meniran herbs, Potassium oxonate p.a. (Aldrich Chemical Company), Allopurinol p.a. (Sigma), NaCl 0.9%, distilled water and uric acid reagent kit FS * TBHBA (DyaSys).

Animals: male white mice Balb-C strain with an average weight of 30-40 g and aged 2-3 months.

Plant material and extraction

Salam (Syzygium polyanthum Walp) and Meniran (Phyllanthus niruri L.)were collected fresh from the regionin Surakarta areas, Central of Java, Indonesia and dried in the shade and then powdered. The plant was identified by experts team from the pharmaceutical biology, Pharmacy Faculty, Muhammadiyah University of Surakarta. Extraction of samples of Salam and Meniran herbs wereboiled with water solution until the volume becomes half of its initial volume, then filtered and the filtrates evaporated at the rotary evaporator (RE) in order to obtain a thick extract. Then the condensed extract obtained was dried in a Vacuum Dryers and Vacuum Ovens to be a dried extract.

Hyperuricemia induction

High uric acid levels (hyperuricemia) is made by the intraperitoneal injection of potassium oxonate 250 mg / kg or 5 mg / 20 g BW in mice (Zhao et al., 2005).

Introduction preclinical testing

Preliminary tests was conducted for the purpose of obtaining data on the extract dose, blood sampling time, and the single active extracts in lowering uric acid levels.

Treatment for animal tests

Test animals were divided into treatment groups, which include: a negative control group/hyperuricemia (potassium oxonate dose of 250 mg/kg b.w.), positive control (allopurinol dose of 10 mg/kg b.w.), water extract of Salam leaves and Meniran with single dose (200 mg/kg b.w.). Administration of the test preparation performed one hour after the induction with potassium oxonate dose of 250 mg/ kg b.w.

Intake of blood serum

Blood sampling is done one hour after giving extract treatment or two hours after induction potassium oxonate. Blood was taken through the eyes of mice by piercing the ophthalmic vein branches located in the median saccus orbitales the capillary tube. Blood flowing through a capillary tube is collected in Eppendorf tubes, centrifuged after a blood clot to obtain serum.
Determination of uric acid levels

Uric acid levels are set based on the enzymatic reaction of uric acid reagent F5-TBHBA. Blood serum was mixed homogeneously with the uric acid reagent F5-TBHBA incubated for 10 min at 37OC. Then a solution of the sample, standard and blank absorbance is read using a spectrophotometer Start Dust FC*15 at a wavelength of 546 nm.

Standardization of Extract

Standardization of extract were done with following standard procedures based Materia Medika Indonesia and Parameter Extract. Plant Medicine General Standards recommended by Badan POM RI, including specific and non-specific parameters.

Results and Discussion

Preliminary test was conducted to determine how the model of hyperuricemia in male white mice, is to find an effective dose of potassium oxonate for increasing uric acid levels of normal conditions.

The results of the preliminary testing can be seen that the potassium dose of 250 mg / kg was able raised the levels of uric acid from normal level with an average of 1,433 mg / dl to 3,067 mg / kg. This is consistent with the theory that if the mice said hyperuricemia blood uric acid levels ranged from 1.7 to 3.0 mg / dl. Based on the results of the statistical test result that between normal controls and a group of very different potassium oxonate significant that the significance value of 0.205 (p> 0.05). Thus, from the results of the research method can be continued further research is adapted to the laboratory conditions for 7 days.

Male sex selection is based on the consideration that male mice do not have the hormone estrogen, jikalaupun there are only relatively few in number and condition of the male hormone more stable when compared with the female mice because female mice experiencing hormonal changes at certain times such as the the estrous cycle, pregnancy and lactation in which these conditions can affect the psychological condition of the test animals. In addition, the level of stress in female mice was higher than the male mice that may be annoying at the time of testing.

Potassium oxonate is used as an inductor hyperuricemia because potassium oxonate is a competitive inhibitor urikase for increase uric acid levels by preventing uric acid into allantoin changes. Where is water soluble allantoin and can be excreted through the urine, so the denial rikase enzyme by potassium oksonat the uric acid will accumulate and are not eliminated in the form of urine.

Allopurinol as a positive control used was the one of the common drug for gout. Allopurinol is the only uricostaticum are currently used therapeutically, which works to reduce the formation of uric acid. While working to increase the elimination of uric acid called uricosuric (Mutschler, 1991). Allopurinol is substrate xantine oxidase and eliminated through the kidneys primarily as oxyipurinol (often also referred to as the one that is aloxantine) (Schunack and Mayer, 1990). Allopurinol and oxyipurinol, inhibits xantine and uric acid, which in low doses went competitive inhibition mechanism and in high doses it does not work competitively. Allopurinol which has a plasma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. HU</th>
<th>Uric acid level (mg/dL)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control(without treatment)</td>
<td>1</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.7</td>
<td>1.433</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium oxonate dose 250 mg/kg b.w.</td>
<td>1</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>3.067</td>
<td>0.950</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1- Preliminary test data modelling hyperuricemia
half of the approximately 40 minutes, is hydrolysed by xantine oxidase into metabolites (Mutschler, 1991). Metabolite of allopurinol-1-ribonucleotide, which can be expressed in a small organ extracts, may be responsible for the additional inhibition of the de novo purine synthesis (Schunack and Mayer, 1990). Through inhibition of xantine oxidase hypoxantine and xantine then excreted in the urine so that more levels of uric acid in the blood and urine decreased (Mutschler, 1991).

Test preparations are used to lower uric acid levels in this study were water extracts of the leaves starfruit. Penyarian method used is solvent extraction with water, wherein the method is similar to the use of plant materials as traditional medicine (herbal medicine) is by boiling the material and taking the concentrates to be taken so that the traditional equality of treatment in the study and treatment identical. The difference in this study concentrates obtained after boiling evaporated in a vacuum dryer to dry the extract tebentuk. It is intended to maintain stability during storage as if it is stored in liquid form is very vulnerable to contamination and quickly overgrown by fungi.

Figure 1- Reaction Mechanism of Formation Kuinonimin (Schunack et al., 1990)

Determination of uric acid levels determined by enzymatic methods using reagents Uric acid TBHBA F5 * (2,4,6-tribromo-3-hydroxybenzoic acid) using a spectrophotometer StarDust FC 15. mechanism that occurs is uric acid is oxidized by the enzyme urikase with the help of H2O and O2 into allantoin, carbon dioxide and hydrogen peroxide. The hydrogen peroxide formed reacts with 4-aminoantipirin and TBHBA be kuinonimin pink where the reaction catalysed by the enzyme peroxidase (POD). The magnitude of the intensity of the colour produced by the kuinonimin equivalent to the levels of uric acid in the blood. The reaction mechanism can be seen in Figure 1.

Data uric acid levels in the serum of mice after induction with potassium oxonate extract the test preparation and administration of a single dose of 200 mg / kg is presented in Table 2.

From the table 2 can be created histograms between treatment groups with an average blood uric acid levels male white mice as follows:

Based on the results of the ANOVA testing can be known that the levels of uric acid of the negative control and the positive control extract group showed significantly different results with a significance value of 0.000. This suggests that allopurinol and Salam leaf extract, or combination Salam-Meniran and Meniran extract can reduce ofblood uric acid levels in white male mice Balb-C strain compared to the negative control (Potassium oxonate).

When compared with the positive control, decrease produced by extracts and extract Meniran greetings differ significantly from the values respectively 0.029 and 0.000 (P <0.05). from the results obtained it can be said that the extracts with a single dose can lower uric acid levels in white male mice Balb-C strain but decrease significantly different given each other with a significance value of 0.009.
Figure 2. Histogram of treatment groups Vs average blood uric acid levels (mg / dl).

Table 2- Data Serum Uric Acid Levels After Extract Treatment

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>BB (Gram)</th>
<th>Uric acid levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post Testing</td>
</tr>
<tr>
<td>1</td>
<td>Negative control(KCl 250 mg/kgBB)</td>
<td>39.3</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>30.5</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>Positive control(Allopurinol 10 mg/kgBW)</td>
<td>36</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>39</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>38.5</td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td>Salam leaves extract(200 mg/kgBB)</td>
<td>36</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>38.5</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>Meniran herbs extract(200 mg/kgBB)</td>
<td>36.5</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>36.5</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>36.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1</td>
<td>Extract ofSalam – Meniran(200 mg/kgBB)1:1</td>
<td>33.5</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>36</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>36.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

While combined, the result is compared with the positive control was also significantly different with $P_{value} < 0.001$ ($P < 0.05$), and not significantly different when compared with single Salam leaf extract. This means that the decreasing produced by a combination of Salam extracts and Meniran could not equivalent with allopurinol. Percentage decrease by a combination of extracts Salam-Meniran almost equal to the decrease by Salam as a single extract. Therefore it can be concluded that the Meniran extract less potent in reducing blood uric acid levels male white mice.

In the determination of standardized extracts, tests performed include analysis of the non-specific analysis of drying shrinkage, specific gravity, moisture content, ash content, the content of residual solvents, pesticide residues, heavy metal contamination, microbial contamination, and specific analysis that includes the identity of the extract, compounds dissolved in certain solvents, also test the chemical content of the extract. Results of standardized test bay leaf extract can be seen in the following table 3.

Results and Discussion ofStandardisation Extract
Table 3- The results of standardized extracts of non-specific parameters

<table>
<thead>
<tr>
<th>Extract</th>
<th>drying shrinkage</th>
<th>water content</th>
<th>Ash content</th>
<th>ash content</th>
<th>Acid insoluble</th>
<th>Heavy metal contamination (µg/kg)</th>
<th>microbial contamination (aflatoxin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salam</td>
<td>12.287</td>
<td>6.733</td>
<td>28.537</td>
<td>22.110</td>
<td></td>
<td>2.782 (Pb)</td>
<td>n.d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.d (Cd)</td>
<td></td>
</tr>
<tr>
<td>Meniran</td>
<td>10.206</td>
<td>0.550</td>
<td>28.860</td>
<td>19.636</td>
<td></td>
<td>5.194 (Pb)</td>
<td>0.0096 (Cd)</td>
</tr>
</tbody>
</table>

Remarks: n.d: no detection

Table 4- The results of standardized extract of specific parameters

<table>
<thead>
<tr>
<th>Extract</th>
<th>water-soluble extract</th>
<th>total phenolics</th>
<th>total flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salam</td>
<td>64.656</td>
<td>1.083</td>
<td>0.196</td>
</tr>
<tr>
<td>Meniran</td>
<td>56.195</td>
<td>7.154</td>
<td>0.786</td>
</tr>
</tbody>
</table>

**Compound Identity** Compound identity: Fluoretine (Salam) and phyllantine (Meniran) Structural formula:

1. Salam and Meniran leaves extract, single dose of 200 mg/kg b.w proved to potentially reduce levels of uric acid in the blood of male white mice Balb-C strain that been induced potassium oxonate where the percentage reduction in uric acid levels provided by Salam extracts approximately 79.35% and 61.94% for Meniran extracts whereas a decrease of 93.55% by allopurinol.

2. The combination of Salam and Meniran extracts with the same ratio (1: 1) gave results not significantly different with positive control is the percentage decrease of 70.97%.

3. Based on the results of standardize common testing medicinal plant extracts, both materials under study had met the recommended requirements.

4. Compound identity of the Salam leaves extract is fluoretine while Meniran extract is phyllantine. The difference is exactly what distinguishes active compound potentiation decreased levels of blood uric acid male white mice.

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**References**


