The Efficacy of Sidaguri (Sida rhombifolia) Extract in Hyperuricemia Induced Wistar Rats

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ARTICLE INFO

Keywords:
Sidaguri
Uric Acid
Xanthin Oxidase
Rat

ABSTRACT

Background: Uric acid is a metabolic product of exogenous (brought in with food) or endogenous purine bases. Sida rhombifolia is a traditional plant in Indonesia known as sidaguri, empirically in traditional medicine was proven as a cure of hyperuricemia. Aim of The Study: This study was carried in order to investigate the component active of the plant that have an ability to inhibit further uric acid formation in the hyperuricemia wistar rat. Methods: An experimental study with pre-post test-control group design. Male Wistar rats (weight, 180-230 g) were induced by potassium oxonate (280 mg/kg BW) and randomized into five groups (6 rats/group). Group 1: negative group. Group 2: positive group (allupurinol 50 mg/kg BW). Group 3,4 and 5: treatment with extract, each group 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW. Uric acid level were assayed by spectrophotometry and Xanthin oxidase were assayed by ELISA. Result: Treatment with extract (250, 500 and 1000 mg/kg b.w) and allupurinol 50 mg/kg b.w showed a significant decrease in uric acid level compare with the negative control rats with a reduction of 59.26%, 53.25%, 64.56% and 67.53%, respectively. Treatment either extract combination or allupurinol in hyperuricemia rats, significantly decreased Xanthin Oxidase Level (p<0.05) compared with negative control. Conclusion: The Sidaguri (Sida rhombifolia) extract showed the ability to decrease uric acid level by decrease the activity of xanthin oxidase.

1. Introduction

Background: Uric acid is a metabolic product of exogenous (brought in with food) or endogenous purine bases. This acid in most physiologic fluids is an end product of purine degradation. The serum urate level in a given patient is determined by the amount of purines synthesized and ingested, the amount of urate produced from purines, and the amount of uric acid excreted by the kidney (and, to a lesser degree, from the gastrointestinal tract). Gout is an inflammatory arthritis caused by the deposition of monosodium urate crystals in tissues. This condition typically occurs after years of sustained hyperuricemia. It is estimated to affect 5.1 million people in the United States according to the most recent National Health and Nutrition Examination Survey (NHANESIII). Gout affects approximately 2% of men older than 30 years and 2% of women older than 50 years, and is the most common form of inflammatory joint disease in men older than 40 years. Serum uric levels are, on average, 0.5 to 1.0 mg/dL higher in men than women, making male sex a risk factor for hyperuricemia and gout. Lower serum uric levels in women are associated with the presence of estrogen, which is thought to act as an antihyperuricemic.

In Indonesia, based on Health Survey in the year of 2005, there were around 10-20% men and postmenopausal women who have a higher levels of uric acids than normal person. It was proven that, celery seed is often used in treating this condition, as it possesses many anti-inflammatory compounds. Other helpful herbs include turmeric, boswellia, cayenne, colchicum and hyssop were also potent to treat hyperuricemia. Distortion of this metabolism leads to elevate level of uric acid and known as hyperuricemia.

Sida rhombifolia is a traditional plant in Indonesia known as sidaguri, empirically in traditional medicine was proven as a cure of hyperuricemia. This study was carried in order to investigate the component active of the plant that have an ability to inhibit further uric acid formation in the hyperuricemia wistar rat.
Methods

Plant Materials

Sidaguri were collected from Bantul Plantation in Bantul District, Yogyakarta, Indonesia, in the month of August-September, identified and authenticated by the Indonesia Science Institute (LIPI). The collected plant material was made free from foreign organic matter.

Chemicals Reagents

Allupurinol obtained from Dexa Medica PT in Palembang, South Sumatera Province. Spectrophotometer Bio Rad® and Uric acid Diasys reagent for measuring uric acid levels in hyperuricemia rats. ELISA reader Bio Rad® was used in ELISA analysis for Xanthin oxidase, using the RatXanthin Oxidase ELISA kit from Abcam. Potassium oxonate were produced by sigma-aldrich®. All the other chemical used for the experiments were of analytical grade.

Preparation of Extract

The collected Sidaguri (Sida rhombifolia) were washed, rinsed, blotted, sliced and ground. The extraction process was carried out at 90°C for 15 minutes in ratio of plant to water 1:10. The extract was filtered, concentrated, and evaporated in rotary evaporator.

Animals

Ten-weeks old Male Wistar rats (170-230 grams) purchased with animal health certificate from the veterinarian in the Department of Agriculture, Bandung, West Java. All of them maintained in an air conditioned room (25±1 °C), with a 12 h dark cycle and fed with standard diet and water ad libitum. Those were housed in the Animal House Faculty of Medicine, Sriwijaya University (Palembang, Indonesia) for 7 days before starting the experiment. The study approved by Health Research Review Committee of Mohammad Hoesin Central General Hospital and Faculty of Medicine Sriwijaya.

Experimental Procedure

Hyperuricemia rats were induced by potassium oxonate 280 mg/kg b.w./intra peritoneal using modification the method as describe by Zhu et al, and then uric acid level, collected from the orbital sinus puncture were checked. After two hours latter, rats with uric acid level over 7,0 mg/dL were used in study and given treatment.

ELISA Assay

This assay used to measure level of xanthin oxidase from tissue sample (liver tissues were placed on a separate micro tube, washed 3 times with PBS 1%, homogenized, add PBS, centrifuge for 20 minutes at the speed of 3000 rpm, supernatant collected) as described manufacturer’s instructions of ELISA kit.

Uratic acid level

Uric acid level estimated by kits as mentioned by the manufacturer’s instructions.

Phytochemical Analysis

Specimen from each fraction was examined to check the presence of bioactive phytochemicals. Thin layer chromatography (TLC) GF254 was used as stationary phase. Solvent n-hexan : ethylacetate : format (6:4:0,2) was used to examine flavonoid. Solvent Chloroform: metanol :water (64:50:1) was used to examine saponin. Solvent n-hexan : ethylacetate (93:7) was used to examine terpen. Solvent Toluene: ethylacetate:dietylamine (7:2:1) was used to examine alkoidal. Solvent : Etylacetate: format acid : toluene : water (6:1:5:3:0.5) was used to examine fenolik. After that, Citroborat was sprayed to TLC to examine fenoloid. Lieberman- Bourchart was sprayed to TLC to examine saponin. Anisaldehide-Sulphat acid was sprayed to TLC to examine terpen. Dragendorf was sprayed to TLC to examine alkoidal. FeCl3was sprayed to TLC to examine fenolnic.

Statistical analysis

Statistical analysis was performed using SPSS software package version 18. The values were analysed by paired t test, unpaired ttest, one-way analysis of variance (ANOVA) followed by bofferroni pos-hoc test. All results were expressed as mean ± SD. A value of p<0.05 was considered statistically significant.

Results

The uric acid level of hyperuricemia rats were significant increase (p<0.001) more than 7,0 mg/dL, and then treatment with extract (250, 500 and 1000 mg/kg bw) and allupurinol 50 mg/kg b.w showed a significant decrease in uric acid level compare with the negative control rats with a reduction of 59,26%, 53,25%, 64,56% and 67,53%, respectively. But, there was no differences between allupurinol group and treatment groups in uric acid level after treatment, p>0,05 (table 1).

Table 1. The Efficacy of Extract Sidaguri on Uric Acid Level in Hyperuricemia Rats

<table>
<thead>
<tr>
<th>Group per 9 each group</th>
<th>Uric Acid (mg/dL)</th>
<th>Uric Acid (mg/dL)</th>
</tr>
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<tbody>
<tr>
<td>Negative control</td>
<td>7,6± 1,33</td>
<td>7,5± 2,32</td>
</tr>
<tr>
<td>HR-ES-250 mg/kgbw</td>
<td>5,5± 1,95</td>
<td>5,5± 1,32</td>
</tr>
<tr>
<td>HR-ES-500 mg/kgbw</td>
<td>3,5± 1,37</td>
<td>3,5± 1,27</td>
</tr>
<tr>
<td>HR-ES-1000 mg/kgbw</td>
<td>7,3± 1,31</td>
<td>7,3± 1,21</td>
</tr>
<tr>
<td>Allupurinol 50 mg/kgbw</td>
<td>2,5± 1,67</td>
<td>2,5± 1,67</td>
</tr>
</tbody>
</table>

HR= hyperuricemia rats, ES= extract sidaguri; Paired t test, a p<0.05; Unpaired t test, b p<0.05 VS allupurinol; c p<0.05 VS negative control; Significance level was determined by one way ANOVA followed by bonferroni pos-hoc test.
Table 2 shows Xanthin Oxidase Level in hyperuricemia rats induced potassium oxonate as negative control and subjected to extract combination (250,500 and 1000 mg/kgBW) and allopurinol 50 mg/kgBW. Treatment either extract combination or allopurinol in hyperuricemia rats, significantly decreased Xanthin Oxidase Level (p<0.05) compared with negative control. But, there was no differences between allopurinol group and treatment groups (groups IV and V) in Xanthin Oxidase after treatment, p>0.05.

Table 2. The Efficacy of Extract Sidaguri on Xanthin Oxidase in Hyperuricemia Rats

<table>
<thead>
<tr>
<th>Group (n=6 each group)</th>
<th>Xanthin Oxidase (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>665.3±14.74</td>
</tr>
<tr>
<td>HR+ES 250 mg/kgBW</td>
<td>548.9±3.902</td>
</tr>
<tr>
<td>HR+ES 500mg/kgBW</td>
<td>604.6±5.004</td>
</tr>
<tr>
<td>HR+ES 1000mg/kgBW</td>
<td>522.0±27.25</td>
</tr>
<tr>
<td>Allopurinol 50 mg/kgBW</td>
<td>515.3±34.90</td>
</tr>
</tbody>
</table>

HR= hyperuricemia rats, ES= extract sidaguri; Paired t test, a p<0.05; Unpaired t test, b p<0.05 VS allpurinol; c p<0.05 VS negative control; Significance level was determined by one way ANOVA followed by bonferroni pos-hoc test.

Table 2 shown the main bioactive phytochemicals in extract sidaguri was flavonoid. There were also fenolic and saponin in extract sidaguri.

Table 3. Bioactive Phytochemicals in Extract Sidaguri

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Fr. Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+++</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Fenolic</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Alcaloid</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) : containing compound tested, (-) : not containing compound tested

Discussion

Gout and hyperuricemia are metabolic disorders associated with abnormal uric acid levels in the body, resulting in the deposition of urate crystals in the joints and kidneys that lead to inflammation, as well as gouty arthritis and uric acid nephrolithiasis. The enzyme, xanthine oxidase, which is present in significant levels only in the liver and intestine, oxidizes hypoxanthine and xanthine to uric acid in the purine catabolic pathway. Xanthin oxidase inhibitors could be used clinically to block the final step in uric acid synthesis, thereby reducing the production of uric acid. Allopurinol is the xanthin oxidase inhibitor currently in clinical use, but its use can result in a number of adverse side effects. A possible alternative to allopurinol treatment is phytochemicals, such as flavonoids.

Potassium oxonate, a selective, competitive uricase inhibitor, blocks the effect of hepatic uricase and produces hyperuricemia in rodents. Potassium oxonate-treated mice can serve as a useful animal model of hyperuricemia to evaluate drugs that affect serum uric acid levels and also to evaluate possible therapeutic agents in certain disorders associated with abnormal uric acid levels.

In the present study, Sidaguri extract (mainly flavonoid) have a significant effect on Xanthin Oxidase activities in hyperuricemic rat. Xanthin Oxidase is the key enzyme in the catabolism of purines and has a critical role in the endogenous production of uric acid. Several in-vitro studies confirmed the Xanthin Oxidase inhibitory activity of some flavonoids. These compounds are structurally similar to Xanthin Oxidase substrate and so can inhibit the enzyme activity. Therefore the hypouricemic property of sidaguri extract (flavonoid), observed in this study, could be explained at least in part by the inhibitory effects of them on Xanthin Oxidase activity. The extent of reduction in Xanthin Oxidase activity elicited by allopurinol was much higher than that observed with the sidaguri extract groups. Similar results have been reported by others. According to these studies, the involvement of other possible mechanisms such as enhanced uric acid clearance or actions on other purine metabolizing enzymes cannot be ruled out. Further study by the existence of some hypouricemic compounds including natural products that are devoid of Xanthin Oxidase inhibitory activity.

Conclusion

The Sidaguri (Sida rhombifolia) extract showed the ability to decrease uric acid level by decrease the activity of xanthin oxidase.

Acknowledgments

This study was supported by research foundation from Sriwijaya University, Palembang, Indonesia. We thank to Yeni Agustin for her assistance with ELISA assay and Suparman for his assistance with animal handling.

References
