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Anti-Hyperuricemic Effects of Water-Soluble Fraction of Leaf Extract of Sukun (*Artocarpus Altilis*) on Mice Fed Purine-Rich Foods

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Sukun (Artocarpus altilis), the breadfruit, has been known to contain phytochemicals that have inhibitory activity against xanthine oxidase, however little is known about their efficacy in lowering uric acid levels. This study aims to determine the effect of water-soluble fraction of breadfruit leaf extract on blood uric acid levels in purine-rich food-induced mice. Twenty-five male mice received beef liver extract once daily for 7 days and were grouped into five. The first group receives only solvent as the normal control. At day 8, mice of first group were sacrificed for taking their blood and measuring its uric acid levels. As for the group 2, 3, and 4, in the period of day 8 to day 14, consecutively received sukun leaves extract at the dosage of 58.5, 117 and 234 mg/kg BW. Group 5 received allopurinol at the dose of 13 mg/kg BW as the positive control. At the day 15 all mice were sacrificed and their blood uric acid levels were determined. The results showed that the water fraction of sukun leaf extract significantly decreased blood uric acid levels of test mice. At the dose of 234 mg/kg BW the anti-hyperuricemic properties of the plant leaf extract equals the standard drug, the allopurinol.

Keywords: Artocarpus Altilis; Breadfruit; Hyperuricemia; Sukun; Uric Acid.

Hyperuricemia is a condition characterized by high levels of uric acid in the blood mainly due to its metabolic inefficiency[1]. Hyperuricemia is associated with many human diseases such as stroke incidence, chronic kidney disease, cardiovascular disease and mainly gout, an inflammatory disease, with usually targets joint[2,3]. The most common strategy applied to treat hyperuricemia is by inhibiting xanthine oxidase (XO) which targeted to reduce the uric acid contents of plasma and urine[4, 5]. Until now, allopurinol remains the most recommended agents for urate-lowering therapy (ULT) due to its efficacy, availability, and low cost[6].

However, long-term use of allopurinol has been known to cause side effects. In patients with asymptomatic hyperuricemia accompanied by renal or cardiovascular diseases, for instance, long-term uses of allopurinol increased the risk of hypersensitivity reactions[7]. For this reason, the search for natural ingredients, especially

herbs, which have antihyperuricemia activity like allopurinol is still ongoing.

There are many plants already known to contain phytochemicals showing inhibitory activity against xanthine oxidase (XO), among others Crateva adansonii, Piper betle, Phyllanthus niruri, and Aster glehni. The active chemicals contained in these plants that are thought to have urate-lowering properties are alkaloids, phenolics, flavonoids, saponins, and terpenoids[8, 9, 10].

One of the plants that have been known to contain alkaloids, phenolics, flavonoids, saponins, and terpenoids, that are thought to have efficacy in lowering blood uric acid levels, is sukun (breadfruit), Indonesia vernacular name of Artocarpus altilis[11, 12, 13]. However, the efficacy of breadfruit extract against diseases associated with hyperuricemia is not well known. In this study, we have tested the effect of water fraction of sukun leaf extract on uric acid levels in mice fed purine-rich food.

MATERIALS AND METHODS

Plant Material and Extraction

Plant leaves sample of sukun (Artocarpus altilis (Parkinson) Forberg) were collected from Sub District of Jatimulyo, the District of Southern Lampung, Lampung province, Sumatra, Indonesia. Fresh sukun leaves are finely chopped and then sun-dried under a black cloth cover. The dried-fine leaves sample (350 g) then subjected to maceration in 70% ethanol three times. Macerate were then evaporated using a rotary evaporator at 60oC until a paste form extract were obtained.

To make water fraction of the extract, liquid-liquid fractionation were performed. Into 50 g of the paste was added 50 ml water and 100 ml of n-hexane in a separating funnel. Into the water fraction were then added 100 ml chloroform. The residual water fraction obtained were evaporated at 60oC and dried in an oven at 60oC. The dried leaf extracts of sukun were then diluted serially with water according to the desired dosage level.

Test Animals

Male albino Swiss mice, aged 2.5 – 3 months, weight range of 20-25 g, obtained from Lampung Veterinary Center, Indonesia, were used as experimental animals. Before the experiments, mice were allowed to acclimate for 1 week, fed with standard diet and water ad libitum and housed in a 12?h light/dark cycle at 25 ± 2°C. This study was conducted according to guidelines issued by Institutional Research Ethics Committee of the Faculty of Mathematics and Sciences, University of Lampung, Indonesia.

Purine-Rich Foods Preparation

To make test mice have high blood uric acid levels, the animals need to be induced with purine-rich diets. In this study, we used beef liver as the purine-rich foods. A fresh beef liver of 250 g was roasted, slice into small pieces and blended. The liver extract then suspended in 250 ml of water containing 1% CMC-Na.

Experimental Design and Treatments

After being fasted for 18 h, all mice were developed to hyperuricemia by administering 1 ml of beef liver extract using oral gavage, once daily for 7 days. By using a completely randomized design the hyperuricemic mice (n=25) were divided into five groups of five mice each. The first group was subjected to receive only solvent as the normal control. At day 8, mice of first group were sacrificed for taking their blood and measuring its uric acid levels. As for the group 2, 3, and 4, in the period of day 8 to day 14, consecutively received sukun leaves extract at the dosage of 58.5, 117 and 234 mg/kg BW. Whereas for the last group (Group 5), in the same period of treatment, was received allopurinol at the dosage of 13 mg/kg BW as the positive control. At the day 15 all mice were sacrificed and their blood uric acid levels were determined.

Uric Acid Assay

Blood samples of mice were collected using terminal cardiac puncture technique. Blood sample (1 ml) was centrifuged at a speed of 3500 rpm. The plasma is taken and then analyzed for uric acid content using direct uricase methods. Uric acid FS TBHBA used as the reagent and UV-Vis spectrophotometer used for reading the absorbance at the wavelength of 520 nm.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by least square difference was used for comparing mean values of uric acid levels of test mice. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Summary results of the measurement of blood uric acid levels in mice are presented descriptively in Table 1. The results of one-way variance analysis of the mean values of the data in Table 1 are presented in Table 2. Furthermore, the results of post hoc test (LSD) of the mean values of the blood uric acid levels of mice after treatment are shown in Figure 1.

Based on these data it can be summarized that the water-soluble fraction of sukun leaf extract effectively decreased blood uric acid levels in purine-rich foods-induced hyperuricemic mice. At the dose of 234 mg / kg BW the anti-hyperuricemic properties of the Artocarpus altilis leaf extract equals the standard drug of gout, the allopurinol.

Phytochemical screening efforts reveal the sukun plant (breadfruit) leaves contain water soluble as the highest parts (>21%) with a major component detected are flavonoids, steroids, saponins, terpenoids, tannins, phenolics and anthraquinone glycosides[14, 15, 16].

Various testing of antihyperuricemia effects of plant extracts with positive results showing the presence of above phytochemicals.

Table 1. Descriptive data showing effect of treatments on the blood uric acid levels of test mice

Treatments	N1	N2	N3	N4	N5	Mean	Std. Error
Solvent (negative control)	2.90	2.70	2.30	2.40	2.60	2.58	.10677
Allopurinol	0.90	1.20	0.90	0.70	0.90	0.90	.08367
Extract of 58.5 mg/kg	1.50	1.40	1.50	1.30	1.40	1.42	.03742
Extract of 117 mg/kg	1.20	1.40	1.30	1.30	1.20	1.28	.03742
Extract of 234 mg/kg	1.10	1.20	0.90	1.10	0.90	1.04	.06000

Table 2. One-way ANOVA against mean difference between treatment groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.886	4	2.221	89.573	.000
Within Groups	.496	20	.025		
Total	9.382	24			

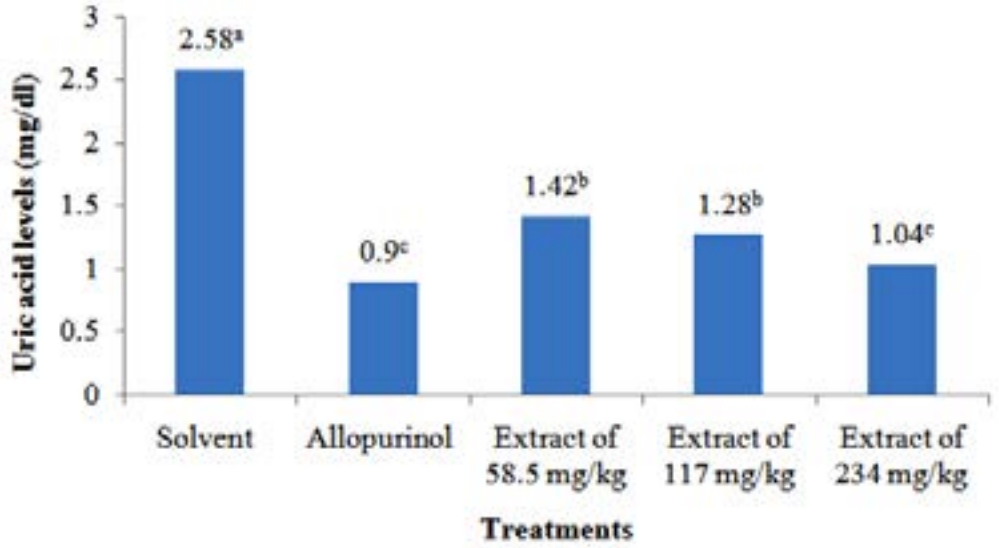

Fig. 1. Diagram showing mean values of blood uric acid levels of mice after treatment followed by LSD test significance notification. Values above bars followed by the same superscript are not differ at p<.05

Plant leaves extract of Crateva adansonii in an in vitro study, which has been shown to be effective in inhibiting the enzyme xanthine oxidase (XO), has been shown to contain alkaloids, phenolics, flavonoids, and saponins[17].

Flavonol, a flavonoids extracted from parsley (Petroselinum crispum) in hyperuricemic rats significantly reduced the serum uric acid levels in a time-dependent manner[18]. In an in vivo study using oxonate-induced hyperuricemic mice flavonoid constituents of plant extract Biota orientalis, quercetin and rutin, siginifcantly inhibit the xanthine dehydrogenase/xanthine oxidase activities[19].

In addition to flavonoids other bioactives similar to those contained in leaves extract of Artocarpus altilis such as alkaloids, phenolic compounds and tannins also showed inhibitory activity against XO and therefore these phytochemicals have the potential to be used as anti-gout[20]. Among such compounds, phenolics seem to have the most prominent role in reducing uric acid production and inhibiting the activity of XO enzymes[21]. Phenolic compouds extracted from Tabebuia roseoalba, the caffeic and chlorogenic acids, was known to reduce serum uric acid levels in hyperuricemic mice, allegedly because it can inhibit activity of liver xanthine oxidase[22].

CONCLUSION

Water-soluble fractions of leaves extract Artocarpus altilis are revealed to reduce serum uric acid levels in purine-rich food- induce hyperuricemic mice. It suggests that water extract of the breadfruit, the sukun, is potential to be use as anti-gout ingredients.

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