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Research Article

ANTIHYPERURICEMIC ACTIVITY OF THE KEPEL [*Stelechocarpus burahol* (Bl.) Hook. F. & Th.] LEAVES EXTRACT AND XANTHINE OXIDASE INHIBITORY STUDY

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ABSTRACT

Kepel or *Stelechocarpus burahol* (Bl.) Hook. F & Th. is one of the original plants from Indonesia and has been traditionally used for gout by the Indonesian communities. Kepel has synonyms name as Burahol and Turalak, and some studies showed that the extract and isolated flavonoid from Kepel leaves have antihyperuricemic and antioxidant activity. The aim of this research is to evaluate the antihyperuricemic potency of the ethanol and hexane extract of Kepel leaves as compared to allopurinol. The experiment was divided into two parts, *in-vivo* and *in-vitro* studies. For *in-vivo* study, sixty five rats were used in the study and the rats were divided into 13 groups (n=5 per group). The groups were assigned as follows and the test substances were given orally. Group 1: rats were given 10 ml/kg b.w. of 0.5% CMC-Na; Groups 2-5: rats were given 4.5 to 36 mg/kg b.w. of allopurinol; Groups 6-9: rats were given ethanol extract of Kepel leaves (50 to 400 mg/kg b.w.); and Groups 10-16: rats were given hexane extract of Kepel leaves (50 to 400 mg/kg b.w.). The blood of the test animals were taken through the eye vein on days 15, 17 and 19 post-treatment. Serum uric acid levels was determined by using TBHBA [*2,4,6-tribromo-3-hydroxy benzoic acid]* method and the percentage of the degradation was calculated. Inhibitory potency of the ethanol extract to the xanthine oxidase, was done by using procedure Fried & Fried (1993) and compared to allopurinol. Based on this study, it could be concluded that the ethanol and hexane leave extracts of Kepel possess significant antihyperuricemic potency in rat. The antihyperuricemic activity of the ethanol (60.86 to 78.33 %) and hexane extracts (78.23 to 88.52 %) are almost equivalent to that of the allopurinol (50.82 to 91.16%). While i*n-vitro* study showed that the ethanol extract has the inhibitory activity lower than allopurinol.

Keywords: I*n-vivo* study, I*n-vitro* study, *Stelechocarpus burahol* (Bl.) Hook. F & Th., Antihyperuricemic activity

INTRODUCTION

Hyperuricemia is the biochemical abnormalities in clinical practice signed by the serum uric acid in high level (greater than 7.0 mg/dL), occurs as a result of overproduction or underexcretion of uric acid or combination of both. It has been reported to affect almost 10% of the adults at least once in their lifetime[1,2]. Uric acid is the final product of the puryne metabolism, that is a catabolism of dinucleotide or ribonucleotide acid[3,4,5]. Prevalence of gout in Taiwan is 11.7% from 41.4% patients of hyperuricemia[6]. From the one study, the prevalence of gout and or hyperuricemia increased by about 2 cases per 1000 enrollees over 10 years (1990-99) in the overall population[7] and gout patients always increase in Cipto Mangunkusumo Hospital, Indonesia[8] .

Hyperuricemia is not only a known risk factor for gout, but has been strongly linked with other diseases such as hypertension and kidney failures[2,9]. From the prospective study, Choi and Curhan (2007)[10] concluded that men with gout had a higher risk of nonfatal myocardial infarction than men without gout, and among men without preexisting coronary heart disease (CHD), the increased mortality risk is primarily a result of an elevated risk of cardiovascular disease (CVD) death.

Currently, the pharmacotherapeutic agents for treatment of hyperuricemia and gout are very limited. Natural products, mainly of plant origin, have long been used in traditional medicine for the treatment of gout and hyperuricemia[11,12]. Kepel or *Stelechocarpus burahol* (Bl.) Hook.F & Th. comes from Java and Malaya, it has synonym names as Burahol or Turalak[13]. Kepel has been traditionally used by the Indonesian communities for gout, diuretic, cosmetic, renal inflammation and sometimes as abortifacient[13,14].

Some studies have also been conducted, the water extract of Kepel leaves decreased plasma uric acid levels in rats and chicken[15,16], soluble and not soluble fractions of petroleum eter from Kepel leaves have an antihyperuricemic activity on chicken[17] and isolated flavonoid from Kepel leaves has an antioxidant activity[18]. The aims of this research are to evaluate the antihyperuricemic potency of the ethanol and hexane extract of Kepel leaves as compared to allopurinol and to examine the inhibitory activity of the ethanol extract.

MATERIALS AND METHODS

Plant material

The main samples are the ethanol and hexane leave extracts of Kepel. The Kepel leaves [*Stelechocarpus burahol* (Bl.) Hook. F & Th.] were collected from Samigaluh resident, Yogyakarta, Indonesia, have been identified and it's extracts have been standardised by Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Indonesia.

Chemical and reagents

TBHBA (*2,4,6-tribromo-3-hidroksi benzoat)* [Diagnostic System Internasional (Diasys) Halzheim Germany], potassium oxonate [Sigma Chemical, St Louis, MO, USA], allopurinol [PT. Kimia Farma, Jakarta, Indonesia], urea and CMC-Na [E. Merck, Darmstadt, Germany], chicken liver and melinjo (*Gnetum gnemon*) [Yogyakarta, Indonesia], $Na_2HPO_4.7H_2O$, NaOH, Dimethyl sulphoxide (DMSO) and HCl [E. Merck, Darmstadt, Germany], phenasin methosulphate (PMS), nitro blue tetrazolium (NBT), xanthine, xanthine oxidase (Sigma Chemical.Co., St. Louis, MO, USA).

Animals

Male *Sprague-Dawley* rats of age 2.5-3.5 months, weighing initially about 150-200 g, were kept in the animal house of Faculty of Pharmacy, Gadjah Mada University, Indonesia. This project was cleared by Institutional Animal Ethical Committee. The animals were randomly divided into groups of five each and maintained under standard conditions. All animals were fed the standard rodent pellet (Comfeed, Indonesia) and water *ad libitum.*

Antihyperuricemic evaluation

Sixty five rats were used in the study and the rats were divided into 13 groups (n=5 per group). The groups were assigned as follows and the test substances were given orally. Group 1: rats were given 10 ml/kg body weight (b.w) of 0.5% CMC-Na, groups 2-5: rats were

given 4.5 to 36 mg/kg b.w. of allopurinol, groups 6-9: rats were given ethanol leave extract of Kepel (50 to 400 mg/kg b.w.) and groups 10-16: rats were given hexane leave extract of Kepel (50 to 400 mg/kg b.w).

The blood of the test animals were taken through the eye vein on day 0. Briefly, hyperuricemia was induced in the animals by oral administration the mixture of chicken liver juice 25 ml/kg b.w. 2 times per day, added by urea 1 mg/kg b.w., potassium oxonate 0.15 g/kg b.w. and melinjo 2 g/kg b.w. per day), for 18 consecutive days. The serum uric acid levels were measured on days 6 and 9, then the test substances were administered started by the day-10 until 18 orally. The test animal's blood were taken on days 15, 17, and 19 post treatment. The uric acid levels of serum was determined by using TBHBA [*2,4,6-tribromo-3-hydroxy benzoic acid]* method and the percentage of the degradation was calculated.

Uric acid assay by using TBHBA [*2,4,6-tribromo-3-hydroxy benzoic acid]* method

The blood of the test animals were taken through the eye vein (1-2 ml), were then transferred into non-heparinized centrifuged tubes and waited for 1 hour. The blood samples were centrifuged at 10000 rpm for 15 min, and the serum was next separated and the analysis was design by Table 1. A uric acid level was calculated by equation 1 and the percentage of the degradation by equation 2.

$$\text{Uric acid level (mg/dl)}: \frac{ASx - ASb}{ASs - ASb} x6mg/dl \qquad \textbf{(equation 1)}$$

(AS_x = Test absorbance, AS_b = Blank absorbance, AS_s = Standard absorbance)

Table 1: Design of uric acid assay by using TBHBA *[2,4,6-tribromo-3-hydroxy benzoic acid]* method

Composition (µl)	Tubes		
	Blank tubes	Standard tubes	Test tubes
Distilled water	20	-	-
Uric acid standard	-	20	
Serum	-	-	20
Reagent I	1000 (each tube)		
Incubated at room temperature of 27 °C for 5 minutes			
Reagent II	250 (each tube)		
Homogenized for 15 second and incubated at room temperature of 27 °C for 20 minutes			

$$\text{Percentage of the degradation uric acid} = \frac{AU_9 - AU_x}{AU_9 - AU_0} x100\% \qquad \textbf{(equation 2)}$$

(AU_9 = Uric acid level on day-9, AU_x = Uric acid level on days -15,17, dan 19, AU_0 = Normouric acid level)

Xanthine oxidase (XO) inhibitory activity study

Three concentrations of ethanol extract or allopurinol were used in this study as test substances: 5, 50 and 500 µg/ml. The XO activities with xanthine as the substrate was measured spectrophotometrically using the procedure of Fried and Fried (1993) [19]. From the absorbance data was calculated the volume activity xanthine oxidase (XOV) by using equation **3** and then the percent inhibition of xanthine oxidase to the test substances was counted by (B/A) x 100, where B is the changes in XOV (XOV_{blank} – XOV_{sample}) and A is the blank XOV (XOV_{blank}). Inhibitory activity of test substances was expressed as percent inhibition of xanthine oxidase.

$$XOV = \frac{0{,}417 \text{ X } F}{V} \text{ x } \Delta A/\text{menit (U/L)} \qquad \textbf{(equation 3)}$$

(XOV = volume activity xanthine oxidase (U/L), ΔA = changes in absorbance, F = dissolution factor, V = volume of sample)

Data analysis

The results were presented as the mean ± standard error of the mean (SEM) of the percentage of the degradation serum uric acid levels (%) in the groups of animals (n = 5). The statistical significance of difference ($P<0.05$) was evaluated by the analysis of variance (ANOVA) followed by the Scheffe *post hoc* test. For *in-vitro* study, the percent inhibition of the ethanol extract was compared to allopurinol by independent t-test. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Uric acid is formed through hipoxanthine oxidation pathways, xanthine oxidase and guanase will change guanine to xanthine. Then, xanthine will be oxidased by xanthine oxidase to uric acid. So that, xanthine oxidase is an essential target for pharmacology intervention in hyperuricemic or gout patients[20].

The test animals were increased the serum uric acid levels by giving the mixture of chicken liver juice 25 ml/kg b.w. 2 times per day, added by urea 1 mg/kg b.w., potassium oxonate 0.15 g/kg b.w. and melinjo 2 g/kg b.w. per day). The condition was to know, the test substances have the effect as antihyperuricemic agent or not. The mixture contains xanthine in high level, therefore will increase the serum uric acid levels. Increasing the serum uric acid levels has been checked in the preeliminary study (Figure 1). If the ethanol and hexane extracts have an activity to inhibit the xanthine oxidase, giving the both extracts will inhibit the activity, followed by inhibition of the uric acid forming and decreasing the serum uric acid levels. In this study, the doses, sampling time, and time of giving the mixture to increase serum uric acid levels were determined from the preeliminary study.

Antihyperuricemic activity of the Kepel extracts and allopurinol in the hyperuricemic rats are shown in Figure 2 and Table 2.

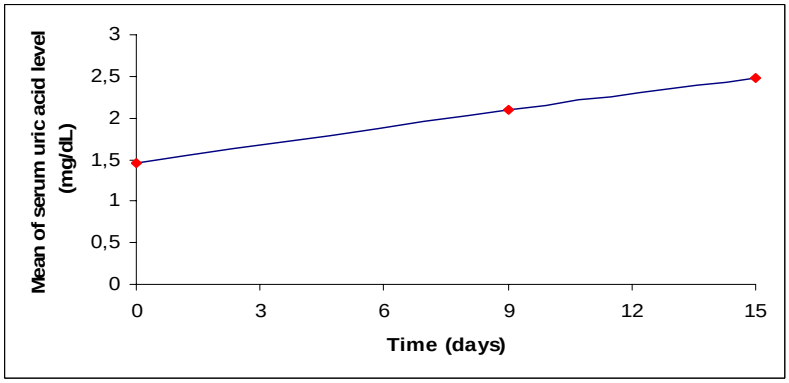

Fig. 1: Mean of serum uric acid level-day curve of male rats on days 0, 9 and 15 after treatment with a mixture of chicken liver 25 ml/kg b.w. 2 times per day, added by urea 1 mg/kg b.w., potassium-oxonate 0.15 g/kg b.w. and melinjo 2 g/kg b.w per day on preliminary study.

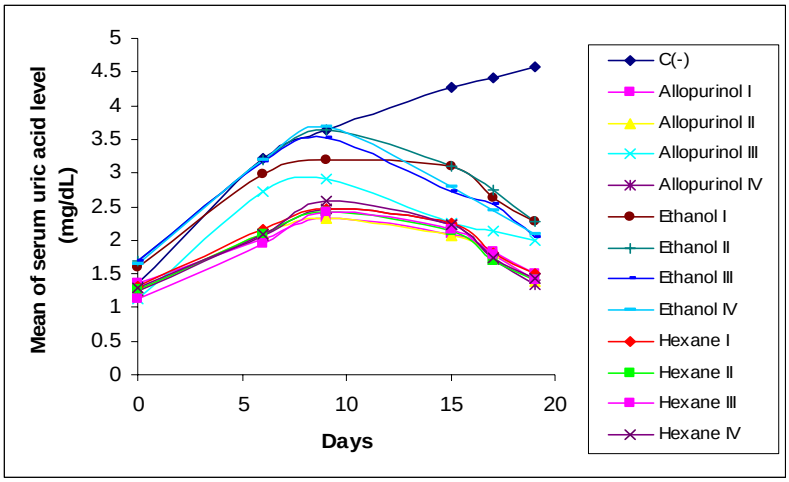

Fig. 2: Serum uric acid level-day curve of male rats on days 0, 6, 9, 15, 17 and 19 after treatment: negative control of 0.5% CMC-Na 10 ml/kg b.w. [C(-)], 4.5 to 36 mg/kg b.w. of allopurinol [Allopurinol I-IV], ethanol leave extract of Kepel (50 to 400 mg/kg b.w.)[Ethanol I-IV] and hexane leave extract of Kepel (50 to 400 mg/kg b.w.))[Hexane I-IV].

Figure 2 showed that the curve of the negative control did not show a decreased, it means the negative control did not have the antihyperuricemic effect, because of the percentage of the degradation serum uric acid levels was negative. The positive control (allopurinol) showed the percentage of the degradation serum uric acid levels more than 50% (50.82% to 91.16%) at the end of day post treatment (Figure 3) for all doses, even though the predicted effect was 20 to 90% and we hoped the effective dose-50% (ED-50) could be counted. Unfortunately, the ED-50 could not be calculated due to the effect did not dependent on the used doses.

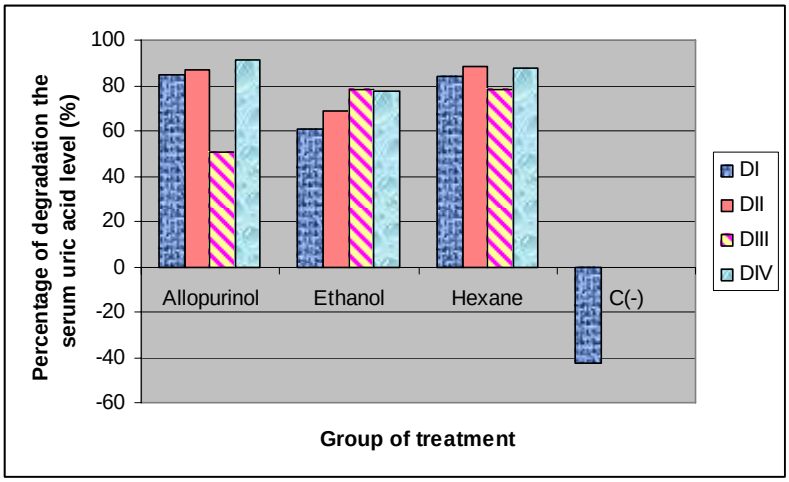

Fig. 3: Histogram of percentage the degradation serum uric acid levels in the groups of animals (n = 5) on day-19: negative control of 0.5% CMC-Na 10 ml/kg b.w., [C(-)], 4.5 to 36 mg/kg b.w. of allopurinol [DI-IV], ethanol leave extract of Kepel (50 to 400 mg/kg b.w.)[DI-IV] and hexane leave extract of Kepel (50 to 400 mg/kg b.w.))[DI-IV].

Percentage of degradation the serum uric acid levels increased along with the day of treatment (Table 2) for all doses. A significant reduction of 60.86 to 78.33% and 78.23 to 88.52% in serum uric acid of the hyperuricemic rats ($P<0.05$), as compared to that of negative control, was observed after nine days of oral treatment with 50, 100, 200 and 400 mg/kg b.w. of ethanol and hexane extracts, respectively. Similarly, treatment for nine days with clinically used drugs allopurinol (4.5, 9, 18 and 36 mg/kg b.w.) also showed a significant degradation of 50.82 to 91.16%, in serum uric acid ($P<0.05$). Inhibitory study just was done for ethanol extract even though both ethanol or hexane extracts have a similar effect with allopurinol. Kepel is one of the traditional herbal medicines from Indonesia that will be developed as phytopharmaceutical, therefore the quality and efficacy should be confirmed by scientific methods. Hexane extract has a higher risk for the human healthy, and the cost is more expensive for production scale. Because of that, the ethanol extract was choiced for future developing.

Xanthine oxidase (XO) inhibitors have been found in a wide variety of plants used in traditional herbal medicines for the treatment of gout and rheumatism[21,11] and we postulate that this may contain XO inhibitors. Allopurinol is a poten xanthine oxidase inhibitor and is prescribed for the treatment of hyperuricemia and gout widely[1]. It will be compared the inhibitory potency of ethanol extract to allopurinol as a positive control. Table 3 showed the inhibitory activity of the ethanol extract, it had a lower activity as compared to allopurinol in the same concentration. The inhibitory potency of allopurinol was 3-4 times higher than the ethanol extract ($P<0.05$). Flavonoids has been reported to be potent XO inhibitors[22,23]. From phytochemical screening, *S. burahol* contained flavonoid compounds and one of them has been identified as 3, 7, 3',4'-tetrahidroxy-5-methyl-flavone[18]. The total flavonoid compound in ethanolic fraction was 6.84. It was suggested that the inhibitory activity of ethanol extract was contributed by flavonoid compounds.

Table 2: Mean ± standard error of the mean (SEM) of the percentage of the degradation serum uric acid levels (%) in the groups of animals (n = 5) on days 15,17 and 19.

Groups		Percentage of the degradation serum uric acid levels (%), on days-		
		15	17	19
		Mean ± SEM	Mean ± SEM	Mean ± SEM
C (-)		-27.49 ± 4.48	-35.11 ± 4.89	-42.87 ± 6.03
Allo	I	15.91 ± 7.54	45.53 ± 9.18	84.37 ± 3.76*
	II	26.17 ± 2.07	46.58 ± 5.41	86.61 ± 2.52*
	III	36.22 ± 2.32	42.57 ± 1.82	50.82 ± 3.06*
	IV	19.93 ± 2.67	60.55 ± 7.34	91.16 ± 2.10*
Eth	I	2.70 ± 6.59	33.10 ± 6.04	60.86 ± 5.91*
	II	26.83 ± 7.47	44.34 ± 8.38	68.59 ± 9.21*
	III	38.74 ± 8.88	49.56 ± 6.04	78.33 ± 6.39*
	IV	39.17 ± 10.87	58.64 ± 7.78	77.79 ± 7.28*
Hex	I	18.06 ± 6.49	55.37 ± 6.25	83.91 ± 3.16*
	II	23.49 ± 3.38	62.61 ± 3.12	88.52 ± 1.54*
	III	19.23 ± 3.27	64.29 ± 7.75	78.23 ± 0.78*
	IV	27.67 ± 4.79	67.29 ± 6.12	87.90 ± 3.21*

Each value is the mean ± S.E.M. for 5 rats, * P< 0.001 compared with negative control. Data were analyzed by using One-way ANOVA followed by Scheffe test, C(-): negative control CMC-Na 0.5% at dose 10 ml/kg b.w., Allo I-IV: allopurinol at doses 4.5; 9; 18 and 36 mg/kg b.w., Eth I-IV: ethanol extract at doses 50; 100; 200 and 400 mg/kg b.w., Hex I-IV: hexane extract at doses 50; 100; 200 and 400 mg/kg b.w.

Table 3: XOV activity and percent inhibition of xanthine oxidase by ethanol extract of Kepel leave and allopurinol

Concentration (µg/ml)	XOV activity (U/L) (Mean ± SEM)	Percent inhibition (%) (Mean ± SEM)
Ethanol extract		
Blank (DMSO)	5.24 ± 0.06	---
5	4.71 ± 0.08	10.08 ± 1.56
50	4.43 ± 0.12	15.50 ± 2.33
500	4.31 ± 0.14	17.78 ± 2.69
Allopurinol		
Blank (DMSO)	3.11 ± 0.02	---
5	1.80 ± 0.04	42.08 ± 1.43*
50	1.53 ± 0.11	51.21 ± 3.33*
500	0.76 ± 0.03	75.65 ± 0.96*

Each value is the mean ± S.E.M. for 5 rats, * P<0.001 compared with ethanol extract at the same dose. Data were analyzed by using independent t-test.

CONCLUSION

Based on the statistical analysis the percentage of degradation serum uric acid levels was significant difference from negative control (P<0.05) and no significant difference from positive control (allopurinol) (P>0.05). The antihyperuricemic activity of the ethanol (60.86 to 78.33 %) and hexane extracts (78.23 to 88.52 %) of Kepel leaves are almost equivalent to that of the allopurinol (50.82 to 91.16%) at the end of day post treatment. The ethanol extract had a lower activity as XO inhibitor if compared to allopurinol in the same concentration, it was suggested that the inhibitory activity of ethanol extract was contributed by flavonoid compounds.

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