Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage : www.journal.uii.ac.id/index.php/JKKI

The effect of kersen's skin infusion (*Muntingia calabura* L.) on blood uric acid levels of the rats (*Rattus novergicus*)

Asril Burhan*[1], Usmar[2], Zulham[1], and Ana Andarwiyati[3],

[1]Akademi Kebangsaan Farmasi Makassar, Makassar, Indonesia
[2]Universitas Hasanuddin, Makassar, Indonesia
[3]Sekolah Tinggi Ilmu Farmasi Makassar, Makassar, Indonesia

Original Article

ABSTRACT

ARTICLE INFO

Keywords:
Ripe Kersen's,
Muntingia calabura L.,
uric acid,
Rats,
Rattus novergicus

***Corresponding author:**
asrilburhan@gmail.com

DOI : 10.20885/JKKI.Vol9.Iss3.art7

History:
Received: March 21, 2018
Accepted: September 10, 2018
Online: December 31, 2018

Background: Uric Acid or Gout is the results of purine metabolism, i.e. the enzymatic reshuffle of body cells from dinucleotide or ribonucleotide acids. High uric acid levels can be settled in the central joints and mesenchymal tissues such as the kidney.
Objective: To determine an effect of administering the Kersen's (*Muntingia calabura* L.) ripe skin infusion against uric acid levels in the rats which have been induced with caffeine.
Methods: Eighteen rats was divided into six groups, i.e. three as a control group (negative, positive and normal) and three as a treatment group (0,5%, 1%, and 2% doses). The measurements of blood uric acid levels were performed before induction, after induction and after treatment on 9th, 12nd, and 15th days.
Results: There was a reduction of the uric acid levels in the rats which administered by ripe Kersen's fruit skin infusion at 1% with 3,43 mg/dL and 2% with 3.32 mg/dL doses. However, this reduction is not equivalent to the effect of allopurinol control decreased at 2.9 mg/dL. Statistical analysis results with Complete Randomized Design (RAL) revealed that positive control group, infusion in 1% and 2% dose did not have a significant difference with normal controls, that means allopurinol and both doses test give an effect to return the uric acid to normal conditions.
Conclusion: The ripe Kersen's (*Muntingia Calabura*) fruit skin infusion provides a reduction effect on blood uric acid levels in the rats (*Rattus Novergicus*) that have been induced by caffeine. Concentration of infusion that reduced effect of blood uric acid levels in the rats are at 1% (1 g/ 200 gBW) and 2% (2 g/200 gBW). However, the reduction is not equivalent to the positive control of Allopurinol.

Latar Belakang: *Asam urat merupakan hasil akhir dari metabolisme purin, yaitu perombakan enzimatis sel-sel tubuh dari asam dinukleotida atau asam ribonukleotida. Kadar asam urat yang tinggi dapat mengendap pada persendian sentral dan jaringan mesenkim seperti ginjal.*
Tujuan Penelitian: *Tujuan penelitian ini adalah untuk mengetahui efek pemberian infusa kulit buah kersen (matang) terhadap kadar asam urat pada tikus putih (rattus novergicus) yang telah diinduksi kofein.*
Metode: *Hewan uji yang digunakan sebanyak 18 ekor yang dibagi menjadi 6 kelompok yaitu 3 kelompok kontrol (negatif, positif, dan normal) dan 3 kelompok perlakuan (dosis 0,5%, 1%, dan 2%). Pengukuran kadar asam urat darah dilakukan sebelum induksi, setelah induksi dan setelah perlakuan pada hari ke 9, 12, 15.*

Hasil: *Hasil menunjukkan bahwa terjadi penurunan kadar asam urat pada tikus putih yang diberikan infusa kulit buah kersen matang pada dosis 1% sebesar 3,43 mg/dl dan dosis 2 % sebesar 3,23 mg/dl, namun penurunan kadar asam urat tersebut belum setara dengan efek penurunan kontrol allopurinol yaitu sebesar 2,9 mg/dl. Hasil analisis statistik dengan Rancangan Acak Lengkap (RAL) menunjukkan kelompok kontrol positif, infusa dosis 1% dan infusa dosis 2% tidak berbeda nyata dengan kontrol normal, artinya allopurinol dan kedua dosis uji memberikan efek untuk mengembalikan asam urat dalam kondisi normal.*

Kesimpulan: *Infusa kulit buah Kersen (Mutingia calabura) memberikan efek penurunan asam urat pada tikus (Rattus norvegicus) yang telah diinduksikan kafein. Konsentrasi infusa yang menurunkan kadar asam urat adalah 1% (1 g/ 200 gBW) and 2% (2 g/200 gBW) namun, penurunannya tidak setara dengan kontrol positif allupurinol.*

INTRODUCTION

Uric acid or gout is the result of the body's metabolism, so that, its presence in the blood and urine is normal. But, when its production becomes too excessive, it can cause uric acid levels in the blood increases and settled in the central joints and mesenchymal tissues, such as the kidney.[1]

There is two type of pharmacotherapy drugs to reduce uric acid levels, include uricosuric and uricostatic drugs. Uricosuric drugs such as Probenecid, Sulfinpyrazone, and Benzbromarone increase the uric acid eliminations. Uricostatic drug reduces uric acid levels by inhibiting the uric acid forming enzyme (Xanthin Oxidase). Allopurinol is the only therapeutically used uricostatic drug.[2] The alternative way to overcome gout disease is by using a traditional medicine which uses several types of medicinal plants containing antioxidant compounds.[3]

Some indonesia's Origin Medicinal Plants (OAI) content a high Flavonoid compounds, safe to used and easily obtained to prevent the uric acid formation in the body. "Kersen" or "Talok" (*Muntingia calabura* L.) is one of an Indonesia's Origin Medicinal Plants containing Flavonoid.[4]

Based on the description above, need to be conducted a research to verify an effect of Kersen's ripe skin infusion to decrease uric acid levels in blood by using the rats (*Rattus novergicus*) as an animal test.

METHODS

Sampling Preparation

Ripe fruits of Kersen were collected from Perumahan Departemen Agama Makassar, Sulawesi Selatan. The initial stage in sampling preparation included washing, sorting, and stripping processto take only the skin of kersen fruit.

Infusion Preparation

Infusion was prepared in three variations of concentration i.e. 0,5% (500 mg/ 200 gBW), 1% (1 g/ 200 gBW) and 2% (2 g / 200 gBW). a 0,5% concentration was started by adding 100 mL Aquadest to 500 mg Kersen's skin in the infusion pot. After some aquadest was added twice as much as the Kersen's skin weight, the infusion were heated for fifteen minutes (start counting when the temperature reaches 90°c while stirring it at the same time). Then, the infusion was covered by flannel while it was hot. This similar procedure was applied to make 1% and 2% concentration by using 1 g and 2 g Kersen's ripe skin powder.

Preparation of allopurinol suspension

Manucturing of an allopurinol suspension was performed by adding an allopurinol powder into the developed 0,5% of Na-CMC solution. Crush it to homogeneous then add 15 ml Aquadest.[5]

Gout inducing

Increasing uric acid level was performed by inducing caffeine suspension orally at 27 mg dose for six days.

Animal test treatment

Eighteen rats were applied in this research as an animal test (subject). Firstly, subjects were adapted with a research environment for two weeks with a new environment. During the adaptation process, conducted an observation of

subject general conditions and weight weighing.

Furthermore, rats were divided into six groups; each group consisting of three mice, wherein the first treatment was administering Na-CMC solution as a normal control, caffeine 27 mg/200 gBW in 1% Na-CMC as negative control, and 5,4 mg/200 gBW Allopurinol suspension with 27 mg/200 gBW of caffeine suspension as a positive control, and the infusion control of the Kersen's skin is performed in three treatments i.e. 27 mg/200 gBW of caffeine suspension and doses infusion in 0,5%, 1% and 2%.

In Testing Phase, the effort to increase uric acid levels has been done by inducing the rats with 27 mg/200 g BB of caffeine. After that, mice's blood uric acid levels were controlled and measured at the zero (0) day to convince the induction caused hyperuricemia. Then, all the rats were saved in their cages and got the feeding and drinking as usual. On the first day after treatment, the rats were treated in their every group every day. Themeasurement of the mice's blood uric acid levels was done on the third (3rd), sixth (6th) and ninth (9th) days after treatments.

Blood uric acid measurement

Before the blood collection process, the rats were swabbed by ethanol 70%. Blood was taken by injuring or cutting the mice's tail with a small knife (cutter). Then, the blood was dripped on the uric acid strip.

Blood uric acid levels were measured by using a uric acid strip kit, an Easy Touch GCU (Glucose, Cholesterol, Uric Acid). GCU is designed for quantitative measurements of uric acid levels in the blood based on the determination of the current changes which caused by the uric acid reaction with the reagent on the strip electrode. When the blood reaches the target sample area on the strip, the blood automatically is drawn into the reaction zone of the strip. The results will be displayed on the screen after twenty (20) seconds.

Data Analysis

Data analysis that obtained in this research in the form of blood uric acid levels were analysed into a Variance Analysis (ANOVA) for a Completely Randomized Design (RAL) with unequal replications at the 5% significance level. If P< 0,01, 0,05, will continued the follow-up test based on the coefficient of diversity.

RESULTS

This research used the ripe Kersen's (*Muntingia calabura*) fruit skin infusion with three variants of concentration i.e. 0,5% (500 mg / 200 gBW), 1% (1 g / 200 gBW) and 2% (200 mg / 200 gBW). Infusion is an extraction process which is generally used to extract an active substance in plant preparations which protracted in the water and vegetal ingredients.

On zero-day (0) before induced by caffeine, the initial measurement of blood uric acid level was done to find out all ratgroups revealed normal levels. Then, on the 6th day, rats have primary hyperuricemia after caffeine induction for six days.. On seventh days, the treatment was given based on each group - performed similar treatment for nine days. After that, blood uric acid levels are measured on the 9th, 12th day until the final measurement on 15th.

Table 1. The results of average levels of Uric Acid During Experiments (mg/dL).

Period (day)	Negative control group	Control (5%)	Control (1%, 1 g / 200 gBW)	Control (2%,2 g / 200 gBW)	Positive control group
0	3,13	2,87	3,00	2,93	2,43
6	4,87	4,17	3,87	4,17	4,03
15	7,13	5,33	3,43	3,23	2,90

The table 1 shows the average of initial blood uric acid level on the zero-day, on sixth day (after induced by caffeine), and on fifteenth day 15 (after treatment).

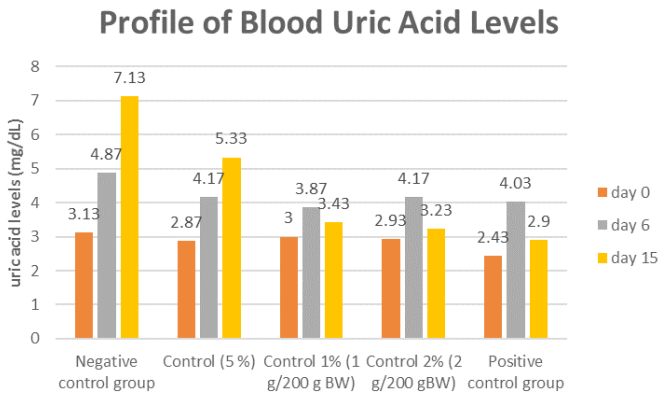

Figure.1 The graphic of the average value of uric acid Level

The graphic reveals that positive control group (Allopurinol) had a uric acid level higher than control dose of 1% group (1 g / 200 gBW) and control dose of 2% group (2 g/200 gBW). These results showed that the infusion of ripe kersen fruit at a dose of 2% (2 g / 200 gBW) and a dose of 1% (1 g / 200 gBW) could reduce uric acid levels in the blood but did not equivalent to the positive control group (Allopurinol). In addition, the group dose of 0,5% (500 mg / 200 gBW) was not affected by decreasing uric acid levels.

Determining the comparison of decreasing of uric acid levels between the groups, analysis of Variance (ANOVA) method was carried out for Completely Randomized Design (CRD) with unequal replications at the significance level α=0,05. The data of uric acid levels on the fifteenth day were taken from all treatment groups for analysis.

Table 2. The analysis of variance (ANOVA) of the effect of treatment in uric acid levels after induced for 15 days

Groups	Uric Acid Levels (mg/dL) Day Of 15*			Total	Mean
	1	2	3		
Normal group	2,0	2,2	2,4	6,6	2,20
Negative group	6,8	6,3	8,3	21,4	7,13
Control (0,5%)	5,2	5,5	5,3	16	5,33
Control (1%)	3,2	3,6	3,5	10,3	3,43
Control (2%)	3,5	2,4	3,8	9,7	3,23
Positive control group (Allopurinol)	2,6	2,8	3,3	8,7	2,90
Total	23,30	22,80	26,60	72,70	24,23
Mean	3,88	3,80	4,43	12,12	4,04
SD					1,84

*replicated measurements

Table 3. Analysis of variance (ANOVA) of the effect of treatment in uric acid levels after induced for 15 days

SK	Degree of Freedom	Number of quadrant	Quadrant count	F_{count}	F_{Table}	
					$Ft_{0.05}$	$Ft_{0.01}$
Treatment	5	50,84	10,168	32,08**	3,11	5.06
Galat	12	3,73	0,310	-	-	-
Total	17	54,57	-	-	-	-

** : Significantly

According to the data analysis above, treatment groups affected significantly based on value of $F_{count}>F_{table}$ at significant level 5% and 1%. The investigation continued to Least Significance Difference (LSD) as post hoc test to ensure the difference between groups.

The LSD Analysis showed that there was significant difference uric acid levels between all control dose groups of extract infusion and positive control groups with the negative control group so that allopurinol and all control dose groups of extract infusion had effect in coffein-induced rats.

DISCUSSION

The positive control group were not significantly different to normal control, meaning that allopurinol can restore gout in normal conditions. The control group dose of 2% (2 g / 200 gBW) 1% (1 g / 200 gBW) were not significantly different from the positive control at the test level of 5%. The control group dose of 2% (2 g / 200 gBB) and 1% (1 g / 200 gBB) had an effect on decreasing uric acid levels but was not comparable with the positive control (Allopurinol). Kersen fruit juice was able to reduce blood uric acid levels of male Wistar rats induced by potassium oxonate, but the decreasing levels were not equivalent to normal control (Allopurinol).[6]

The mechanism for reducing uric acid levels in this study is not yet known. The effect of lowering uric acid levels from ripe Kersen fruit skin infusions is thought to be due to the inhibition of enzyme activity of xanthine oxidase by flavonoids as has been done in previous studies or can be caused by an increase in excreting urine or a combination of both. Therefore, it is necessary to do further research on the mechanism of reducing uric acid levels by administering ripe Kersen fruit skin infusion. Uric acid levels increase depending on the causes. A high purine diet can trigger hyperuricemia in people who have congenital abnormalities in purine metabolism increasinguric acid production. Treatment of hyperuricemia is an attempt to minimise uric acid production or increase uric acid excretion by the kidneys. The drug allopurinol works to reduce the formation of uric acid by inhibiting xanthine oxidase, an enzyme that converts hypoxanthine to xanthin which then becomes gout.[2,7]

CONCLUSION

The ripe Kersen's (*Muntingia calabura*) fruit skin infusion provides a reduction effect on blood uric acid levels in the rats (*Rattus novergicus*) that have been induced by caffeine. Concentration of infusion that reduced effect of blood uric acid levels in the rats are at 1% (1 g/ 200 gBB) and 2% (2 g/200 gBW). However, the reduction is not equivalent to the positive control of Allopurinol.

CONFLICT OF INTEREST

Non Declared

Acknowledgement

We greatly appreciate the contribution of Akademi Farmasi (AKFAR) Kebangsaan as provider of our research facilities.

We are grateful to expert reviewers for the contribution the make.

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