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INFLUENCE OF GIVING BREADFRUIT *(Artocarpus altilis* (Park) Fosberg.) LEAVES EXTRACT TO DECREASE URIC ACID LEVELS IN WISTARS RATS HYPERURICEMIC

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ABSTRACT

Background: *Hyperuricemic is a condition where the uric acid levels in blood more than 7 mg/dl. In 2010, Hyperuricemic was included as one of non-communicable disease with incidence rate up to 30% and can cause the mortality. This study aims to examine the effective dose of breadfruit (Artocarpus altilis (Park) Fosberg.) leaves extract to decrease uric acid levels in Wistar rats Hyperuricemic.*
Methodology*: This study was an experimental with pre-posttest with control group design. 30 Wistar rats aged three months was divided into 2 control groups (K1 and K2) and 3 treatment groups (K3, K4, and K5). Control groups were given purine (K1= standard, K2=high) and aquadest. Differently, treatment groups were given high purine and breadfruit leaf extract (K3=0.25g /KgBW, K4=0.5g /KgBW, K5=0.75g/KgBW). All the treatments were given one time daily in the morning for 7 days. Data were analyzed by the Shapiro-Wilk test followed by the One-Way ANOVA test and then Post-hoc Tamhane test.*
Result: *The control groups revealed negative average reduction of uric acid in mg/dl (K1= -0.05, K2= -0.09) whereas the treatment groups revealed the positive average reduction of uric acid in mg/dl (K3=2.19, K4= 4.26, K5=5.87). The significant differences ($p<0.05$) of uric acid levels before and after treatment in each treatment groups had meaning that giving the Breadfruit leaf extract could reduce the uric acid level rats. Post-hoc Tamhane test result showed that the mean decrease of each group was significantly different. Particularly, K5 was the most effective treatment compared with others.*
Conclusion: *Breadfruit leaves extract with dose of 0.75g/KgBW was the most effective treatment to reduce the uric acid level in blood. Stakeholders in health field should promote the Breadfruit as the traditional medicine method to reduce the uric acid in blood.*

Keywords: *Breadfruit leaves, uric acid, Hyperuricemic, Wistar rats, flavonoids*

INTRODUCTION

Hyperuricemic is a condition where an increase in blood uric acid levels above the normal value due to over-production or under-excreation or combination of both factors. [1] The value of the normal uric acid in females from 2.4 – 6.0 mg/dL in males from 3.0-7.0 mg/dL. [2] Levels of uric acid in the blood that exceed normal limits cause a build up of uric acid in the joints and other organs.[1]

The prevalence of hyperuricemic in the world tend to have increased According to the WHO (World Health Organization) in 2010 hyperuricemic is included in one of the non-communicable diseases with the incidence rate reached 30% which can cause death in the world. Hyperuricemic in Indonesia ranks second after osteoarthritis, According to the survey of non-communicable diseases in Indonesia is estimated to decline from 31.7% in 2007 to 25.8% in 2015. [3] Based on 2013 (Indonesian Basic Health

Research) Riskesdas The prevalence of joint-related disease obtained from data west Java Province was 17.7%, and shows the results are not much different from the national figure. [4]

Therapy of hyperuricemic can be treated by non-pharmacological and pharmacological. Pharmacological therapy with drugs such as allopurinol consumption with doseage of 200-400 mg, but consumption is continuously can result in some side effects that include gastrointestinal disturbances, hypersensitivity reactions, and skin rashes, therefore other alternatives to treat hyperuricemia such as by using herbal medication. [5,6]

One type of herbal medication by using Breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg.). breadfruit are included in the genus Artocarpus, and the family Moraceae. This is found in tropical regions such as in Southeast Asia and the Pacific islands. [7] Almost all parts of this plant from the roots, flowers, leaves, fruits, stems, and sap can be used for the purpose of human life in the range of disturbance types of health care, Which leaves nutritious trusted by people can lowering uric acid levels in the body. [8] Based on previous research conducted by Nia (2014), breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg .) leaves extract with dose 0.5 g/KgBW can reduce uric acid level in wistar rats, because it is suspected in breadfruit leaf extract to contain flavonoids as antioxidants that can inhibit the acts of the xanthine oxidase enzyme, where the enzyme content is an enzyme that can trigger the process of forming uric acid in the body.[9] the aim of this research to find the effective dose of breadfruit leaf extract to decrease uric acid levels in Wistar rats hyperuricemic.

MATERIALS AND METHODS

Ethical clearance approval 31 / EC / FK / XI / 2018 was Obtained from the Ethical Committee of the Faculty of Medicine Swadaya Gunung Jati University. This research was conducted in the Laboratory of Food and Nutrition, Faculty of Medicine, Gadjah Mada University, Yogyakarta from January to March 2019.

Animal Protocol

Thirty rats divided (of age three months old) whose weight 200-250 grams were divided randomly into 5 groups: K1, K2, K3, K4, and K5. after 7 days of adaptations (all rats were fed routinely with standard alimentation and the cages were cleaned daily) and randomized grouping, the rats were treated differently according to their group during 7 days. All groups received standard feeding and 5 ml aquadest. Group K1 is Negative control were given standard feed and Aquadest, Group K2 is positive control were given high purine feed and aquadest, Group K3 is treatment group were given high purine feed and breadfruit leaf extract 0.25g /KgBW, Group K4 is treatment group were given high purine feed and breadfruit leaf extract 0.5g /KgBW and Group K5 is treatment group were given high purine feed and breadfruit leaf extract 0.75g/KgBW. All the treatments were given one time daily in the morning.

Extraction of breadfruit leaves

Fresh breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) dried in oven at 40°C and then minced. Extraction was done using maceration technique with 70% ethanol as solvent for 48 hours to extract active components. Filtrates were filtered and evaporated using rotary evaporator. The extracted compound in 70% ethanol were then diluted to corresponding concentrations.

Blood sampling and Plasma

Uric acid level measurement was done twice: before the treatment began (pretest) and again after all treatment for 14 days was done (posttest). The blood was taken from retroorbital (ophthalmic venous plexus) by using hematocrit micropipette. Blood is collected in Eppendorf tubes which have been given NaEDTA and sentrifuge at speed of 10,000 rpm 10 ° C for 2 minutes. The resulting plasma is taken slowly using a micropipette and ready to be tested next.

Measurement of uric acid levels

Measurements carried out on the level of uric acid plasma diagnostic reagent kit Dyasis with enzymatic spectrophotometric method. measurements performed before(pretest) and after(posttest) administration of the sample test.

Statistical analysis

The data were analysed statistically by computer program using IBM SPSS Statistics 20. A statistical test used to look normal and homogeneous distribution of the data using the Shapiro-Wilk test for the number of samples is less than 50, then continued using *One way ANOVA* test with advanced test *Post Hoc Tamhane*.

RESULTS

After obtaining the results of research data, then the data was analyzed to see which dose showed the most effective results of breadfruit leaf extract to decrease uric acid levels.

A. Uric acid levels

Uric acid Level Before (pretest) and After Treatment (posttest)

Table 1. Levels of uric acid before and after supplementation of breadfruit leaves extracts.

Group	N	Average uric acid levels (mg/dl)		Average reduction of uric acid (mg/dl)
		Pretest	Posttest	
K1	6	1.65	1.70	-0.05
K2	6	8.07	8.16	-0.09
K3	6	8.04	5.85	2.19
K4	6	8.03	3.77	4.26
K5	6	8.08	2.22	5.87

The Average reduction in negative control (K1) was -0.05mg/dl.Positive control (K2) was -0.09mg/dl, Breadfruit leaves extract group (K3) was 2.19mg/dl, Breadfruit leaves extract group (K4) was 4.26mg/dl and Breadfruit leaves extract group (K5) was 5.87mg/dl.

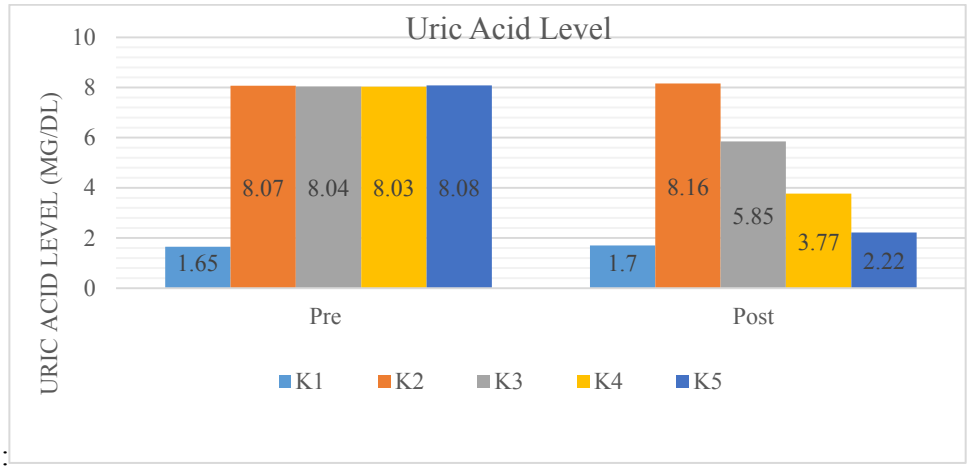

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Figure 1. Levels of uric acid before and after supplementation of the leaf's extracts. Reduction in uric acid levels is due to the antioxidative properties of and breadfruit leaves.

B. One Way Anova Test

Based on One Way Anova Test results showed that giving Breadfruit Leaf Extract provides significant difference in reduction of uric acid levels rats. depending mean decrease in uric acid levels of various treatment groups had p.value of 0.0000 ($p < 0.05$), which means that there are differences in average uric acid levels decrease significantly in each treatment.

Descriptives

Level of uric acid

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
K1	6	.0517	.03061	.01249	.0195	.0838	.02	.10
K2	6	.0917	.05707	.02330	.0318	.1516	.04	.19
K3	6	-2.1883	.41034	.16752	-2.6190	-1.7577	-2.73	-1.59
K4	6	-4.2617	.34982	.14281	-4.6288	-3.8945	-4.77	-3.80
K5	6	-5.8700	.16407	.06698	-6.0422	-5.6978	-6.12	-5.66
Total	30	-2.4353	2.40829	.43969	-3.3346	-1.5361	-6.12	.19

C. Tamhane Post Hoc Test

Table 2 Post-hoc Tamhane Inter-group difference Treatment of Uric Acid Levels Decrease

Group	Group	mean different	*p.value*
K-5	K-1	-5.92167 *	0.000
	K-2	-5.96167 *	0.000
	K-3	-3.68167 *	0.000
	K-4	-1.60833 *	0.000

After further post-hoc test K5 to K1-K4 in Table 2 shows K5 group with giving high purine feed and breadfruit leaf extract dose of 0.75g/KgBW Mean Difference value is negative. Comparison of uric acid all treatment groups had p value <0.000 or <0.05, which means that there are differences between the mean decrease in uric acid levels were significantly between groups. Thus K5 more effective lowering of uric acid in the K1, K2, K3 and K4.

DISCUSSION

Breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) is known have an efficacy for reducing of uric acid levels in the blood because it contains phenolic compounds. Phenolic compounds are compounds found in many plants, especially green plants, Phenolic have aromatic rings one or more hydroxyl (OH) group and the other side groups.[10] Phenolic compounds including flavonoids, is a potent antioxidant to prevent free radicals, many uses of flavonoids known to have biological and pharmacological activities such as antioxidative effect, anti-bacterial, anti-viral, and Antimutagenic. Additionally known potent as an inhibitor of several enzymes such as cyclooxygenase, lipooxigenase, phospoinositide3-kinase and xanthine oxidase.[11]

Flavonoids act as an xanthine oxidase inhibitor which may cause hyperuricaemia because this enzyme as a powerful catalyst in the change hypoxanthine into xanthine and then to uric acid. This reaction is also generated superoxide radicals that react with water to form acid peroxide. [12]

Xanthine oxidase enzyme inhibition by flavonoids occurred in 5.7 which dihydroxy groups on the A ring like on allopurinol as an inhibitor of xanthine oxidase allosteric by reducing the oxidation-reduction reactive group's xanthine oxidase. [13] The ability of flavonoids to inhibit xanthine oxidase activity lasted through competitive inhibition mechanism and the interaction with the enzyme in the side groups. [14].

Flavonoid substances contained in breadfruit leaf extract which has a mechanism to decrease uric acid levels quercetin flavonoid class of plant extracts as an inhibitor of the enzyme xanthine oxidase. [15] The phenolic compounds in breadfruit leaves is 55.416 ± 5.78 mg/g.[16,17] Flavonoid contained in breadfruit leaves is 45.763 QE / g.[18]

Lin & Shieh have isolated four flavonoid antioxidant compounds contained in extracts of the leaves of breadfruit siklokomunol, cyclocommunin, dihydroisocycloartommunin, and cyclommulberin. [19] While the isolation breadfruit leaf extract (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) with the amount of 0.62% flavonoid derivatives tend to have a more simple, 2-geranil-2',4',3,4-tetrahidroksidihidrokalkon and 8-geranil-4',5,7-trihydroxy-flavanones. [20]

Based on this study showed that the breadfruit leaves contain a compound that is potentially as anti-hyperurisemia flavonoid which the flavonoid was able to inhibit the enzyme xanthine oxidase in the process of the formation of hypoxanthine into xanthine and then to uric acid is more potent as anti-hyperurisemia. [21] it is supported by previous research conducted by Rahmatia in 2016 and Azmi in 2010 stated that 50% of xanthine oxidase inhibitors is equal to 50% decrease in the production of uric acid. [13,22]

CONCLUSION

The results showed breadfruit leaf extract (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) dose 0.25g/KgBW, 0.5g/KgBW and 0.75g/KgBW give a significant difference in reduction of uric acid levels Wistar rats. Differences in the mean number of breadfruit leaf extract showed significant p=0.000 has a significant difference ($p < 0.05$) this is seen in the average number of changes before and after a given treatment. because allegedly the content of the extract of leaves of breadfruit *(Artocarpus altilis* (Park) Fosberg.) are flavonoid compounds that play a role in the inhibition of uric acid.

CONFLICT OF INTEREST DECLARATION

The author states that there is no conflict of interest regarding the publication of this paper.

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76
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