DOI: 10.21776/ub.jpacr.2018.007.01.357 *J. Pure App. Chem. Res.*, **2018**, 7(1), 19-24
4 January 2018

Optimization Of Elevating Blood Uric Acid Levels With High Purine Diet

Fitria Rahmawati,[1] Putranty Widha Nugraheni,[1] Chanif Mahdi,[1] Arie Srihardyastutie[1] and Sasangka Prasetyawan[1]

[1] Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia

[*]Corresponding email : fitira.rahmawati@gmail.com

Received 9 August 2017; Revised 9 October 2017; Accepted 10 November 2017

ABSTRACT

An exploration of the use of medicinal plants to lower uric acid levels has been widely practiced. Stages of new drug development research are preclinical test using experimental animals, therefore the manufacture of animal model of hyperuricemia is necessary. This study was aimed to determine the optimal induction of uric acid to increase blood uric acid levels by administering high purine diets such as cow's liver, cow's spleen, *Gnetum gnemon*, *emping* and fried peanuts. Eighty male white rats were used individually and divided into 4 groups, they were: (I) cow's liver, (II) cow's liver and cow's spleen, (III) cow's liver, cow's spleen and boiled *Gnetum gnemon* beans, and (IV) cow's liver, cow's spleen, *emping* and fried peanuts. This study applied easy touch GCU to measure blood uric acid level. The result of statistical analysis of uric acid level with 5 times repetition using One Way ANOVA showed that there was a very significant difference between treatments (p <0.01). The results concluded that high purine diet in group I, II and III had not been able to increase uric acid levels significantly. High purine diet group IV was able to increase blood uric acid levels significantly to make the rats experiencing hyperuricemia with the level of 6.54 mg/dL on day 7 and 13.79 mg/dL on day 14.

Key word: optimization, increase, uric acid, hyperuricemia, high purine diet

INTRODUCTION

Currently, there has been an increase in hyperuricemia that cause by unhealthy lifestyles, one of those is consuming high purine foods [1,2]. Hyperuricemia is a condition of increased blood uric acid levels exceeds normal levels caused by abnormal purine metabolism, whether overproduction or underexcretion of uric acid. Normal value of uric acid in women is 1.5 to 6.0 mg/dL and in men is 2.5 to 7.0 mg/dL [3,4]. Uric acid is the end product of purine metabolism (adenine and guanine), which is produced in tissues due to the activity of xanthine oxidase enzymes that contained in the liver and small intestine. Uric acid can be secreted through the kidneys under normal circumstances [3]. Uric acid acts as an antioxidant if the levels are not excessive in the blood. However, if the levels are excessive, uric acid will act as a prooxidant that will lead to crystallization. Crystals are difficult to dissolve in all body fluids and will settle in joints and tissues, and cause inflammation and pain [5,6].

The stage of development of drug research is preclinical testing using experimental models with animals, generally rats. Frequently, making animals model of hyperuricemia rats uses potassium oxonate [7] because it will block uricase enzyme activity that converts uric

The journal homepage www.jpacr.ub.ac.id
p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

acid into allantoin. Allantoin makes uric acid dissolved in water and excreted through the kidneys [8], then with the cessation of enzyme activity, it caused the uric acid excretion lower and blood uric acid levels higher. However, potassium oxonate can not make permanent hyperuricemia condition in rats. This chemical only provides hyperuricemic conditions within 2 hours after intraperitoneal administration in rats and then decreases until the blood uric acid level finally reaches normal after 24 hours. This causes uric acid excretion in the kidney return to normal and uric acid levels in the blood decreases [9]. In addition, the toxicity and the high-cost potassium oxonate can be a problem in making the model of hyperuricemia rats. Therefore, creating a model of hyperuricemia rats needed another alternative that relatively non-toxic, easy to obtain and affordable.

The previous research in making the animal model of hyperuricemia was conducted by giving high purine diet of *Gnetum gnemon* with the dose of 4 g/kg of body weight mixed with chicken's liver in 50 mL/kg of weight dose for 9 days. Those doses resulted in average result of blood uric acid levels 4.74 ± 0.665 mg/dL [10], but results of uric acid levels were not significant. This study aims to combine some high purine foods from animals and plants to be induced to rats in order to increase blood uric acid levels significantly. Some high purine foods may be used as an inducing agent to make hyperuricemic rats, such as *Gnetum gnemon* beans. *Gnetum gnemon* beans have purine content of 222 mg/100 g [11,12]. Peanuts have purine levels of 190 mg/100 g and containing purine bases of adenine, hypoxanthine, xanthine, and guanine can increase blood uric acid levels [13]. High purine foods from animal products such as cow's liver (554 mg/100 g) and cow's spleen (444 mg/100 g) can also increase uric acid levels, especially when it consumed excessively every day [11,12]. Based on these descriptions, it is important to conduct research on optimization test to elevate blood uric acid levels with some high purine foods such as *Gnetum gnemon*, peanuts, cow's liver and cow's spleen and to prove scientifically and statistically.

EXPERIMENT

Chemicals and instrumentation

Materials used in this study were cow liver, cow's spleen, *Gnetum gnemon*, fried peanuts, and distilled water.

The instruments used in this research include analytical balance (Mattler Toledo), oral gavage tool, -20^{o}C freezer, refrigerator, 3 mL syringe (Terumo), and Easy Touch GCU set.

Procedure reaction

Designing high purine diet composition

There were four variations of high purine diet composition for each group. Group I was induced with 100 g liver, group II induced by 50 g liver and 50 g spleen, group III induced by 25 g liver, 50 g spleen, and 50 g boiled *Gnetum gnemon* bean, and group IV induced by 25 g liver, 25 g spleen, 25 g *emping* and 25 g fried peanut. Each composition was blended and dissolved with 120 mL of distilled water. The mixture was filtered.

Preparation of Experimental Animals

Rats used were 80 white male rats (*Rattus norvegicus*) weighing 175-225 grams. Rats were divided into 4 groups, each group consists of 4 rats and in each group was repeated 5 times. All animals were given high purine diet to induce hyperuricemia effect. Administration of high purine diet was carried out for 14 days each group orally and given twice a day as much as 3 mL/rat. All experimental animal procedure have been approved by the ethical

acceptance of UB's Research Commission No. KEP-689-UB. Rats were taken for blood uric acid levels measurement using Easy Touch GCU.

Treatment of Experimental Animals

All experimental animals were adapted for 7 days. On the next day, uric acid levels before high purine induction. On the following day, rats were fed with a high purine diet for 14 days with volume of 3 mL twice a day, at 8 A.M. and 2 P.M. to accelerate the increasing uric acid levels. Measurement of rats uric acid levels was conducted by withdrawing from the tip of the rats tail every week to check the increase in uric acid levels.

Data analysis

Data analysis were obtained using ANOVA analysis, furthermore, equipped with Tukey test to test the difference between treatments. All statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) software and significance test was checked at 99% confidence level (p <0.01).

RESULT AND DISCUSSION

Administration of high purine diet aims to provide the effect of hyperuricemia in rats, characterized by blood uric acid levels increasing in rats. Consuming food with high purine content can lead to the excessive synthesis of uric acid and lead to an imbalance metabolic processes. As a result so uric acid synthesis is higher than its excretion [14]. In this study, high purine diet given to rats derived from animal and plants. The purine content of cow's liver, cow's spleen, *Gnetum gnemon*, and peanuts are 554 mg/100 g, 444 mg/100 g, 222 mg/100 g and 190 mg/100 g respectively [11,12].

The result of statistical analysis of mean with 5 times repetition using One Way ANOVA showed that there was a very significant difference between treatments (p <0.01) in induction of high purine diet for each group of rats. The mean of rats uric acid levels on day 0 for each treatment group were similar, at 2.3 mg/dL. Based on previous studies, the baseline blood-uric acid levels were 2.15 mg/dL and 2.77 mg/dL. This suggests that blood uric acid of rats in this study were categorized as normal [15]. These number in all group of rats did not have significant difference. Therefore, average result of uric acid levels on day 0 can be used as a reference to determine the increasing of uric acid levels.

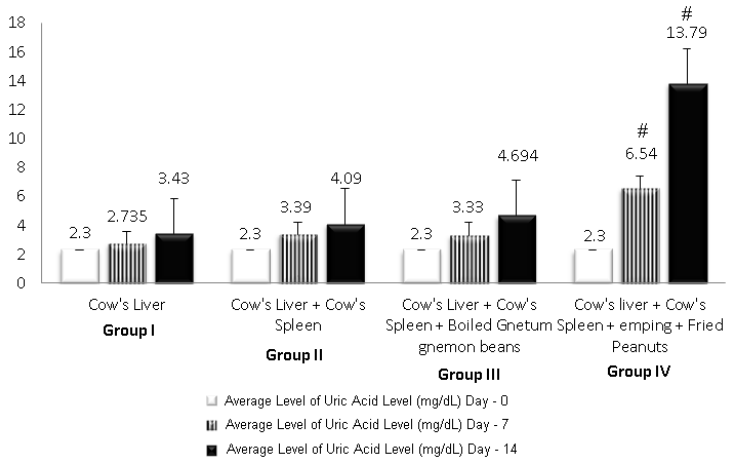

Figure 1. Average of blood uric acid level with 5 times of repetition. There was a very significant difference between group I and group IV at week 7 and week 14 with $^{\#}p$ <0.01.

Uric acid levels of rat in group I, had the lowest levels of uric acid among other groups with a value 2.74 mg/dL on day 7 and 3.43 mg/dL on day 14. There was slight increase in uric acid levels in group II with level on day 7 of 3.39 mg/dL and on day 14 was 4.09 mg/dL. The results did not differ greatly from uric acid levels in group III that 3.33 mg/dL on day 7 and 4.69 mg/dL on day 14. The highest levels of uric acid were group IV with value of 6.54 mg/dL on day 7. Moreover, there was a very significant increase on day 14, with uric acid levels of 13.79 mg/dL. Uric acid levels in rat groups I, II and III showed that rats had not been experienced hyperuricemia, while rats group IV already had. Rats with hyperuricemia are characterized by an elevated concentration of uric acid in the blood until above normal. This suggests that the provision of high purine diet in group IV was able to increase blood uric acid levels significantly compared with other groups.

Tabel 1. The highest levels of blood uric acid were rats group IV with 5 times repetition

No	1			2		3		4		5	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
1	2.3	6.5	19.7	5.8	19.7	6.4	11	6.1	10.2	6	20
2	2.3	6.5	18.3	6.8	13	6.9	9.8	5.9	9	6.5	9.8
3	2.3	7.9	20	7	10.7	5.8	9.2	6	20	6.3	13.5
4	2.3	8.1	20	6.9	11.6	6.7	9	6.6	9.8	6.1	11.6
Average	**2.3**	**7.2**	**19.5**	**6.6**	**13.7**	**6.45**	**9.7**	**6.1**	**12.2**	**6.2**	**13.7**

In this study, giving high purine diet in making the animal model of hyperuricemia with some dose variations based on preliminary tests that have been done. The previous research in making the animal model of hyperuricemia was conducted by giving high purine diet cow's liver with the dose of 100 grams [11]. Those doses resulted in average result of blood uric acid levels 4 mg/dL, and other research giving high purine diet of *Gnetum gnemon* with the dose of 4 g/kg of body weight mixed with chicken's liver in 50 mL/kg of weight dose for 9 days. The result of blood uric acid levels 4.74 ± 0.665 mg/dL [10], but results of uric acid levels were not significant. Hence, in this study showed that blend some high purine foods from animals and plants to be induced to rats able to increase uric acid levels significantly compared with previous studies.

Administration of High Purine Diet With Cow's Liver (Group I)

The maximum dose of purine that humans can consume is 600 - 1000 mg/day [11], but if it converted to rats that had average weigh of 200 grams, then the maximum purine intake in rats is 3.3 mg/day. Purine content that were given in group I was 554 mg/100 g and purine dose given to rats in one day was 27.7 mg. This means that purine intake in this study was above the normal intake. Theorically, administering a high purine diet with cows liver for 14 days consecutively can increase uric acid levels of rats. However, in this work, result showed either way around. This can be due to the metabolism system of rats still running smoothly, thus, the process of uric acid excretion still normal. Purine metabolism process that occurs continously by giving high purine diet on the rats will cause increasing levels of purine. Purines will be converted into hypoxanthine, xanthine and uric acid by the activity of xanthine oxidase enzymes [3]. The renal function in this group is still normal. Therefore, high level of uric acid, the purine will convert them into uric acid and the excessive will be secreted by the kidneys and removed in the form of urine, as a result uric acid levels in the blood decreased or back to normal.

Administration of High Purine Diet With Cow's Liver and Cow's Spleen (Group II)

Purine contained in diet given in group II were 499 mg/100 gram and purine dose that given to rats in one day was 24,95 mg. There was a slight increase in uric acid levels in group II compared with group I. This is because a high purine diet was given for 14 days orally and twice a day make some imbalance between uric acid synthesis and secretion in the body [14]. Excessive uric acid levels lead to an imbalance in metabolic processes, causing much more uric acid synthesis than its excretion and cause increased levels of uric acid in the blood. The administration of high purine diet in group I and II that derived from animal and has not been able to increase uric acid levels significantly.

Administration of High Purine Diet With Cows Liver, Cow Spleen and *Gnetum gnemon* Rats (Group III)

The feeding of high purine diet in group III contained 416 mg/dL purine and purine dose in rats in one day was 20.8 mg. The diet that had been given in group III were the mixture of purine that were derived from animals and plants. There are *Gnetum gnemon* beans that can increase uric acid levels if consumed continuously. The average result of the uric acid level of rat group III did not have a significant differences with group IV. It is because the bean of *Gnetum gnemon* has been boiled, causing the decrease of purine. Boiling and heating process can dissolve purine, as is known that purine is a compound that is easily soluble in water [16]. Beans that are softened and watery due to the boiling process can lead to the releasing of purine compounds during bean swelling due to beans outer shells inability hold much heat and water, and makes purines out dissolved in water. This process makes boiled *Gnetum gnemon* bean contained in high purine diet can not increase uric acid levels significantly.

Administration of High Purine Diet With Cows Liver, Cow Spleen, Emping, Fried Peanuts Rats (Group IV)

Purine contained in high purine diet given in rats group IV is 352.5 mg/dL and purine dose in rats in one day that is 17.625 mg. The purine content given to the group IV was the lowest purine content compared to the other groups. But rats group IV had the highest levels of uric acid, it is caused by the diet given contained fried peanuts and *emping* which is one form of processed *Gnetum gnemon* bean. Purines contained in emping will not decrease as shown in group III that including boiled *Gnetum gnemon* beans, due to the differences in food processing. The beans of *Gnetum gnemon* heated directly on the roster (frying using sand), Hence, there was no boiling process in processing of *Gnetum gnemon* beans to be emping; thus, there were no dissolution occurs that cause purines dissolve in boiling water and reduce the purine levels. Similar thing occur in peanuts diet preparation there were no interaction of peanut with water that could result in purine degradation from the cell, since peanuts were fried. Peanuts also contain purine bases of adenine, hypoxanthine, xanthine and guanine which can increase blood uric acid levels. Purine base content in peanuts that influence increase of blood uric acid levels mostly are adenine and hypoxanthine. Adenine and hypoxanthine altogether increase blood uric acid levels, but hypoxanthine has the rapid effect to increase blood uric acid levels compared to adenine. The difference in velocity occurs because of the differences of each purine base metabolism. Adenine undergo phosphorylation process before into uric acid, whereas xanthin does not. The purine guanine and xanthine bases show no effect on increasing uric acid levels [14].

CONCLUSION

Administration of a high purine diet in rats groups I had the lowest levels of uric acid among other groups with a value 2.74 mg/dL on day 7 and 3.43 mg/dL on day 14. The results of uric acid levels between group II and III did not differ greatly. Group II with uric acid level on day 7 of 3.39 mg/dL and on day 14 was 4.09 mg/dL, and group III that 3.33 mg/dL on day 7 and 4.69 mg/dL on day 14. Uric acid levels of group I, II and III for 14 days was not able to increase uric acid levels significantly. The highest uric acid levels have been shown in rats group IV that received high purine diet of cow's liver, cow's spleen, *emping* and fried peanuts. The uric acid levels reached 6.54 mg/dL on day 7 and had very significant increase on day 14 at 13.79 mg/dL, indicating that rats have experienced hyperuricemia. Because of its significance, the composition of high-purine diet can be used as an alternative optimization to create an animal model of hyperuricemia.

REFERENCES

[1] Torralba, K.D, De Jesus, E., and Rachabattula, S., **2012**, *Int. J. Rheum. Dis.*, 15(6), 499–506.
[2] Yamanaka, H., **2012**, *Japan Med. Assoc. J.*, 55, 4, 324–329.
[3] Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C., and Mollace, V., **2016**, *Int. J. Cardiol.*, 213, 8–14.
[4] Gliozzi, M., Malara, N., Muscoli, S., and Mollace, V., **2016**, *Int. J. Cardiol.*, 213, 23–27.
[5] Choi, H. K, Mount, D. B, and Reginato, A. M, **2005**, *Ann. Intern. Med.*, 143(7), 499–516.
[6] Baker, J. F, and Shumacher, H. R, **2010**, *Int. J. Clin. Pract.*, 53(3), 557–558.
[7] Al-azzawie, H. F, and Abd, S. A, **2015**, *Int. J. Adv. Res.*, 2(6), 55–61.
[8] Werner, A. K, and Witte, C. P, **2011**, *Trends Plant Sci.*, 16(7), 381–387.
[9] Zhao, X., Zhu, J. X, Mo, S. F, Pan, Y., and Kong, L. D, **2006**, *J. Ethnopharmacol.*, 103, 357–365.
[10] Manuaba, P., **2012**, *Adv. Pure Appl. Chem.*, 2(1), 86–90, 2012.
[11] NHS, N., "*Dietary Advice for People with Gout*", **2017**, Dep. Nutr. Diet. Norfolk Norwich Univ. Hosp. NHS Found. Trust James Paget Univ. Hosp. NHS Found. Trust, no. November, pp. 1–2.
[12] Kaneko, K., Aoyagi, Y., Fukuuchi, T., Inazawa, K., and Yamaoka, N., **2014**, *Biol Pharm Bull.,* 37, 709–721.
[13] Settaluri, V. S, Kandala, C. V., Puppala, N., and Sundaram, J., **2012**, *Food Nutr. Sci.*, **2012**, 1644–1650.
[14] Gowda, S., Desai, P. B, Kulkarni, S. S, Hull, V. V, Math, A. K, and Vernekar, S. N, **2010**, *N. Am. J. Med. Sci.*, 2(4), 170–3.
[15] With, T., and Versus, F., **2014**, *Prof. Med. J.*, 59.
[16] Johns, C. O, **1996,** *JBC,* 1, 273–276.