EFFECT OF ETHANOL EXTRACT OF SNAKE FRUIT'S PEEL ON URIC ACID SERUM AND CRP

**Fajar Wahyu Pribadi[*], Setiawati[*], Viva Ratih Bening Ati[*], Catharina Widiartini[**], Hilmi Puguh Panuntun[***]**

[*]Departement of Pharmacology and Therapy, Faculty of Medicine, Jenderal Soedirman University

[**]Departement of Anatomy, Faculty of Medicine, Jenderal Soedirman University

[***]Faculty of Medicine, Jenderal Soedirman University

## Abstract

Unhealthy diet that consisted of high purine, derived from protein, had caused an increasing hyperuricemia-related disease in community. Anti-oxidant activity of snake fruit's peel had anti-hyperuricemia effect due to the flavonoid content. This study aimed to investigate the effect of ethanol extract of snake fruit's peel in reducing uric acid serum & *C-Reactive Protein* (CRP) level and to find out the most effective dosage of the activity. The rats were grouped into 6 groups (negative control, positive control, Allopurinol, dosage I (105 mg/kgbw extract), dosage II (210 mg/kgbw) and dosage III (420 mg/kgbw). All, except negative control groups were fed by standard diet pellet mixed with 20 mg/day goat brain for 15 days. Serum uric acid and CRP level of all groups was measured on day 0,9 and 16. The result showed that the uric acid serum level of extract groups was lower than control groups and there was CRP reduction in extract groups. The most effective dosage for reducing serum uric acid level was 420 mg/kgbw.

Keyword : snake fruit's peel, uric acid serum, C-Reactive Protein (CRP)

## INTRODUCTION

Unhealthy diet that contains of high purine, derived from protein, has caused an increasing hyperuricemia-related disease in society, such as arthritis gout. Hyperuricemia means that plasma uric acid is more than 7,0 mg/dL. This condition is not life-threatening, yet reduce the quality of life due to episodes of pain, swollen and even disability of hand and foot joints. The pain is caused of peri-articular tissue irritation by monosodium urate chrystal depositing from saturated uric acid.[1]

Uric acid is the final product of purine (adenine,guanine) in tissue that have xanthine oxidase enzyme, especially liver and intestine. Normally, it is excreted in urine. However, when the synthesis of uric acid is too much or the excretion is impaired, then its plasma level will increase. Whenever the saturation level is reached, the monosodium urate chrystal begins to deposit and irritate the tissue, including kidney[2].

Clinically, hyperuricemia is treated with uricostatic synthesis agents such as Allopurinol. However, this agent may cause harmful side effects such as exzematous dermatitis, liver hypersensitivity reaction, liver failure, hepatitis, hyperbilirubinaemia, jaundice and disorders of stomach, intestine and blood[3]. Therefore, alternative medication that does not have these side effects is needed.

A study by Herliani has proven that snake fruit ethanol extract has potential for reducing uric acid serum level and increasing its excretion[4]. Aralas showed that the flesh and peel of snake fruit has antioxidant potential due to its flavonoid content[5]. Antioxidant may neutralize free radicals and stops chain reaction of lipid peroxidation. Therefore, this study aims to investigate whether antioxidant activity of snake fruit peel ethanol extract may reduce serum uric acid level and CRP in hyperuricemic samples.

## RESEARCH METHODS

This study used male Wistar strain white rats (Rattus norvegicus) which are 2 month-old, weight 150-200 grams from the Preclinical Services and Animal Model Development Laboratory of Faculty of Medicine, Padjajaran University as the subject. The rats were separated into 6 groups, namely Group A: negative control (standard diet); Group B: positive control (high protein diet); Group C: high protein diet + 2,52 mg/kgbw Allopurinol; Group D: high protein diet +snake fruit peel ethanol extract dosage I (105 mg/kgbw); Group E: high protein diet + snake fruit peel ethanol extract dosage II (210 mg/kgbw); Group F:high protein diet + snake fruit peel ethanol extract dosage III (420 mg/kgbw). Each group consisted of 5 rats. Materials used were ethanol extract of snake fruit peel with 3 dosage, i.e 150 mg/kgbw, 210 mg/kgbw and 420 mg/kgbw prepared in Facuty of Medicine, Padjajaran University. Antihyperuricemia agent used for comparison was 2,52 mg/kgbw Allopurinol. During acclimatization, all groups were fed with standard diet 521 diet. Before dietary treatment, all of groups were fasted in 3 days. After the first uric acid serum level were measured (on day 0), the negative control group continued to be fed with standard diet 521 pellet for 15 days. The other groups were fed with the same pellet mixed with 20 grams per day of goat brain for 15 days. The second uric acid serum level of all groups was measured on day 9. From day 9 to day 15, all groups except negative and positive control groups were given antihyperuricemic agents (Allopurinol or snake fruit peel ethanol extract). The last uric acid serum and CRP level of all group was measured on day 16. Each of the measurement used 2 ml orbital venous plexus blood samples. For uric acid level were used strip test kit (Easy Touch®) and *sandwich ELISA* for CRP level. The independent variables were the dosage of antihyperuricemic agents (allopurinol and graded dosage of snake fruit peel ethanol extract) and the dependent variable was serum uric acid level. Statistical analysis used F test with 5% significanct, followed by post hoc test. We also kept the note of body weight change.

## RESULTS

Figure 1 showed that the changing of body weight.

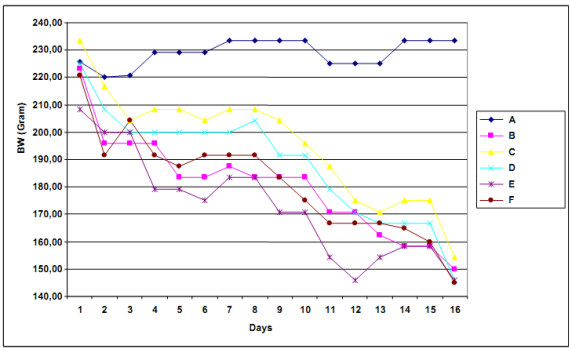


**Figure 1. Change In Body Weight Of Each Group**

(Data is shown as mean). Group A: negative control (standard diet); Group B: positive control (high protein diet); Group C: high protein diet + 2,52 mg/kgbw Allopurinol; Group D: high protein diet +snake fruit peel ethanol extract dosage I (105 mg/kgbw); Group E: high protein diet + snake fruit peel ethanol extract dosage II (210 mg/kgbw); Group F:high protein diet + snake fruit peel ethanol extract dosage III (420 mg/kgbw).

**Uric acid serum level of the 6 groups at 3 times of measurements as shown in figure 2.**

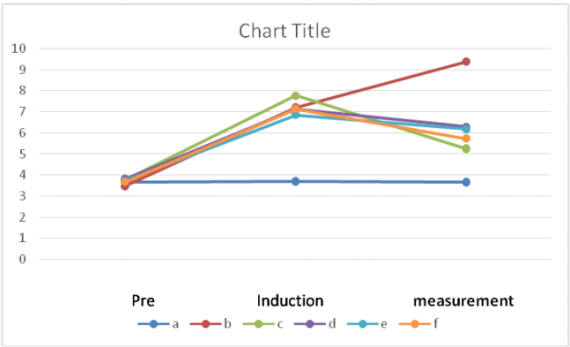


**Figure 2. Serum Uric Acid Level Of The 6 Groups At 3 Times Of Measurements.**
(Data is shown as mean). Pre: measurement on day 0 (before dietary treatment); Induction: measurement on day 9 (after dietary treatment); measurement on day 16 (after antihyperuricemic agent) a: negative control (standard diet) group; b: positive control (high protein diet) group; c: high protein diet + 2,52 mg/kgbw Allopurinol group; d: high protein diet +snake fruit peel ethanol extract dosage I (105 mg/kgbw) group; e : high protein diet + snake fruit peel ethanol extract dosage II (210 mg/kgbw) group; f: high protein diet + snake fruit peel ethanol extract dosage III (420 mg/kgbw) group.

CRP level of the 6 groups were showed in figure 3.

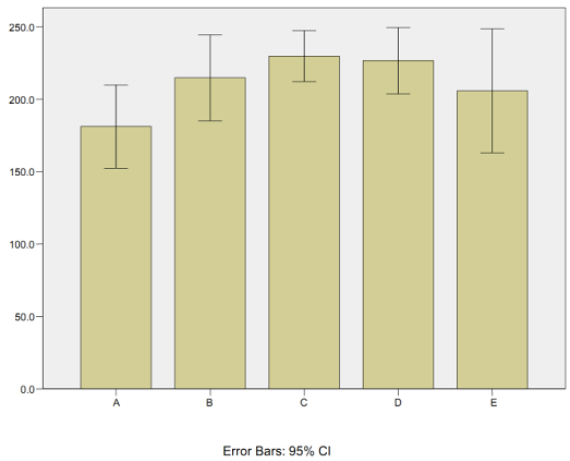


**Figure 3. CRP level**
(Data is shown as mean). A: negative control (standard diet); B: positive control (high protein diet) group; C: high protein diet + 2,52 mg/kgbw Allopurinol group; D: high protein diet +snake fruit peel ethanol extract dosage I (105 mg/kgbw) group; E : high protein diet + snake fruit peel ethanol extract dosage II (210 mg/kgbw) group; F: high protein diet + snake fruit peel ethanol extract dosage III (420 mg/kgbw) group. SD : Standard Deviation, $p_{value}$<0,05/95%CI; *Error bars* = ± 1 SD

## DISCUSSION

As seen in the figure 1, all of groups, except the negative control group showed the significant decrease of body weight. High protein diet consumption was expected to increase body weight. In this study, the decreasing of body weight due to decrease the absorption was confirmed by amount and consistency of the feces, that was more voluminous, softer and stinkier odor. As seen in figure 2, the uric acid serum of all 5 groups that they were fed with standard diet

pellet mixed with goat brain were higher than negative control group that they were fed with only standard diet pellet. Thus the higher uric acid level was found in high the group with protein diet addition treatment.

Goat brain was one of high protein diet source. Purine (adenine, guanine), as the product of protein degradation was metabolized into the final product, namely uric acid. Nucleic acid was degraded into guanine nucleotidase and adenine nucleotidase by nuclease enzyme. Guanine nucleotidase was transformed into guanine by purine nucleoside phosphorilase enzyme. Adenine nucleotidase was transformed into adenosine and IMP, then into inosine. Inosine was transformed into hypoxanthine by purine nucleoside phosphorilase. Guanine and hypoxanthine were transformed into xanthine by guanase and xanthine oxidoreductase enzymes. Xanthine was then transformed into uric acid by xanthine oxidoreductase enzyme[6]. If the synthesis of uric acid was too much or the excretion was impaired, its plasma level would increase. Whenever the saturation level was reached, the less- water-soluble monosodium urate chrystal began to deposit and irritated the tissue, including kidney[2,7].

The statistical analysis with ANOVA test ($\alpha$ = 0,05), obtained the F =113,950 and p= 0,000; (p< 0,005) value. Thus Ho was denied, or mean of uric acid serum levels of the 6 groups were significantly different. Homogenous subsets test revealed that the uric acid serum levels of the 3 groups of snake fruit peel ethanol extract (group D,E,F) were significantly lower than positive control group. The uric acid serum level of group F (protein diet + snake fruit peel ethanol extract dosage III (420 mg/kgbw)) was the lowest, yet still significantly higher than group C (high protein diet + 2,52 mg/kgbw Allopurinol).

Anti-hyperurisemic effect of snake fruit peel of ethanol extract could be caused by its anti-oxidant and anti-inflammatory effects. Its work is like Allopurinol in prevent the production of uric acid by inhibiting xanthine oxidase enzyme. It also increased the formation of uricase enzyme that transformed uric acid into water-solluble allantoin which then increased its excretion through urine[7,8,910].

The administration of ethanol extract of snake fruit peel at least with the dosage of 105 mg/kg bw was shown to significantly reduce uric acid serum & CRP level in high protein diet induced hyperuricemic Wistar rats. The most effective dosage to get this effect was 420 mg/kgbw.

## ACKNOWLEDGEMENT

This study was funded by BLU fund Jenderal Soedirman, Purwokerto. Thank you for the supports of funding.

## REFERENCES

1. Dipiro, JT. *Pharmacotherapy Handbook seventh edition*. Mc Graw Hill Companies, Inc, New York. 2009.
2. Murray, R. *Biokimia Harper, edisi 29*. EGC, Jakarta. 2007.
3. Gerald, K. *AHFS Drug Information*, American society of Health-System Pharmacists, Bethesda. 2005.
4. Herliani, L. Antihyperuricemic Effect of Ethanol Extract of Snake Fruit (Salacca endulis Reinw.) var. Bongkok On Wistar Male Rat. *Journal of Food Science and Engineering*. 2012;2:271-276.
5. Aralas, S.. Antioxidant properties of selected snake fruit (Salacca zalacca) varieties in Sabah, Malaysia. *Nutrition and Food Science*. 2009;39 (3) 243-250.
6. Edwards, NL. The role of hyperuricemia and gout in kidney and cardiovascular disease. *Cleveland Clinic Journal of Medicine*. 2008;75 (5): 513-6.
7. Baker, J.W., Schumacher, E.H. *Uric Acid Transport and Disease*. Philadelphia : W. B. Saunders Company. 2010.
8. Barr, DJ. *Biosynthesis and Degradation of Nucleotides*. Elsevier's Health Sciences Rights Department, USA.2007.
9. Fitrianingsih, SP., Lestari, F., Aminah, S. Uji Efek Antioksidan Ekstrak Etanol Kulit Buah Salak (Salacca zalacca (Gaertner) Voss) Dengan Metode Perendaman DPPH. *SNaPP2014 Sains, Teknologi dan Kesehatan*. 2014;4 (1): 49-54.
10. WHO. C-Reactive Protein Concentrations as a Marker of Inflamation or Infection for Interpreting Biomarkers fo Micronutrient Status. 2014.