Journal of Nutraceuticals and Herbal Medicine

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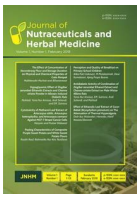

The effect of Salam Leaves Extract (*Syzygium Polyanthum* Wight.) on Urine Volume in The Potassium Oxonic-Induced Hyperuricemia Mice

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ABSTRACT

The research objective is to determine the effect of Salam (*Syzygium polyanthum* Wight.) leaves extracts on urine volume in the potassium oxonic-induced hyperuricemia mice. Fifteen Swiss male mice were divided into 3 groups. Group I (normal group) were given intraperitoneal aquadest 1 mL/20g.bw, group II (negative group) and group III (Salam leaves extracts group) were given intraperitoneal 250 mg/kg.bw of potassium oxonic. After 1 hour of the treatment, group I and group II were given aquadest 0.5 mL/20g.bw orally, while group III was given Salam leaves extracts 200 mg/kg.bw orally. Mice are included in metabolic cage. The cumulative urine volume data were analyzed by Kruskal-Wallis and and Mann-Whitney test with a confidence level of 95%. In the urine volume profile test, the negative group did not experience a significant increase in urine volume when compared with the Salam leaves extracts group at hyperuricemia for 3 days ($p > 0.05$), while the negative group had higher urine volume when compared to the normal group ($p = 0.007$). The research results indicated that Salam leaves extracts were not able to increase urine volume in hyperuricemic conditions.

Keywords: *hyperuricemic; potassium oxonic; Syzygium polyanthum* Wight; *urine volume.*

1. Introduction

Hyperuricemia is a condition where there is an increase in uric acid concentration (Carter, 2005). Uric acid is derived from purine compounds that cannot be utilized by the body (Priyanto, 2008). Uric acid is formed by the breakdown of purines and by direct synthesis of 5-phosphoribosyl pyrophosphate (5-PRPP) and glutamine. In humans, uric acid is excreted in urine, but in other mammals, uric acid is oxidized again to allantois before being excreted (Ganong, 2002).

The prevalence of gout in Europe and the US is estimated at 2.6 per 1,000 people to 10% in Maori men in New Zealand (Tjay and Rahardja, 2007). According to Darmawan, (1992) cit Kurniari, et al. (2011) the prevalence of hyperuricemia in the population in Central Java was 24.3% in men and 11.7% in women.

At present most people use herbal medicines to treat gout, one of the herbal medicines used is Salam leaves. Based on phytochemical tests, secondary metabolites contained in Salam leaf water extract include flavonoids, saponins, and tannins (Muhlifat, 2008). In the study (Muhtadi, et al., 2010), the identity compounds found in Salam leaves were Fluoretin. In the previous study stated that Salam leaves have the effect of increasing urine output under normal conditions. (Retnosari, et al. (1996) cit (Sudarsono, et al., 2002), and Muhlifat (2008). According to the study of Apriono, et al. (2008) cit (Sumono and Wulan, 2008), Salam leaves in dose 0,5 mg can increase urinary acid excretion in Wister strain mice.

This study aims to determine the effect of Salam (*Syzygium polyanthum* Wight.) leaves extracts on urine volume in the potassium oxonic-induced hyperuricemia mice. The research on this topic has never been reported by other researchers before.

2. Materials and Methods

Tools

The tools used in this study were injection syringe volume 1 ml (Terumo), 18 gauge oral syringe, flucon, 2610 gram capacity scales (Lark, China), analytical scales (Presica A-SCS), glassware (Pyrex), metabolic cage, UV-Vis spectrophotometer (Star Dust FC*15).

Materials

The materials used in this study were Salam leaves extract, aquadest, potassium oxonic (Aldrich Chemical Company), white mice (Mus musculus) Swiss strains weighing 20 -30 grams and aged 2-3 months were obtained from the Pharmacology laboratory of the Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, as well as Uric Acid FS*TBHBA (2,4,6-tribromo-3-hydroxybenzoid Acid) (DyaSys) Reagents.

Making Salam leaves extract

The extract is made by decocta, by weighing 1 kg of simplicia and then put it into the infusion pan. The aquadest is put in the pan until the simplicia is submerged and boiled for 30 minutes, the time is calculated when the temperature reaches 90 ^{o}C. The boiled simplicia is then separated by centrifuge and the top is taken, then put in the evaporator at 60 ^{o}C for 8 hours so that the viscous extract is obtained. The viscous extract is then put into the vaccum dry oven to dry.

Making hyperuricemia

Making hyperuricemia is done for 3 days, 1 hour before administration of the test preparation by intra peritoneal injection of potassium oxonic a dose 250 mg/kg (Haidari, et al., 2009).

Determination of the period of hyperuricemia

Fifteen mice were weighed, divided into 5 groups per group of 3 tails; 3 mice were first taken for 0.5 mL ophthalmic venous blood at hour to start. Then induced potassium oxonic i.p 250 mg/kg.bw. One hour later was given aquadest p.o 0.5 mL/kg.bw. The blood is then taken through the ophthalmic vein at 2.4, 6, 8 hours after administration of potassium oxonic 250 mg/kg. Blood is left for 30 minutes at room temperature. The blood is then centrifuged at a speed of 12,000 rpm for 5 minutes. 20 µL of serum was taken, put in

cuvette and added 1,000 µL of mono reagent [uric acid FS * TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid)]. The mixture of serum and reagent was then incubated for 10 minutes at 37^{o}C. Reads the absorbance using a UV-Vis spectrophotometer (Star Dust FC * 15) at λ 546 nm.

Test urine profile

Fifteen Swiss male mice were divided into 3 groups. Group I (positive group) was given intraperitoneal aquadest 1 mL/20g.bw, group II (negative group), and group III (Salam leaves extracts group). Group II and III were given potassium oxonic 250 mg/kg.bw intraperitoneally. After 1 hour, group I and group II were given aquadest 0.5 mL/20g.bw orally, while group III was given Salam leaves extracts 200 mg/kg.bw orally. Mice were put into metabolic cage to measure urine volume for 3 days.

Data analysis

Data on uric acid levels were tested for normality. All data were normally distributed and homogeneous variances were uniform. The data were then tested for significance with one way ANOVA and the data obtained had significantly different differences so that it was continued with the LSD (Least Significant Difference) test with a confidence level of 95%.

Data on urine volume of mice obtained is cumulative urine volume for 3 days. Cumulative urine data were tested by parametric statistical tests, but were not normally distributed and the uniformity of the variants was not homogeneous, so the data were analyzed by nonparametric tests namely the Kruskal-Wallis test and for significantly different data followed by the Mann-Whitney test to find out which data was significantly different, the data was tested with a confidence level of 95%.

3. Results & Discussion

Test results for the period of hyperuricemia

Tests of uric acid levels were carried out to determine how long male white mice induced by potassium oxonic at a dose of 250 mg/kg experienced hyperuricemia. From the test results of uric acid levels, potassium oxonic 250 mg/kg.bw can increase uric acid levels from 1.067 ± 0.088 to 3.4 ± 0.265 at 2 hours (hrs). Uric acid levels decrease at 6 hrs, and close to normal at 8 hrs. According to Huang, et al., (2008) 250 mg/kg.bw of potassium oxonic can increase uric acid levels and reach a maximum concentration of 2 hours after administration of potassium oxonic.

The results of uric acid levels were significantly different only at 2 and 4 hrs ($p < 0.05$), whereas at the 0, 6 and 8 hrs, there were no significant differences ($p > 0.05$), so it can be concluded that administration of potassium oxonic 250 mg/kg gives the effect of hyperuricemia up to the 4th hour. In a study by Huang, et al., 2008, uric acid will return to normal at 8 hrs, but on the curve, uric acid levels drop dramatically between at 2 and 4 hrs, then slowly fall to normal levels at 8 hrs. The measured uric acid level in the blood will be smaller if the distance of blood extraction time after induction of potassium oxonic is longer, this is probably due to the short half-life of potassium oxonic or the rapid elimination process of potassium oxonic (Ariyanti, et al., 2007).

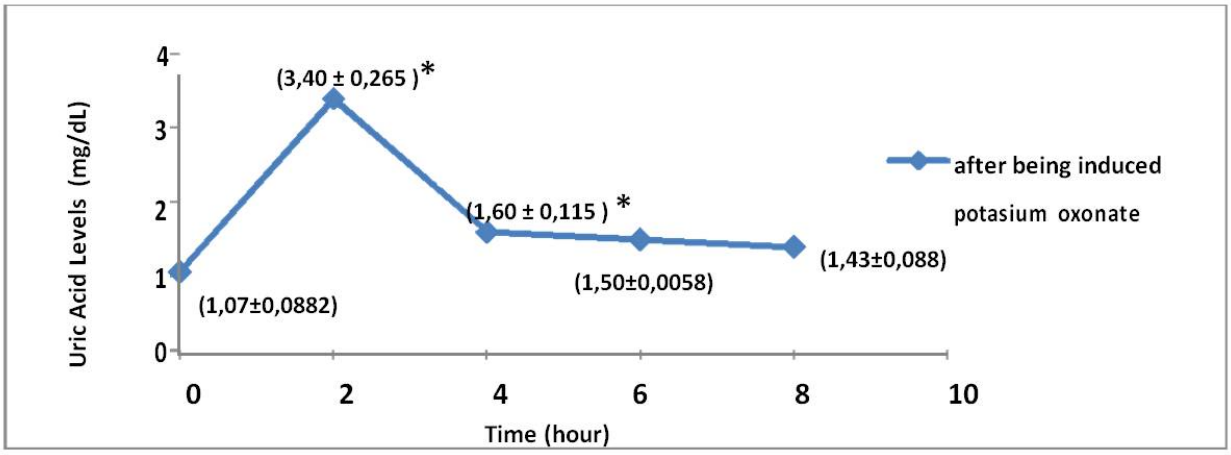


Figure 1. Graph of uric acid levels after administration of potassium oxonic

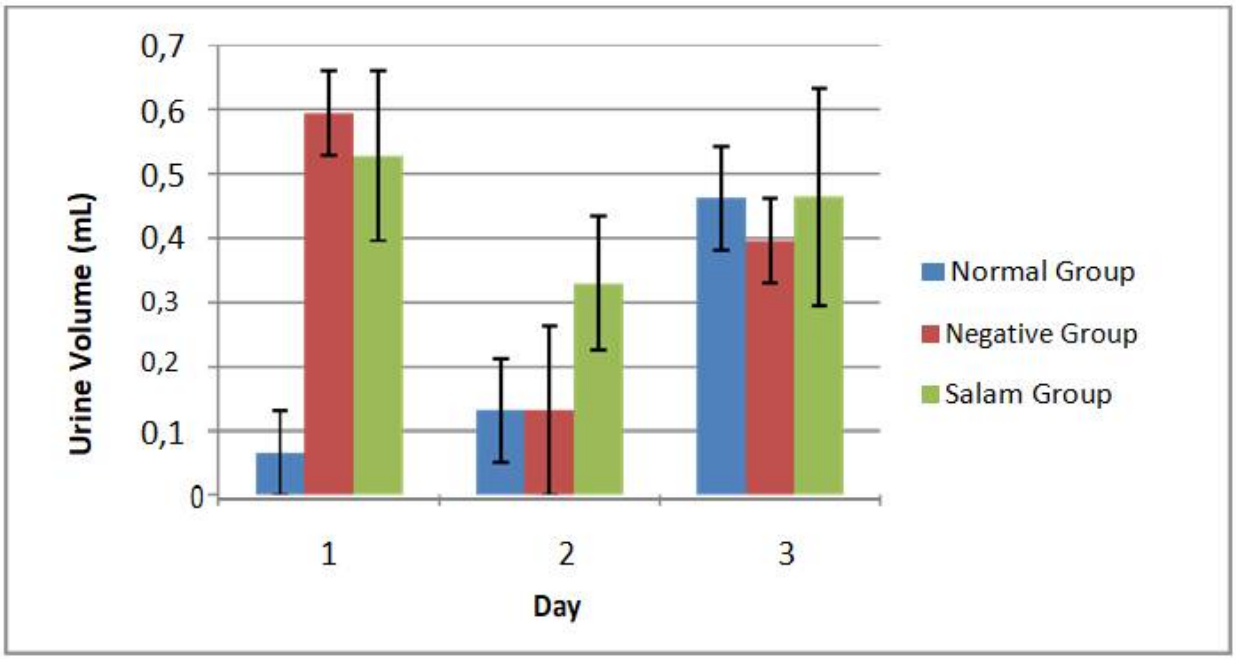
Profile of Urine Volume at Hyperuricemia

The urine volume profile at 1 to 4 hours (figure 2a), on the first day showed that the negative group had more urine volume than the normal group (p = 0.007), so it can be concluded that the increase in urine volume in the negative group was caused by the condition of hyperuricemia in the negative group and not due to drinking factors and eating factors. This conclusion is supported by research by Yonetani and Iwaki (1983) who said that potassium oxonic at a dose of 250 mg/kg can increase urine volume. The negative group had the same urine volume when compared to the Salam leaves extract group on the first day (p = 0.881), as well as the drinking volume and feeding weight between the normal group, the negative group, and the Salam leaves extract group which had no significant differences (p> 0.05). These results indicate that Salam leaves extract cannot increase urine volume under hyperuricemia.

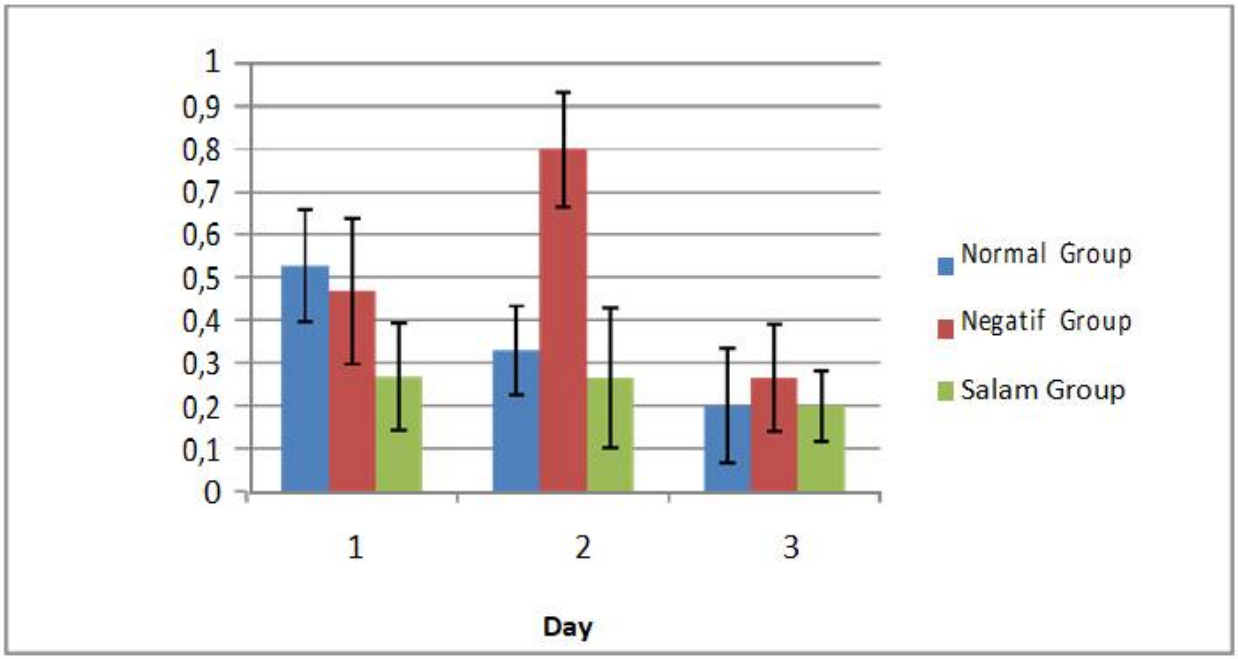
On the second day there was a decrease in urine volume in the negative group when compared with the negative urine excretion volume on the first day (p = 0.028), drinking volume and negative group feeding weight on the second day tend to be the same compared to drinking volume and feeding weight on the first day (p> 0.05). Urinary, drinking volume and feeding weight on the third day tended to be equal when compared with urine volume, drinking volume, and feeding weight on the first day (p> 0.05). The comparison between urine volume, drinking volume, and feeding weight the second day with urine volume, drinking volume, and meal weight on the third day showed results that tended to be equivalent (p> 0.05).

This study did not include measurements of uric acid levels in urine; this was a weakness in this study, so it was suggested in subsequent studies to measure uric acid levels excreted in urine.

a.


0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Urine Volume (mL)
1
2
3
Day
Normal Group
Negative Group
Salam Group

b.


1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1
2
3
Day
Normal Group
Negatif Group
Salam Group

c.

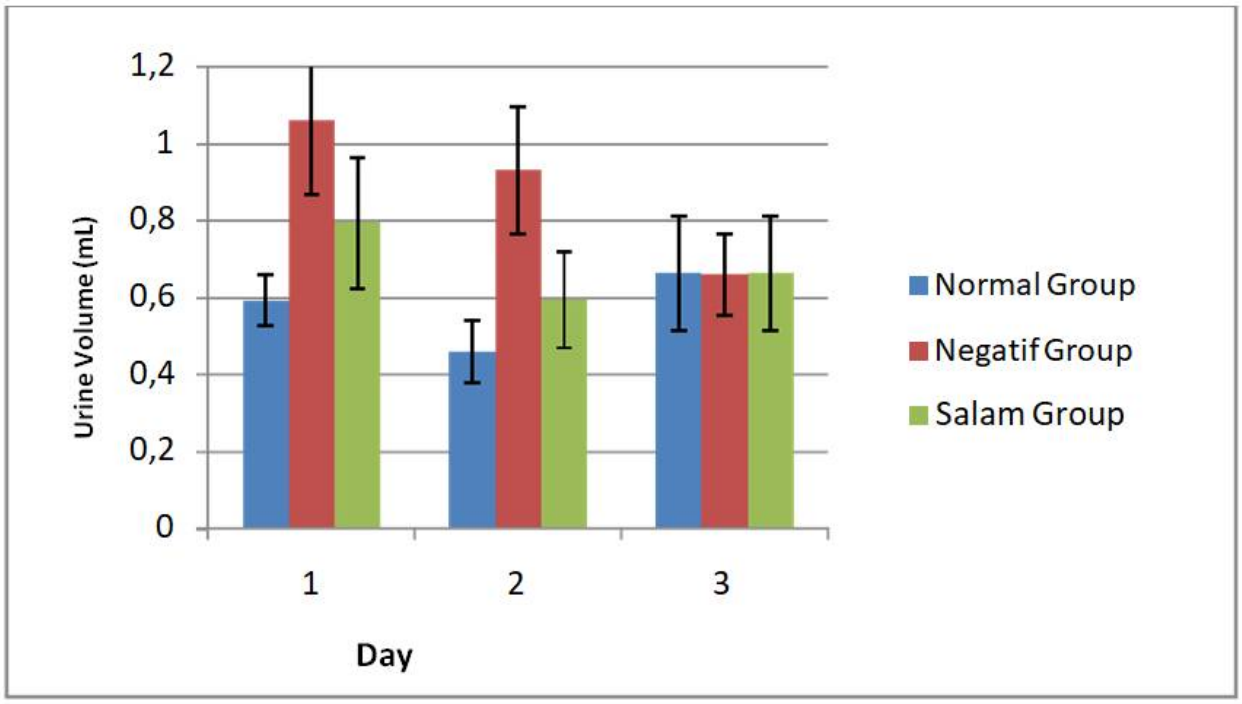


Figure 2. The cumulative average urine profile at 1 to 4 hour (a); Cumulative average urine profile at 4 to 24 hours (b); Cumulative average urine profile at 1 to 24 hours (c)

Profile of Urine Volume after Hyperuricemia

At observations at 4 to 24 hours (Figure 2b), urine volume and drinking volume on the first day between the negative group, normal group, and Salam leaves extract group both did not have a significant difference ($p > 0.05$), while the feed weight in the negative group was smaller when compared to the normal group and Salam leaves extract group ($p < 0.05$). On the second day, urine volume in the negative group was higher compared to the normal group and Salam leaves extract group ($p < 0.05$), while drinking volume and feeding weight between negative groups, normal groups, and Salam leaves extract groups tended to be equal ($p > 0.05$). On the third day, there was no significant difference in urine volume, drinking volume, and feeding weight between the negative group, normal group, and Salam leaves extract group ($p > 0.05$).

Urinary volume and drinking volume in the negative group showed no difference for 3 days ($p > 0.05$) with increased food weight between the first day and feeding weight on the second day ($p = 0.028$) and increased feeding weight between the first day and the weight eat on the third day (0.028).

In the negative group, the second day's urine volume at hours 4 to 24 experienced an increase when compared with the urinary excretion volume at hours 1 to 4 and on the third day urine volume at the 4th to 24th hour in the negative group decreased compared to with urinary excretion volume at 1 to 4 hours, this is probably due to impaired kidney function due to administration of potassium oxonic for 3 days. The drinking volume of the negative group at 4 to 24 hours did not differ for 3 days ($p = 0.079$). Feed weight in the negative

group increased on the second and third days when compared to the first day ($p < 0.05$), while the feeding weight between the second day tended to be the same as the feeding weight on the third day ($p = 0.602$).

In the Salam leaves extract group, urine volume and drinking volume at 4 to 24 hours for 3 days tended to be the same ($p > 0.05$). Feed weight on the second and third days decreased when compared to the first day ($p < 0.05$), while on the second day with the third day feeding weight in the Salam leaf extract group from 4 to 24 hours tended to be equal ($p = 0.754$) .

On a cumulative urine profile for 24 hours (figure 2c), the negative group experienced a decrease in urine volume for 3 days, this is because the administration of potassium oxonic carried out for 3 days can cause sub chronic hyperuricemia. According to Schlesinger (2005), sustained hyperuricemia can be at risk for kidney dysfunction and can increase the risk of kidney failure significantly (Edwards, 2008). According to Venkataraman and Kellum (2007), a decrease in urinary excretion volume is one of the clinical signs of the development of acute renal failure.

4. Conclusion

The administration of Salam leaves extracts (*Syzygium polyanthum* Wight.) at a dose of 200 mg/kg.bw has no effect on urine volume in the potassium oxonic-induced hyperuricemia mice.

5. Acknowledgments

The authors wish to thank to Universitas Muhammadiyah Surakarta for PINPRU Research Grant Scheme for financial support.

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