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EFFECT OF LEMON PEEL EXTRACT ON URIC ACID LEVELS AMONG HYPERURICEMIA MALE WISTAR RATS

Revi Ardiansyah[1], Noor Wijayahadi[2], Endang Mahati[2], Yora Nindita[2]*
[1]Undergraduate Program, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Departement of Pharmacology and Therapy, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
*Corresponding Author: E-mail: nindita.yora@fk.undip.ac.id

ABSTRACT

Background: Purine metabolism, carried out in the liver, results in the production of uric acid. Hyperuricemia is a condition of elevated level of uric acid in the blood that can lead to gout if untreated. Lemon (*Citrus Limon*.) contains flavonoids which act as antioxidants and have the potential role as antihyperuricemia. **Aim:** To determine the effect of lemon peel extract on uric acid levels. **Methods:** Thirty-six male wistar rats from Farmhouse Ungaran were randomly divided into 6 groups, consists of healthy control group (K0), hyperuricemic control (K1), allopurinol control (K2), and 3 treatment groups (P1, P2, P3). Hyperuricemia induced with administration of block broth and potassium oxonate for 4 weeks. Lemon peel extract doses of 17,5, 35, and 70 mg/kg BW, and allopurinol 90 mg/kg BW was given orally for 2 weeks. Statistical analysis was conducted to evaluate differences among groups before and after the intervention. **Results:** There was a significant difference (p<0,05) in uric acid levels before and after intervention of lemon peel extract in groups K1, K2, and P3. Meanwhile, in groups K0, P1, and P2, there were no significant differences observed. In the pre-test uric acid levels, there was a significant difference (p<0,05) between the K0 group and K1, K2, P1, P2, and P3. **Conclusion:** There were no significant differences observed in the uric level acid of the treatment and control group after intervention, but there were significant differences in the effects of graded dosages between the treatment groups.

Keywords: Lemon, Rats, Uric Acid

BACKGROUND

Uric acid is the metabolic result produced by each individual through the process of protein breakdown, especially purines. Due to its significant antioxidant role, uric acid levels must be maintained within normal ranges. If the metabolism and excretion of uric acid is not optimal, it may cause the blood's uric acid level to increase, known as hyperuricemia.[1]

The total cases of hyperuricemia keep on increasing throughout the world. In hyperuricemia research that was conducted in China in 2006, there were a total of 25.3% cases. Hyperuricemia is also increasing and affects as many as 43.300.000 people (21%) in the US. Although exact data on hyperuricemia in Indonesia is still unknown, surveys have been conducted in several part of Indonesia to gather the information. The hyperuricemia prevalence where it is highest in Indonesia is in Minahasa at 29.2% and Denpasar at 18.2%.[2]

The habit of consuming high purine-containing food will increase the risk of hyperuricemia. Some examples of high purine-containing foods are offal, shellfish, crab, and anchovies. Consuming meals high in purines will raise blood uric acid levels, with an inadequate treatment, and can lead to gout.[3]

Treatment for hyperuricemia is very necessary, whether it is chemical/conventional medicines or herbal medicines. An alternative way of using herbal medicine for hyperuricemia is needed to reduce the side effects.[4]

Lemon (*Citrus limon*) can be used to make herbal medicine. One of the parts of lemon that can be used is the peel. Lemon peel contains antioxidants, vitamin C, magnesium, potassium, calcium and flavonoids. Lemon peels can be used as herbal remedy to lower blood uric acid levels.[5]

Research by Sulistyowati (2019) using lemons mixed with water making infused lemon water that concluded that lemons contain flavonoids which have a similar working mechanism to allopurinol, which is to slow down the action of xanthine oxidase (XO) enzyme.[6]

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Other research done by Rakhman and Chen, *et al.* (2019) also explain that consuming lemon juice is an efficient way to maintain vitamin levels. Lin Chen's research also explains that water-soluble lemon extract has an effect on reducing uric acid levels.[7,8]

Based on the explanation above, we are interested to know the effect of lemon peel extract on hyperuricemia male Wistar rats uric acid levels.

METHODS

This study is experimental pre and post test randomized controlled group design, to compared between the treatment groups (K0, K1 and K2) and the control groups (P1, P2, and P3). K0 was given standard feed only; K1 for hyperuricemic control was given standard feed and block broth potassium oxonate; K2 was given standard feed, block broth potassium oxonate and allopurinol 90 mg/kg BW; P1 was given standard feed, block broth potassium oxonate and lemon peel extract 17,5 mg/kgBW; P2 was given standard feed, block broth potassium oxonate and lemon peel extract 35 mg/kgBW; P3 was given standard feed, block broth potassium oxonate and lemon peel extract 35 mg/kgBW.

This research was done in July – August 2023 with a total of 6 groups of rats each plus 1 rat as a back up if there are samples that dropped out. The samples used in this study were Wistar rats (*Rattus novergicus* L.) male with a normal body weight of 150-200 grams, aged 6 - 8 weeks, physically healthy, active and do not have anomaly in anatomy. This research samples were obtained from Ungaran Farmhoues in Semarang, Central Java.

Shapiro-Wilk test was used to verify normality of data distribution. A paired t-test was used to assess significant difference in the uric acid levels between groups before and after administration of lemon peel extract.

The homogeneity was assessed using Levane's test to obtain homogeneous data before the treatment, which was then used for a comparative test of the amount of uric acid in each group, while the after-treatment data was not homogeneous. After that, the samples were analyzed using the Anova plus post hoc test. LSD post hoc test performed on homogeneous data, while Games-Howell post hoc test used on inhomogeneous data.

RESULTS

The rats were acclimatized before treatment for 7 days and given standard food. Two rats died due to stress which caused loss of appetite in groups K2 and P1. During treatment period, 3 rats died, 1 in each group of K1, P2, and P3 due to illness, but the number of samples still met the inclusion criteria that was 31 rats.

From the results of the paired difference test between pre-test uric acid and post-test uric acid using the paired-t test, statistically differences in groups K1, K2 and P3 were found.

Results of the unpaired difference test in the pre-test uric acid with One Way Anova test, found that $p<0.05$, means there was a significant difference and the data variance was homogeneous ($p>0.05$). Post-test uric acid using One Way Anova test, obtained $p<0.05$, means there was a significant difference and the data variance was not homogeneous. Difference in uric acid using the One Way Anova test, found that $p<0.05$, means there was a significant difference and variance homogeneous data.

Table 1. Uric Acid Differential Test Results

Group	Uric Acid		p	Uric Acid Difference
	Pre test	Post test		
K0	3,13 ± 0,45	2,88 ± 0,76	0,385¶	-0,26 ± 0,67
K1	6,44 ± 1,44	8,22 ± 1,85	0,049¶*	1,77 ± 1,42
K2	6,32 ± 1,21	3,46 ± 0,59	0,002¶*	-2,86 ± 0,85
P1	6,20 ± 1,94	5,09 ± 1,87	0,205¶	-1,12 ± 1,65
P2	5,85 ± 1,48	3,64 ± 1,28	0,078¶	-2,22 ± 2,10
P3	5,80 ± 1,71	3,49 ± 0,99	0,042¶*	-2,31 ± 1,75
p	0,005§*	<0,001§*		<0,001§*
Lavene	0,121**	0,007		0,579**

*Significant ($p<0,05$); ** Homogeneous ($p> 0,05$)
¶ Paired t; § One Way Anova

Post LSD test for homogeneous data variants and Post Hoc Games-Howell test for non-homogeneous data variants were used to determine the differences between treatment groups.

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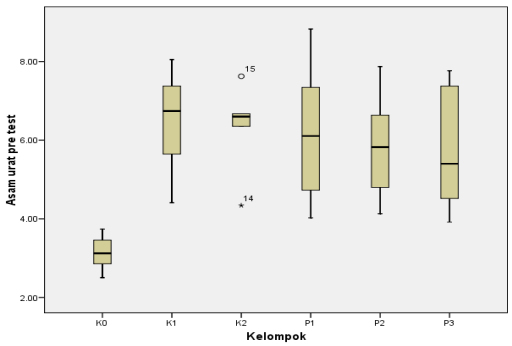
Table 2. Uric Acid Post Hoc Test Results

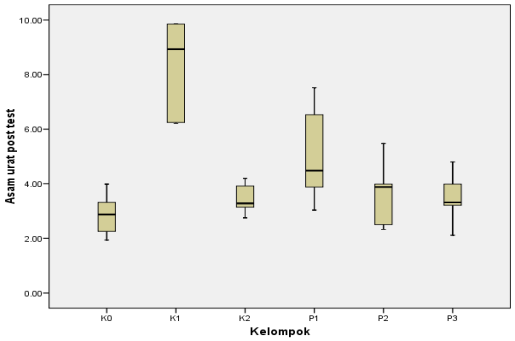
Group		Uric Acid		
I	**II**	**Pre test**	**Post test**	**Difference**
K0	K1	0,001*	0,011*	<0,001*
	K2	0,001*	0,709	0,468
	P1	0,002*	0,279	0,010*
	P2	0,004*	0,838	0,346
	P3	0,005*	0,857	0,446
K1	K2	0,889	0,020*	<0,001*
	P1	0,792	0,188	0,001*
	P2	0,516	0,020*	<0,001*
	P3	0,477	0,017*	<0,001*
K2	P1	0,901	0,511	0,059
	P2	0,698	1,000	0,832
	P3	0,653	1,000	0,973
P1	P2	0,698	0,710	0,090
	P3	0,653	0,580	0,063
P2	P3	0,950	1,000	0,859

Information : * Significant ($p < 0,05$)

Post Hoc LSD test on pre-test uric acid result found that there were significant differences between group K0 and groups K1, K2, P1, P2 and P3.

Meanwhile from Post Hoc Games-Howell test on post-test uric acid, found that a significant difference in group K0 and K1, in group K1 and K2, also in P2 and P3. Post Hoc LSD test on the difference in uric acid, found that there was a significant difference between group K0 and groups K1 and P1; and between group K1 and groups K2, P1, P2 and P3.





Picture 1. Differences in Uric Acid Levels Before (up) and After (bottom) Treatment

Groups K1, K2, and P3 showed a significant decrease ($p<0.05$) in uric acid levels after the treatment.

DISCUSSION

Based on the research data, uric acid levels were measured after hyperuricemia induction or before the treatment (21st day) and also after the treatment (35th day). The rats' uric acid levels before treatment or after induction of hyperuricemia (21st day) had an average of 6.12 mg/dl. The groups of experimental rats that were induced by hyperuricemia included K1, K2, P1, P2, and P3. Normal uric acid levels in rats are around 1.2 – 5 mg/dl.[9]

The ingredients used to induce hyperuricemia are Maggie® broth blocks made from yeast extract, disodium inosinate, guanylate, and monosodium glutamate (MSG). Disodium inosinate and guanylate are purine nucleotides. The main end product of purine metabolism is uric acid.[10] Consuming excessive amounts of purine nucleotides can cause purine metabolism to not be optimal, causing an increase in blood uric acid levels.[11] This is similar with the research done by Addinurrahmat (2017) which assessed the uric acid levels of hyperuricemic rats. In that study, 1 g/ml beef broth per animal were used for 11 days and showed average increase in uric acid levels (6.57 mg/dl) for all groups, so it can be concluded that the conditioning of hyperuricemic rats was successful.[12]

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Hyperuricemia inducers, such as potassium oxonate, is an inhibitor activity of the enzyme uricase, which is responsible for converting uric acid to allantoin.[13,14] Induction of potassium oxonate in hyperuricemia is not very effective, because potassium oxonate works as an inhibitor of the uricase enzyme and the effect of potassium oxonate is not permanent so it is require to use other ingredients to increase uric acid levels. That is why Maggie® broth block was chosen as a more stable induction of hyperuricemia.[13]

On the 35th day, blood samples were obtained to assess uric acid levels after the treatment. This is done to determine whether or not there is a decrease in the amount of uric acid in the sample. Experimental rats in groups K0, K2, P1, P2, and P3 had lower uric acid levels in their bodies, but group K2 had higher uric acid levels. The K2 group experienced the opposite.

Comparison of uric acid levels before and after treatment in each group showed significant results in groups K1 (p=0.049), K2 (p=0.002), and P3 (p=0.042) so it can be interpreted that the administration of lemon peel extract dose of 70 mg/kg can reduce the uric acid levels of hyperuricemia mice significantly. The highest decrease was in the K2 group with an average decrease of -2.86 mg/dL and followed by the P3 group with an average decrease -2.31 mg / dL.

Allopurinol, a gout medicine, inhibits xanthine oxidase because it has the same structure as xanthine, as a substrate.[15] Allopurinol inhibits purine catabolism which converts hypoxanthine into xanthine and uric acid, and can reduce uric acid significantly.[16] This is the same as research that was done by Addinurrahmat (2017) which showed a decrease (p=0.00) when administering allopurinol at a dose of 90 mg/kg to Wistar rats for 12 days from 8.07 mg/dl to 3.03 mg/dl. This phenomenon arises due to the pharmacological action of allopurinol, a drug that functions as an inhibitor of the xanthine oxidase enzyme. By inhibiting this enzyme, allopurinol interferes with the process of uric acid formation, causing a decrease in the concentration of uric acid in the bloodstream.

Uric acid levels after the treatment of lemon peel extract decreased in each treatment group, proving that the compounds in lemon peel can affect the synthesis of uric acid synthesis activity in the blood. Lemons are known to contain flavonoids which have been proven to be useful in reducing uric acid levels. Flavonoids are antioxidant compounds that have the potential to act as xanthine oxidase inhibitors. Flavonoids that are useful for reducing uric acid are flavones and flavanols.[17,18] Flavonoids reduce uric acid levels in the area as free radical reducers.[19]

Comparison of uric acid levels after treatment between groups to determine the reduction in uric acid levels showed significant results between the comparison of groups K0 with K1 (p=0.001) and in groups K1 with K2 (p =0.020), P2 (p =0.020) and P3 (p =0.017). These findings indicate a disparity in post-treatment uric acid levels between the healthy group, the hyperuricemia-induced group, and the group given Allopurinol at a dose of 90 mg/kg. In the group given lemon peel extract, there was no difference in uric acid levels after treatment with the healthy group, so if the uric acid levels of the K0 group were assumed to be normal values, then giving lemon peel extract was able to reduce uric acid levels to normal values. Apart from that, post-treatment uric acid levels were not significantly different between group K2 and groups P1, P2, or P3. This shows that uric acid can be reduced by administering lemon peel extract at doses of 17.5 mg/kg, 35 mg/kg, and 70 mg/kg, but this dose is less effective than Allopurinol 90mg/kg.

The relationship between drug dosage and effects produced in the clinic usually complex and influenced by many factors. In animals and patients, the response to affects usually increases within proportion of high dose drug administered. If the taken drug is too low, it will not be able to provide the expected therapeutic effect. This will result in the ineffectiveness of the drug.[18] This may occur when given lemon peel extract at a dose of 17.5 mg/kg, 35 mg/kg and 70 mg/kg. The reduction in uric acid levels was not significant compared to Allopurinol 90 mg/kg.

In the comparison of uric acid levels after treatment between group K1 and the treatment group (P1) there were no significant results, but between group

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K1 and the treatment groups (P2 and P3) there were significant results, this showed that there was no significant difference between the group that was only induced by hyperuricemia (K1) and the group that was given lemon peel extract (P1). Also there was a significant difference between the group that was only induced by hyperuricemia (K1) and the group that was given lemon peel extract (P2 and P3). It showed that there is a graded dose of lemon peel extract as a minor hypothesis in this study. The weakness of this study is the absence of phytochemical examination which is needed to determine the flavonoid levels in lemon peel.

CONCLUSION

There is a statistically significant difference in uric acid levels after the treatment between the treatment group and the control group. There are differences in the multilevel dose effects between each treatment groups, although it is not statistically significant.

ETHICAL APPROVAL

The ethical clearance certificate issued by The Health Research Ethics Committee of Universitas Diponegoro No. 61/EC-H/KEPK/FK-UNDIP/VI/2023

CONFLICT OF INTEREST

The authors affirm that there isn't any conflict of interest with this article's publishing.

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