

Effects of Sidempuan Salacca (*Salacca sumatrana*) Vinegar on Hyperuricemia: Histopathological Assessment

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Abstract. This research aimed to ascertain the effectiveness of Sidempuan salacca vinegar on improving kidney degeneration in hyperuricemia male mice. The mice were grouped into untreated mice, hyperuricemia mice without treatment, and hyperuricemia mice treated with Sidempuan salacca vinegar. The blood uric acid was measured by Easy Touch GCU, and the histopathology of the kidney was observed using the paraffin method and HE staining. The results demonstrated that Sidempuan salacca vinegar has the ability to decrease blood uric acid significantly. Sidempuan Salacca vinegars have the ability for regeneration in addition to kidney cells. However, the capacity of P3 to regulate hyperuricemia seemed to be better than that of other treatments. Sidempuan salacca vinegar has the potential to be used therapeutically by reducing tissue damage in hyperuricemic mice.

1 Introduction

Uric acid is the end result of purine metabolism in the body. Uric acid will be excreted through the kidneys in the form of urine [1]. Uric acid is the end product of purine metabolism that can be deposited in tissues and can cause inflammation, known as gout [2]. Gout is an inflammatory joint disease caused by the buildup of monosodium urate crystals in or around the joints [3]. Excessive uric acid production or lack of uric acid excretion will increase uric acid levels in the blood, called hyperuricemia [4]. Diet plays an important role in increasing or decreasing uric acid levels in the blood [5]. Consuming foods high in purines can increase uric acid levels in the blood [2]. A high purine diet will cause xanthine oxidase activity to increase 20 times compared to normal conditions, causing hyperuricemia and inflammation [6].

Allopurinol is the standard treatment therapy for patients with hyperuricemia to lower uric acid levels; however, long-term use of allopurinol can have adverse effects on the body, including gastrointestinal disorders, allergies, poisoning, peripheral neuritis, depression of spinal cord elements, aplastic anaemia, intestinal nephritis, fever, hepatitis, and kidney disease. Therefore, it is necessary to develop anti-hyperuricemia drugs from natural ingredients to minimise the side effects of using allopurinol drugs [4]. Based on the results of previous research, antioxidant compounds in sidempuan salacca vinegar can reduce blood

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uric acid levels in male hyperuricemia mice [7]. Antioxidants are compounds that can inhibit free radical reactions in the body [8]. The ability of Sidempuan salacca vinegar to regenerate kidney cells in hyperuricemic mice was examined more in this study.

2 Materials and methods

This research is based on true experimental design: pre- and post-test with control group design. Twenty-five male mice were divided into five groups. There were normal group (K-), hyperuricemia group (K+), hyperuricemia + salacca vinegar 0.2 mL (P1), hyperuricemia + salacca vinegar 0.4 mL (P2), hyperuricemia +salacca vinegar 0.8 mL group (P3). Mice strain Balb-C (*Mus musculus* L) were given an offal and egg yolk diet to create hyperuricemia in mice treated with K+, P1, P2 and P3 for 14 days. The study on animals and diet was approved by the Ethical Committee (No. 12.02/KEP-UNP/IV/2023) of Universitas Negeri Padang. Uric acid levels are measured triple using easy touch GCU before a high uric acid diet, after a high uric acid diet, and after treatment with salacca vinegar. Histological observations of the kidney were carried out after administering salacca vinegar for 14 days to hyperuricemia mice using the paraffin method with Haematoxylin-Eosin staining.

Uric acid level data was analysed using the ANOVA test, followed by the Duncan test. Observation of kidney histopathology preparations refers to the method carried out by Kamaliani [9] namely by observing changes that occur in kidney histopathology preparations and then scoring the kidney condition. The criteria for the changes observed were fatty degeneration and necrosis in the kidney tissue. Histopathology scoring data were then analysed through the Kruskal-Wallis test. If the results obtained $p < 0.05$ (significantly different), then further tests were carried out using the Mann-Whitney test.

3 Result and discussion

3.1 Blood uric acid level

The administration of salacca vinegar significantly affected the uric acid levels of male mice (*Mus musculus*) hyperuricemia, as seen in Fig. 1.

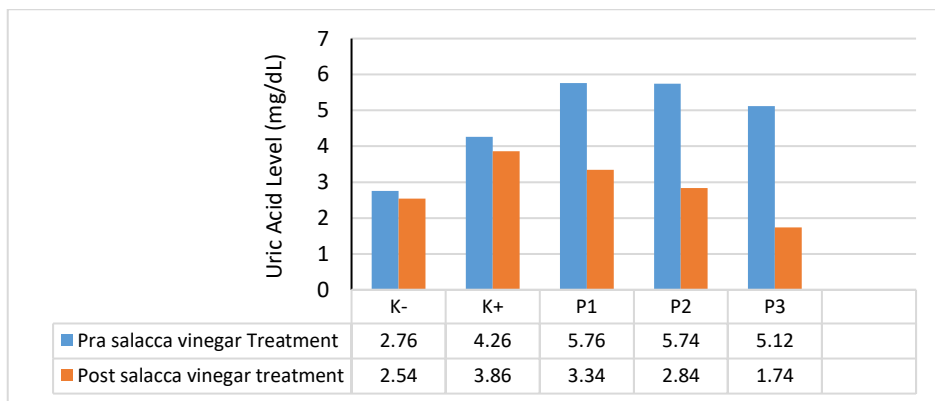


Fig. 1. Uric acid levels in hyperuricemic mice pre-and post-treatment with Sidempuan salacca vinegar

This research showed that all mice had hyperuricemia, as marked in the blue bar graph. Then, there was a decrease in uric acid levels after being given salacca vinegar (orange bar graph). Mice are said to have hyperuricemia if their body's uric acid level reaches 1.7-3.0

mg/dl. The highest reduction was in the P3 treatment, namely 3.38 mg/dL from 5.12 to 1.74 mg/dL, and the lowest was in the K- treatment, namely 0.22 mg/dL from 2.76 to 2.54 mg/dL. This is in accordance with the results of the Duncan advanced test, which showed that P3 was statistically significantly different from other treatments (Table 1)

Table 1. Result of the DMRT test, the mean of decreasing uric acid levels

Group	Sign
K-	0.22 ^a
K+	0.4 ^a
P1	2.42 ^b
P2	2.9 ^{bc}
P3	3.38 ^c

Salacca vinegar has natural antioxidant compounds that can help neutralise free radicals. Reducing high or excessive uric acid levels can be reduced by consuming antioxidants. In salacca vinegar, its antioxidant activity is influenced by the high content of phenols and organic acids [11]. Apart from antioxidants, salacca vinegar also contains phenols, tannins, vitamin C and flavonoids [12]. Salacca extract has antihyperuricemia activity [13]. This experiment was carried out on mice that had been induced with sodium urate. In this study, doses of salacca extract of 100 and 200 mg/kg bw could reduce uric acid by 7.9 and 8.3%. The flavonoid content in salacca meat can reduce uric acid levels in mice. Flavonoids work by inhibiting the xanthine oxidase enzyme [14].

3.2 Kidney histopathology

The results of histopathological observations of the kidneys of hyperuricemia male mice in all treatment groups can be seen in Fig. 2. Determination of the level of kidney damage based on research by Hilmawan namely focal kidney damage (mild), if the damage is found in the same location, multifocal kidney damage (moderate), if the damage is found in several locations, and diffuse kidney damage (severe), if the damage is found evenly [15].

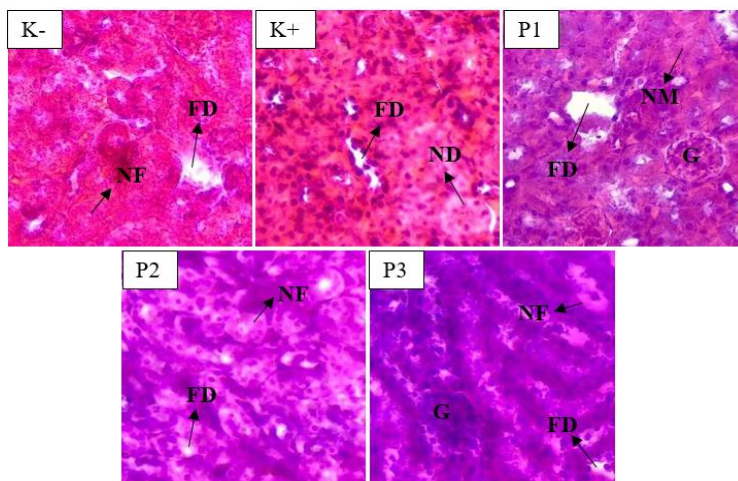


Fig. 2. Histopathology of kidney cells (400x magnifications). K- = normal diet, K+ = high uric acid diet without salacca vinegar, P1 = high uric acid diet + salacca vinegar dose of 0.2 ml, P2 = high uric acid diet + salacca vinegar dose of 0.4 ml, P3 = high uric acid diet + salacca vinegar dose of 0.8 ml. G: Glomerulus, FD: Fatty Degeneration, NF: Focal Necrosis NM: Multifocal Necrosis, ND: Diffuse Necrosis

Data histopathological observations of hyperuricemia mice kidneys show that in K- there was fatty degeneration and focal necrosis. This damage is classified as mild. This is thought to be due to the food given to the mice. This is thought to be due to the diet given to the mice. Mice were fed *ad libitum*. Diet plays an important role in increasing or decreasing uric acid levels in the blood. Eating pattern is a permanent pattern of relationship with food based on the type of food ingredients and their frequency [4]. In the K- treatment group, mice were given food in the form of B2 pellets. B2 pellets are a type of feed made from highly nutritious ingredients [15]. Thus, B2 pellets can increase uric acid levels in mice. If uric acid production exceeds normal limits, uric acid can become free radicals in the body [16]. The K+ treatment group showed fatty degeneration and diffuse necrosis. This damage is classified as severe. This is thought to be because mice consume high-purine foods, namely offal. Consuming high-purine foods can increase uric acid production and increase free radicals that could damage cells.

The P1 treatment group showed fatty degeneration, and multifocal and diffuse necrosis were seen. This indicates an improvement in kidney damage in the P1 treatment group compared to the K+ treatment group. However, the improvement was not optimal. This is thought to be due to the low dose of salacca vinegar given to the P1 treatment group. The P2 treatment group showed fatty degeneration and focal and multifocal necrosis. This indicates an improvement in kidney damage in the P2 treatment group compared to the P1 treatment group. In the P3 treatment group, fatty degeneration and focal necrosis were seen in the focal classified. This also indicates an improvement in kidney damage in the P3 treatment group compared to the P2 treatment group.

Under normal circumstances, uric acid production produces byproducts in the form of superoxide anions. Superoxide anion is a type of free radical that is highly reactive and can cause damage to cell membranes. Superoxide anion is produced from the work of the enzyme xanthine oxidase, which converts hypoxanthine and xanthine into uric acid using oxygen as a catalyst [5]. If the amount of superoxide anion is high in the body, this can cause damage to cell membranes due to an imbalance between the amount of free radicals and antioxidants [14].

In inhibiting the free radical reaction of superoxide anion, which is a byproduct of the uric acid formation process, that is by donating hydrogen atoms to peroxy radicals to form flavonoid radicals, and they will react with reactive superoxide anions so that they become neutral and do not damage body cells. Therefore, the antioxidant compounds in Sidempuan salacca vinegar improved the histopathology of the kidneys of treated hyperuricemic mice [17].

4. Conclusion

Sidempuan salacca vinegar is effective in improving kidney degeneration in hyperuricemic male mice. The higher the dose given showed better the improvement.

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