Effectiveness of betel leaves (*Piper betle* l.) and breadfruit leaves (*Artocarpus atilis*) in reducing uric acid levels in hyperuricemic male white rats (*Rattus norvegicus*)

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Abstract. Hyperuricemia is a condition where plasma uric acid is elevated. Prolonged elevated uric acid levels increase the risk of gout. Herbal medication is an alternative therapy for gout with minimum side effects. Betel (*Piper betle*) leaves and breadfruit (*Artocarpus atilis*) leaves contain natural substances that may reduce uric acid levels. The study aims to compare the effectiveness of betel leaves and breadfruit leaves extract in reducing plasma uric acid levels in hyperuricemic male white rats using a pre-and-post control group experimental research with 24 hyperuricemic male white rats divided into 4 groups. Negative control group were given normal feed and aquadest. Positive control group were given high-purine feed and aquadest. Group 1 and 2 were given high-purine feed and betel leaf extract of 332mg/200gBW and breadfruit leaf extract of 500mg/200gBW respectively. Data were analyzed using paired t-test.

Positive control group showed uric acid level of 8.01mg/dl and 8.25mg/dl (pre and post-test respectively). Betel leaf extract group showed with post-test uric acid level of 2.02mg/dl from 8.09mg/dl, while breadfruit leaf extract group from 8.12mg/dl to 2.28mg/dl. Betel leaf and breadfruit leaf extract were effective in reducing uric acid levels in hyperuricemic male white rats.

1. Introduction

Hyperuricemia is a condition where plasma uric acid levels are elevated above normal due to over-production or under-excretion or combination of both factors. Prolonged hyperuricemia increases the risk of gout [1]. Incidence of hyperuricemia in the world has continue to increase in the last 40 years and varies with nation [2,3]. The prevalence of hyperuricemia in the general population is estimated to be 20-25%, but only 4-6% in premenopausal women. Progressive elevation of plasma uric acid is caused by increase in obesity levels and excessive consumption of sugary beverages, food with high purine content, and alcohol [4,5].

Based on 2013 Indonesian Basic Health Research (RISKESDAS), the prevalence of joint-related diseases based on medical professional diagnosis is 11.9% and based on symptoms is 24.7% with highest prevalence in East Nusa Tenggara (33.1%), followed by West Java (32.1%) and Bali (30%) [6]. With the recent advance in medical technology, hyperuricemia can be treated with not only pharmacological therapy but also non-pharmacological therapy. Pharmacological therapy of hyperuricemia includes drugs such as allopurinol with a dosage of 100-300mg daily, but with side effects such as nausea, vomiting, diarrhea, bone marrow suppression, aplastic anemia, and peripheral...
neuritis [7]. Therefore, herbal extracts can serve as an alternative therapy such as betel leaves and breadfruit leaves [8].

Betel (Piper betle L.) leaves contains chemical compounds such as betelphenol, sesquiterpenes, diastase, sugars, triterpenoids, flavonoids, as well as tannin and leaves extract of 332mg/200gBW can reduce uric acid level [9]. Breadfruit (Artocarpus altulis) leaves contain flavonoids, phenols, riboflavin, tannin, acetylcholine and potassium and is believed to reduce the effect of xanthine oxidase in uric acid formation [10,11]. Breadfruit leaves extract of 500mg/200gBW has uric acid reducing properties. This research would like to compare to the effective dose of betel leaves extract and breadfruit leaves extract in uric acid reduction in hyperuricemic male white wistar rats.

2. Materials and Methods

2.1. Hyperuricemic induction of male white wistar rats

Hyperuricemic induction were done by feeding the rats with 3mg/200gBW chicken liver juice once daily for 7 days. Chicken liver is a high in purine with 150-1000mg purine per 100g liver and able to induce high uric acid levels. The rats were then divided into groups and measured for pre-test and post-test uric acid levels.

2.2. Extraction of betel leaves

Fresh betel (Piper betle L.) dried in oven at 40°C and then minced. Extraction was done using maceration technique with 70% ethanol as solvent for 48 hours to extract active components. Filtrates were filtered and evaporated using rotary evaporator. The extracted compound in 70% ethanol were then diluted to corresponding concentrations.

2.3. Extraction of breadfruit leaves

Fresh breadfruit (Artocarpus altulis) dried in oven at 40°C and then minced. Extraction was done using maceration technique with 70% ethanol as solvent for 48 hours to extract active components. Filtrates were filtered and evaporated using rotary evaporator. The extracted compound in 70% ethanol were then diluted to corresponding concentrations.

2.4. Data analysis

Data were analysis statistically between pre-treatment and post-treatment results using paired t-test. Statistical significance was set at 95% confidence and p<0.05 is considered statistically significant.

3. Results and discussion

3.1. Extract dosing

The subjects were divided into the following groups: negative control group with 6 rats given standard feed and aquadest, positive control group with 6 rats given high purine feed and aquadest, betel leaves extract group with 6 rats given high purine feed and betel leaves extract of 332mg/200gBW, and breadfruit leaves extract group with 6 rats given high purine feed and breadfruit leaves extract of 500mg/200gBW. No rats dropped out during the experiments.

3.2. Anti-hyperuricemia activity

Positive control were given high purine feed and aquadest, betel leaves extract group given high purine feed and betel leaves extract of 332mg/200gBW, and breadfruit leaves extract rats given high purine feed and breadfruit leaves extract of 500mg/200gBW. Phenolic compound in betel (Piper betle L.) leaves and breadfruit (Artocarpus altulis) leaves is known to reduce blood uric acid levels. Phenolic compounds are abundantly found in plants and they are characterized by an aromatic ring and one or more hydroxyl (OH) group and other side groups. Phenolic compound from betel leaves and breadfruit leaves has antioxidant properties and varies between the two extracts. The phenolic compound in betel leaves is 50.38±0.08mg/g while breadfruit leaves is 55.416 ± 5.78 mg/g [12,13].

Phenolic components occur naturally and have highly varied structure and easy to find in many plants, leaves, flowers, and fruits. Thousands of phenolic compound has been identified including flavonoid, simple monocyclic phenol, propanoid phenil, polyphenol (lignin, melanin, tannin), dan phenolic quinone [11].
Table 1. Levels of uric acid before and after supplementation of betel and breadfruit leaves extracts.

| Group                    | N  | Average uric acid levels (mg/dl) | Average uric acid reduction (mg/dl) |
|--------------------------|----|---------------------------------|-------------------------------------|
|                          |    | Pretest | Post-test |                          |
| Negative Control         | 6  | 1.68    | 1.74      | -0.0600                 |
| Positive Control         | 6  | 8.01    | 8.25      | -0.2400                 |
| Betel leaves extract     | 6  | 8.09    | 2.02      | 6.0700                  |
| Breadfruit leaves extract| 6  | 8.12    | 2.28      | 5.8400                  |

The average reduction in the negative control group was -0.060mg/dl, positive control was 0.240mg/dl, betel leaves extract group was 6.070mg/dl, and breadfruit leaves extract was 5.840mg/dl.

Figure 1. Levels of uric acid before and after supplementation of the leaf’s extracts. Reduction in uric acid levels is due to the antioxidative property betel leaves and breadfruit leaves.

One the phenolic components that acts as an antioxidant is flavonoid. Flavonoid is a secondary metabolite found in abundance in plant tissues. Flavonoid is a member of phenolic compound with chemical structure of C6-C3-C6[14]. Flavonoid acts as an antioxidant by donating its hydrogen atom or by its metal chelating properties, in glucosides from (containing glucose side chain) or in free from known as aglikon.14 Flavonoid functions as an inhibitor in uric acid formation and able to reduce the level by blocking xanthine oxidase, the enzyme responsible for regulating uric acid formation [15]. Flavonoid content varies from plant to plant and betel leaves extract contains 52.16±0.61QE/g [16] while breadfruit leaves extract contains 44.763 QE/g [17] and the difference in flavonoid content may be the reason behind the difference in effectiveness in reducing uric acid levels.

The results showed that both betel leaves extract and breadfruit leaves extract have p value of 0.000, which means both extracts were effective in reducing uric acid levels with betel leaves is better in reducing uric acid levels of as much as 6.070mg/dl compared to breadfruit leaves extract of 5.840mg/dl.

4. Conclusion
Supplementation of both betel leaves (332mg/200gBW) and breadfruit leaves (500mg/200gBW) extracts have significant reduction effect on uric acid levels in white male rats, with betel leaves extract having higher uric acid reduction effect of 6.070mg/dl compared to breadfruit leaves extract with 5.840mg/dl.
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