Uric acid levels on sub-chronic oral administration of Cassava leaf extract

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Abstract. Cassava leaf one of herb in Indonesia needed the safety data to develop as herbal medicine. Cassava leaf contains carbohydrate, protein, flavonoid, triterpenoid, saponin, tannin, mineral, and vitamin C. The cassava leaf contained moderate purine potentially increasing the plasma uric acid. This study aims to analyze uric acid levels on plasma rat that given sub chronically cassava leaf extract. This research was an experimental posttest with control group design. The total of 36 adult rat was randomly divided into 4 groups. The experimental groups were treated Cassava leaf extract orally for 45 days. The doses were 80 mg/kg body weight (P1), 400 mg/kg body weight (P2) and 2000 mg/kg body weight. Peripheral blood was taken from orbital sinus in day 45, then was measured the uric acid level. The average of blood uric acid levels for all groups were K (1.27 mg/dL), P1 (2.13 mg/dL), P2 (1.38 mg/dL) dan P3 (1.43 mg/dL). All of values were in range of normal uric acid value for rat (1.7 - 3.0 mg/dL). The Anova analysis resulted that no difference uric acid level between control and experiment groups. We concluded that the sub chronic giving of cassava leaf extract of 80 mg/kg body weight, 400 mg/kg body weight and 2000 mg/kg body weight did not increase the uric acid levels on rat.

1. Introduction

Indonesia is a tropical country which has high biodiversity. Cassava or often called as Manihot utilissima Pohl. is a plant that endemic in Indonesia. Cassava leaf not only food ingredient, but also use to medicinal herb. Cassava leaf extract contain terpernoids, tannins, flavonoids, carotenoids that potentially to develop as medicine [1]. The ethanol extract of cassava leaves was developed into standardized antiacne [2]. The other study showed that Cassava leaf extract inhibited the COX-2 expression and potentially as an alternative anti-inflammation agent [3]. The Cassava leaf extract was able to inhibit carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats [1].

Cassava leaf extract as a candidate of herb medicine must fulfill the effectiveness and safety data. Many data showed the effectiveness data, but only a little data about the safety. Flavonoid of cassava leaf extract stay in a long time in the plasma before excretion via urine [4]. Cassava leaf extract need more than 48 hours to clearing from the plasma [5]. This condition may increase probability not only the effectiveness but also the toxic effect of extract. Hyperuricemia is one of the toxic effect may lead Cassava leaf when given in high dose or in a long time.

Hyperuricemia is a condition may influence by the daily diet. Previous study showed the high crude protein levels, β-carotene levels and lipid levels of many variant of Cassava leaf [6]. Crude protein was

contained of cassava leaves ranged from 177 to 240 g/kg dry matter [7]. Other study found protein which contained in cassava leaf was 1-10g in every 100g of cassava leaf [8]. This value indicated that cassava leaf has a containing moderate purine. The moderate purine levels in cassava leaf potentially increase the level of uric acid. Uric acid is the end products of purine catabolism. Uric acid mainly synthetized from endogenous, and only a little influence arises from exogenous sources such as foods with purine content, alcohol, and fructose drinks. Uric acid levels increase or abnormal in the body when kidneys are not able to secrete through urine or if the total intake of purine was high. The sub chronic treatment experiment aims to get the safety data of Cassava leaf on uric acid level.

2. Material and methods

2.1. Animal experimental and treatment

The Cassava leaf extract was processed by maceration methods. The extractor solution was aquadest. The posttest control group design experiment was conducted to 36 Wistar rats. The animals for experiment were divided randomly into four experimental groups, that were Control group (K), P1, P2 and P3. The maintain of rats were in the group cage and acclimatized for 14 days before given the treatment. The treatment groups of K, P1, P2, dan P3 were given cassava leaf extract with dose of each was 0, 80, 400 dan 2000 mg /kg body weight [9-11]. The giving of cassava leaf extract into treatment group was done everyday for 45 days using gastric sonde. The blood was taken in the 45^{th} day, and prepared for uric acid level measurement.

2.2. Measurement of uric acid level

The blood was taken from orbital sinus using hematocrit capillary pipettes. The blood centrifuged for 10 minutes with the speed of 4000 rpm. The uric acid levels were measured base on enzymatic reaction using reagen uric acid FS*TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid) produce by DiaSys. The sample solvent was made by taking 20 µL of serum plus 1000 µL of monoreagent. Serum which mixed homgeneous with reactor Uric Acid FS* TBHBA incubated for 6-8 minutes in the tempeprature of 37ºC. Next, the sample solvent, standard, and blank was read by spectrophotometer StartDust FC* 15 on the wavelength of 546 nm.

2.3. Data analysis

The data of uric acid level was analyzed by Anova to know the difference among groups, then followed Duncan test. The data also compared with the normal value of uric acid level in rat.

3. Results

The sub-chronic treatment of Cassava leaf extract orally was important to get the toxicity or safety data. The data result gained the blood serum uric acid levels of rat after giving the cassava leaf extract sub-chronically. The average of uric acid levels of rat after giving the cassava leaf extract was showed in Table.1.

Table 1. The average of uric acid levels of rat after 45 days oral treatment of Cassava leaf extract.

Treatment group	Average of uric acid levels ±SD (mg/dL)
K (0 mg/kg BW)	1.27±0.465
P1 (80 mg/kg BW)	2.13±0.790
P2 (400 mg/kg BW)	1.38±0.477
P3 (2000 mg/kg BW)	1.43±0.630

Before doing the anova test, first implementing the requirement test from that is normality test and data homogeneity. The result of normality test shows that p= 0,406 can be mentioned that it is a normal data distribution. Then, continued with the data homogeneity test where the result shows that p= 0,726 so it

can be said that it is homogeneous. The result of anova test showed the *Sig.* = 0.025, it means that there was a difference of uric acid level depend on the dose was given. The Duncan test showed the difference between P2 group and other groups.

4. Discussion

Uric acid normally exist in the bloodstream. Uric acid levels in blood can be maintained in normal limit by exceeding through the kidney. So, the level of uric acid was influence not only by intake of purine, but also by normally of kidney function. The average of uric acid levels indicated that there was no enhancement of uric acid levels on rat that given cassava leaf extract. All of values were in range normal uric acid levels of rat [12]. Its means that sub-chronic oral treatment of extract didn't influence the purine metabolism of rat.

Uric acid in blood at normal levels act as a natural antioxidant by completing the deficiency of electron and inhibit the occurrence of chain reaction from free radical formation which can cause oxidative stress. If the uric acid is excessive, the uric acid will not be accommodate and metabolized at all by the body. This matter that causes the enhancement of uric acid in blood. Uric acid is formed in liver and released into the blood circulation.

In this study, the uric acid of all groups was in the normal range. This was caused by the formed uric acid is directly released through kidney. The end of uric acid metabolic process, the uric acid was excreted via urine in the normal value:165-335 mg/dL. Normal function of kidney has essential role in managing uric acid levels, because kidney set the excessively uric acid excretion in the body. Uric acid is mainly excreted through kidney where it will be all filtered in glomerulus, reabsorbed in proximal tubule and then excreted and finally it will be partly reabsorbed again; in the amount of 10% it will be excreted [13].

The obtained data shows that the giving of cassava leaf extract at dose of 40,80 and 2000 mg/bb do not increase the white rat blood serum uric acid levels. Cassava leaf contains flavonoid, triterpenoid, saponin, tannin and vitamin C. Flavonoid containing in the high-level plant has function as antioxidant [14]. Plant that has antioxidant activity and flavonoid positive potentially has xanthine oxidase inhibitor activity [15]. Xanthine oxidase is competitively inhibition. In this kind of inhibitor, the one which has competitively inhibitor mechanism is compound that has structure resemble the substrate structure [16]. The compound will compete with xanthine substrate to occupy the position of active enzyme that will cause the decrease and cease of enzyme activity, so the production of xanthine oxidase enzyme which is uric acid is not formed.

The uric acid level of each individuals varies depend on its synthesis and its excretion. The uric acid level did not be a problem if the excretion or the disposal process go balance. The balance between formation and excretion of uric acid are determined by several enzymatic pathways. This enzymatic pathway was in different genetically-defined isoforms being also highly regulated by pathophysiological determinants including metabolic products and free radical species [17]. The disposal that still leave some uric acid will increase uric acid levels because the longer it stays the more uric acid that buried in the body. The enhancement of uric acid also can happen because the disposal of uric acid is slower than the formation. This can happen due to disturbance of kidney function or incapability of kidney to exceed excessively uric acid in the body, so the uric acid levels cannot be exceeded normally and causes problems such as the enhancement of uric acid in blood. Based on the research result proved that the giving of cassava leaf extract in the dose of 80,400 and 2000 mg/ BB do not affect blood uric acid levels of rat.

The study revealed that supplementing cassava peels with cassava leaves and cowpea haulms as protein sources has no negative effects on rumen fermentation and blood biochemical parameters of West African dwarf goats [18]. The similar result found that no changes chemical panel in the blood after 30 days ingestion fresh Cassava leaf in goats [19]. During the normally function of all system in the body, there are nothing a problem foe consumed the Cassava leaf in along time. The reasonable consumption of plant foods with a higher purine, included Casava leaf may therefore be safely tolerated

in normo-uricemic individuals, but additional data is needed in hyper-uricemic individuals, especially those with chronic kidney disease [20].

5. Conclusion

Cassava leaf not only the source of food, but also potentially to develop as herb medicine. Many data showed the cyanide containing in the leaf and root raised the toxic effect, but the other studies found the safety data for long time consumption. Based on the research result, it can be concluded that the giving of cassava leaf extract in varies dose orally for 45 days do not give impact towards uric acid levels of rat.

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Conflict of interest

We declare that no conflict of interest in this article.

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