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Anti-hyperuricemicactivity of granule formulated from *Anonna muricata* L. fruit juice on hyperuricemia induce Sprague-Dawleys rat

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Abstract
The *Annona muricata* or soursop fruit has long been used in folk medicine for the treatment of variable ailments including hyperuricemia. Recent study has successfully formulated the soursop fruit juice into more practical preparation of instant granule drink. The aim of this study was toinvestigate the antihyperuricemic activity of instant granuleformulated from soursopfruit juice on hyperuricemia induce Sprague Dawleys rat. The soursop granulewas produced from fresh fruit juice with the addition of carboxymethyl cellulose (CMC), citric acid, sucralose and maltodextrin. Different doses of granule (90mg/200g b.wt.180 mg/200g b. wt. and 270 mg//200g b. wt.) were administeredorally to potassium oxonate-induced Sprague Dawley rats. The uric acid level of rats wasmeasured subsequently on the day 4, 8, and 12 of treatment period. The soursop fruit juice produced bright white granule with tipically fresh sour sweet taste, 2.9% water content (<5%), 1.91% ashcontent, 3.13 ml/s solubility, 29.3% angle of repose, and stable in the storage at 15^0C for two months. The soursop granule have shown significant antihyperuricemic activityin both male and female Sprague Dawley rats. The highest percentage of uric acid level decrease occurs at day 8in male rats treated with 270 mg soursop granule and at day 12 in female rats treated with 180 mglevel of soursop granule respectively. The overall the results show thatthe soursop granule has a potent as antihyperuricemic natural medicine which works in gender and time dependent manner, and can be developed to substitute synthetic antihypertensive drugs.

Keywords: *Anonna muricata* L, anti-hyperuricemic, hyperuricemia, soursop granule

1. Introduction
Hyperuricemia, commonly known as gout is a metabolic disorder of purine metabolism that affects about 10% of the world population [1]. The desease characterized by irregular elevated in plasma uric acid andthe deposition of monosodium urate crystals in joints and other tissues, cause an acute inflammatory response with appearance of ulceration of the joint cartilage, marginal osteophytosis, erosive lesions and chronic inflammation of synovial membrane followed by a permanent tissue damage [2, 3]. Recent evidences suggest that gout and hyperuricaemia are the risk factors for high blood pressure and cardiovascular disease [4, 5]. The drugs widely used in the therapeutic and clinical management of gout against hyperuricemia is allopurinol, the synthetic xanthine oxidase inhibitor from purine analog group. However, the use of allopurinol proven to cause several side effects typically associated with immunoallergic manifestations such as fever, rash, eosinophilia, lymphadenopathy, lymphocytosis, arthralgias and facial edema [6]. Allopurinol also reported implicated in acute liver injury [7], increases the risk of cataract in elderly people [8] and other metabolite syndromes [9]. The search for naturalxanthine oxidase inhibitors, therefore have became the object of interest in the recent because of their ease of availability, less adverse side effects and cost effective.Many studies reported the natural medicine from plant have shown some inhibitory effect on xanthine oxidase and proved effective in decreasing uric acid levels in blood [10, 11, 12].

Annona muricata, commonly known assoursop fruit,has long been used as a natural remedy for a variety ofillnesses and subject of countless medicinal uses. The bark and leaves of the soursop tree was confirmed to contain antioxidant, insecticide, antipyretic, antidiabetic, antihypertensive and antibacterial activities [13, 14]. Recent studies revealed that the extracts obtained from various parts of annonaceae plant possess antioxidant, anti-lipidemic [15, 16], anti-diabetic [17], xanthine oxidase inhibitor [18], anti-oxidant and anti-hyperuricemic activity onrats [19, 20].

Despite the fact that the pharmacological propertiesof medicinal plants have scientifically been proven and widely used in themodern world, very little work has been done to process these natural ingredientsinto more practical and easy to drink preparation.

Herbs in form of concentrated granules has gain its popularity as hot and cold instant drinks to take medicines in many markets. Individual sachets contain a single dose of granules that dissolves in water, making it easy to dose and stored for subsequent use and other advantages. Granules are agglomerates of powdered materials prepared into larger, free flowing particles or a small compact particle of a substance. The shape of granules is generally irregular with the size typically fall within the range of 850 μm (no. 20 sieve) to 4.75 mm (no. 4 sieve) size. Granules have a smaller surface area than a comparable volume of powders which makes this form more stable physically and chemically than the corresponding powders and are less likely to cake or harden upon standing than are powders [21]. Compare to other preparation such as syrup or powder, the medicinal herbal in form of granule offer many advantages such as having good flow property, better compression characteristics and uniformity, easy to dose and swalow, remain stable in hot water, dissolve quickly and without leaving residues, even in cold water [22]. The granules also easily combined with colorants, flavorants, and other pharmaceutical ingredients, so the resulting solution or suspension has all the desired medicinal and pharmaceutical features of a liquid pharmaceutical. Granulation may be defined as size enlargement process which corverts fine or coarse powder into physically larger and stronger particles. Granulation also produce particle-size uniformity, thus content uniformity.

In relation to the lack of anti-hyperuricemic drugs in the market,the development of natural drug in granule form offer many advantages such as safe to consume in the long term, pleasant to drink, and low production cost. The objective of this study was toinvestgate the anti-hyperuricemic activities of granule obtained from soursop fruit on oxonate-induced hyperuricemia rats.

2. Materials and methods

2.1 Chemicals and apparatus

Tools used in the study were digital scale (AND G-120®),uric acid test scale Easy Touch® GCU ET 301, evaporator (Buchi Heathy Bath B-490, Syncore®),moisture balance(AND MX 50®),freezedryer (GVD-12, Girovac Ltd), granule flow tester (EFT-10, Electrolabindia),powder tapdensitometer(USP Bulk Density Tester® 315-2E), sieved (Forward Filter&Fitting Co. Ltd), oven (Ney®), and glass tools. All chemicals were of analytical grade, product of Merck & Co., Inc. and Sigma-Aldrich Inc.

2.2 Preparation of plant material

Mature soursop fruits was bought from the local market in Bogor, Indonesia. The soursop fruits were then washed carefully under flowing tap water, peeled, cut into halves, and deseeded. Fruit flesh obtained were put in heat resistant containers and steamed for 4 min over boiling water. The steamed fruit flesh then keep in the referigerator for subsequent uses.

2.3 Preparation of instant granule

The soursop fruit instant granule was formulated according to previous study by Djarot and Badar [23] and processed usingwet granulation method. Granulation may be defined as size enlargement process which corverts fine or coarse powder into physically larger and stronger particles, produce particle-size uniformity, thus content uniformity.

The fruit flesh was placed on plain cotton cloth and squeezed using wringer to obtain pure fruit juice. The fruit juice was dried in freeze dryer machine with addition of 20% of maltodextrin as a filler to obtain instant fruit powder (active ingredient). The active ingredient were mixed with carboxymethyl cellulose (CMC), citric acid, sakaralose, and maltodextrin as excipient and flavoring (Table 1).

Table 1: Instant granule formula

Ingredients	Percentage (%)	Weight (g)
Soursop juice powder	63	12.6
CMC	1	0.2
Citric acid	2	0.4
Saccharalose	0.1	0.02
Maltodextrin	33.9	6.78
Total weight	100	20
Active ingredients per sachet 12.6 g (equivalent to 100 g of soursop fresh weight)		

Soursop juice powder, CMC, citric acid, sucralose and maltodextrin were weighed according to the formula, mixed, and sieved through 30-mesh sieve. The mixture was stirred until homogenous before sprayed with 70% ethanol sufficiently to form granule mass and dried in an oven at 40 ºC-50 ºC for 30 minutes. The coarse granule obtained from this process was sieved through 12-mesh sieve, sprayed with 70% ethanol and dried in an oven at 40 ºC-50 ºC for 3 hours and resieved through12-mesh sieve. The obtained granule was evaluated for physical characteristics such as color, water content, ash content, solubility, angle of repose, and stability in storage. Finally, product was packaged in small sachet containing 20 g of soursop instant drink. Based on the formula above, content of the active ingredient in each sachet drink is 12.6 g which was equivalent to 100 g of soursop fresh fruit weight.

2.3 Experimental design and animal acclimatization

The experiment in this study was conductedusing randomized controlled design. Adult male and female Sprague Dawley rats weighing between 200-250g were prepared separately as test animals. The rats were randomly divided into 10 experimental groups (five male groups and 5 female groups, each group consisting of 10 rats). Prior to the experiment, the rats acclimatized for a period of 2 weeks under controlled environmental condition in accordance with the standard of the care and use of laboratory animals. The uric acid levels of ratsduring acclimatitation period were measured to ensure the rats had normal uric acid levelranging from 4 to 6 mg/dL for male rats and 3 to 4 mg/dL for female rats.

2.4 Hyperurcemia induction and animals' treatment

The experimental procedure was carried out according to previous methods of Liu *et al*. [24] with several minor modifications. To induce hyperuricemia condition, the experimental rats were intraperitonial injected with 4,5 mg/200 g b. wt potassium oxonate daily for ten consecutive days.The increaseof uric acid level in rats was then observed and measured**.** After hyperuricemia induction, each group of experimental rats was treated daily with different doses of soursop granule (Table 2).

Group1 administered with 90 mg/200g b. wt.soursop instant granule

Group 2 administered with 180 mg/200g b. wt. soursop instant granule

Group 5 administered with 270 mg/200 g b. wt. soursop instant granule

Group4 (positive control) administered with 0.45 mg/200 g b. wt. captopril (common antihypertensive drug)

Group 5 (normal control) administered with 2 ml aquades/200 g b. wt.

During the treatment period, the uric acid level of rats was subsequently measured using theuric acid test scale kit on the day 4, 8, and 12 of treatment.

Table 2: Grouping of experimental rats based on dose of soursop granule

Groups of rats	Dose of soursop granule
Group 1	90 mg/200g b. wt.
Group 2	180 mg/200g b. wt.
Group 3	270 mg/200g b. wt.
Group 4 (positive control)	allopurinol 2.7 mg/200g b. wt.
Group 5 (negative control)	2 mL aquadest/200g b. wt.

2.5 Statistical analysis

All the values of systolic and diastolic pressure were expressed as mean±SD (Standard Deviation). Statistical differences between the means of various groups were evaluated by Duncan's multiple range test (MRT) where means followed by same letter in the same column are not significantly different at P≤0.05.

3. Results

3.1 Formulation and production of soursop juice fruit instant granule

The soursop juice powder obtained from 10 kg of soursop fruit with addition of 20% maltodextrin was 1.575 kg or equivalent to 1.26 kg pure soursop juice powder without addition of maltodextrin. This data shows that fresh soursop fruit effectively could produce 12.6% dry active ingredients. The instant drink obtained from processing soursop juice powder as main ingredient appeared as bright white granule with tipically fresh sour sweet taste and smell, 2.9% water content (<5%), 1.91% ash content, 3.13 ml/s solubility, 29.3% angle of repose, and stable in the storage at 15^0C for two months.

3.2 Potassium oxonate induction

Uricase inhibitor potassium oxonate treatment caused hyperuricemia in rats, which was indicated by increasing the level of uric acid in rat's blood serum. Average of uric acid level on male and female rats before and after potassium oxonate induction is presented inthe (Table3) and (Table4).

Tabel 3: Uric acid level of male rats after potassium oxonate induction

Experimental groups	Uric acid level (mg/dL) Day 0	Day 10
Group 1	4.6	10.2
Group 2	4.4	10.7
Group 3	4.8	11.4
Group 4	6.1	10.1
Group 5	5.9	11
Average	5.12±0.81	10.89±0.81

Data are expressed as mean±SD of ten rats in each group.

Tabel 4: Uric acid level of female rats after potassium oxonate induction

Experimental groups	Uric acid level (mg/dL) Day 0	Day 10
Group 1	3.8	15.7
Group 2	4.0	15.4
Group 3	4.5	11.9
Group 4	3.7	16.0
Group 5	3.4	16.7
Average	3.8±0.4	15.1±3.02

Data are expressed as mean±SD of ten rats in each group.

3.3 Antihyperuricemic activity of soursop instant granule on male and female rats

The decrease of uric acid level and the percentage of uric acid decrease in maleand femalerats during treatment was presented in (Table 5), (Table 6), (Table 7) and (Table 8).

Table 5: Average of uric acid level (mg/dL) of male rats during treatment period

Experimental groups	Uric acid level (mg/dL)		
	Day 0	Day 4	Day 8
Group 1	10.2±1.27	8.7^{b} ±0.76	6.5^{c} ±0.24
Group 2	10.7±3.06	8.5^{b} ±2.77	5.5^{bc} ±0.96
Group 3	11.4±0.90	8.0^{b} ±0.72	4.3^{b} ±0.36
Group 4	10.1±.07	7.5^{a} ±3.44	4.0^{b} ±1.63
Group 5	11.6±0.66	11.8^{c} ±0.68	11.9^{d} ±0.57

Data are expressed as mean±SD of ten rats in each group, where the means followed by the same letter in the same column are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 6: Percentage of uric acid decrease in male rats

Experimental groups	Percentage of decrease (%) Day 4	Day 8
Group 1	15%	36%
Group 2	20%	59%
Group 3	30%	62%
Group 4	55%	45%
Group 5	-1%	-2%

Table 7: Average of uric acid level (mg/dL) of female rats during treatment period

Experimental groups	Uric acid level (mg/dL)			
	Day 0	Day 4	Day 8	Day 12
Group 1	15.7±2.4	11.8^{b}±1.09	8.3^{b} ±1.53	4.5^{a} ±0.99
Group 2	15.0±1.7	9.1^{a}±2.09	6.1^{a} ±1.47	4.3^{a} ±0.63
Group 3	11.9±5.3	7.4^{a}±1.24	5.9^{a} ±0.54	4.0^{a} ±0.70
Group 4	16.0±2.9	7.5^{a} ±1.71	4.6^{a} ±1.47	3.5^{a} ±0.55
Group 5	16.6±2.2	12.6^{b} ±1.08	10.9^{c} ±0.86	8.1^{b} ±1.77

Data are expressed as mean±SD of ten rats in each group, where the means followed bysame letter in the same column are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 8: Percentage of uric acid decrease in female rats

Experimental groups	Percentage (%)		
	Day 4	Day 8	Day 12
Group 1	23.6%	46%	78.3%
Group 2	38.8%	58.2%	78.8%
Group 3	32.3%	44.4%	71.3%
Group 4	51.7%	70.9%	86.5%
Group 5	23.4%	3.5%	56.4%

4. Discussion

4.1 Potassium oxanate induction

The datas showed that the uric acid levels were significantly increase after 10 days of potassium oxonate administration both in male and famale rats. Average of uric acid level in male rats increased by 53.7%% from 5.12±0,81 to 10.89±0,81mg/dLmeanwhile the average of uric acid level in female rats increased by 73.3% from 3.8±0,4mg/dLto 14.8±1,8 mg/dL. The increasing of uric acid level in female rats turn to be 36% higher compare to the uric acid level in male rats, reveal that female rats tend to be more sensitive to the potassium oxonate intake.

The differences of uric acid levels according to gender

probably associate with insulin resistance and plasma glucose levels [25, 26]. Other studies also revealed the uric acid levels are significantly different depending on age and gender in which uric acid levels more closely associated with metabolite syndromes in females than in males [27, 28].

4.2 Soursop granule treatment

The overall statistical data show that administration of soursop granule from the lowest to highest dose had significant effect to decrease the uric acid levels in bothmale and female rats. In the male rats, the highest percentage of uric acid decrease occur in group 3 (270 mgsoursop granule treatment) and reach its normal level at day 7. Datas on the table (5) show that the decrease of uric acid level in male rats correlated to the increase of soursop granule dose. Different data pattern can be seen in female rats wherethe highest percentage of uric acid decrease occur in group 2(180 mg soursop granule treatment) and reach its normal level at day 14. The results indicated that the male rats need higher dose of soursop granule compare to the female rats but in the other side, it takes shorter time to decrease the uric acid level in male rats compare to the female rats. The differences in length of and time and level of dose required to lowering uric acid levels in male and female rats probably due to the very high level of uric acid in female rats after potassium oxonate induction and the diffrencesin metabolism of male and female rats. However, further study should be conducted to reveal the mechanism of soursop granule in lowering uric acid level and determine optimum dose and duration of treatment since the efficacy of drugs commonly was effected by these factors [29-33].

Other promising fact confirmed by the efficacy of soursop granule to decrease the uric acid level in male rats which higher than the efficacy of allopurinol. At day 7 of the treatment, soursop granule proved decrease uric acid level by 62% compare to 45% decrease of uric acid level with allopurinol treatment. This shows that soursop granule has high capacity in decreasing the uric level. The high capacity of soursop granule to decrease uric acids level probably related to the presence of polyphenol compounds and vitamin C which act as xanthine oxidase inhibitor and antioxidant agent. Xanthine oxidase is the type enzymes that catalyze the oxidation of hypoxanthine to xanthine and further catalyze the oxidation of xanthine to uric acid [34] and considered to bean important biological source of superoxide radicals [35]. Many studies have confirmed the positive linkage between polyphenol and vitamin C as antioxidant agent and the decrease of uric acid level in patients with hyperuricemia [36-40]. The total phenol content found in the extract of soursop fruit pulp was 120 mg/100 g [40] and the citrate concentration in soursop juice was higher (8.82 g/L) than in WHO/UNICEF Oral Rehydration Salt (ORS) preparation standard (2.9 g/L) [41]. Recent study reveal that the soursop fruit pulp extract has scavenging activity and ferric reducing ability in which 1000 ppm of the fruit pulp extract was equivalent to that of 25 ppm of vitamin C and the total phenol content of the extract of fruit pulp was 139 mg Gallic Acid Equivelant /100 g [42]. This redox value indicates that extract of soursop pulp in term of redox has moderate antioxidant capacity. The dual effect of polyphenols as XO inhibitors and free radical scavengers respectively, provide alternative therapeutic strategies to prevent hyperuricemia, based on the use of low doses of synthetic XO inhibitors in combination with natural antioxidants and vitmanin C supplementation to optimize the efficacy of drug and minimize the undesirable side effects [43].

The hypouricemic activity of soursop granule in the other hand possibly caused by the presence of certain uricase-like compounds in the granule. Uricase or urate oxidase is an enzyme that plays an important role in purine metabolism, catalyse the oxidation of uric acid to a water soluble allantoin and extreted in the urine [44]. This enzyme is widely present in most vertebrates but is absent in humans, thus the hyperuricemic problem can be diminish byuricase intake from the food supply [45]. Various natural resources have been found to produce uricase such as bacteria [46] fungi [47] and higher plants [48, 49]. Thus, the presence of certain compounds in soursop granule which probably act like uricase enzyme can be used as a therapeutic agent for the treatment of hyperuricemia and gout.

With many possibities in the mechanism of how soursop granule could decrease the uric acid level in rats, further research should be conducted to elucidate the compounds responsible for antihyperuricemic activity contained in the soursop fruit.

5. Conclusion

The granule produced from the juice of soursop fruit proved effective to decrease uric acid level in both oxonate-induced male and femalerats. The possible mode of action of the granule might be related to high polyphenolic compounds and vitamin C or the presence of uricase-like compounds in the soursop fruit. Further, soursop granule can be produced commercially to substitute the use of allopurinol.

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Conflict of interest

Authors hereby declare that there were no conflict of interests in the accomplisment of this study.

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