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Research Article
Potential of Leave and Fruit Ethanolic Extract of *Etlingera hemisphaerica* as Antihyperuricemic in Mice (*Mus musculus*)

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Abstract

Background and Objective: Hyperuricemia is a disease triggered by disorders of uric acid metabolism. Therefore, this study evaluated the potential of leaves ethanolic extract of *Etlingera hemisphaerica* (LE3H) and fruits ethanolic extract *E. hemisphaerica* (FE3H) to restore hyperuricemia in mice. **Materials and Methods:** Six groups (A0, A1, A2, A3, A4 and A5) each consisted of four male mice. Hyperuricemia in mice was induced by giving 0.3 mL of fresh chicken liver juice (FCLJ) for seven days (A1, A2, A3, A4 and A5). The condition of hyperuricemia in A1 is not neutralized. Meanwhile, hyperuricemia conditions in A2, A3, A4 and A5 were neutralized for seven days by giving 0.01 mg g^{-1} body weight (BW) allopurinol, 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H. The control group (A0) only received double-distilled water in the same way. Blood uric acid levels were measured with the GCU Meter Device before and after the induction of hyperuricemia and after efforts to neutralize the hyperuricemia condition. Six groups (B0, B1, B2, B3, B4, B5) each consisting of four male mice were also provided to test the potential of FE3H. The recovery potential FE3H against hyperuricemia was tested separately in the same way as was done for LE3H. **Results:** Giving FCLJ significantly increased (140.00-187.00%) uric acid compared to the control, so hyperuricemia was achieved. Doses of 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H significantly recovered hyperuricemia as much as 54.09, 56.14 and 60.88%, respectively. Meanwhile, doses of 0.13, 0.26 and 0.36 mg g^{-1} FE3H significantly recovered hyperuricemia as much as 60.37, 62.24 and 65.572%, respectively. The LE3H and FE3H at the same dose showed that FE3H had a higher potential to restore hyperuricemia than LE3H. **Conclusion:** Leave and fruit ethanolic extract of *E. hemisphaerica* can potentially restore hyperuricemia in mice.

Key words: *Etlingera hemisphaerica*, mus musculus, hyperuricemia, leaves ethanolic extract, fruit ethanolic extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the current health problems in the people of Bengkulu, Indonesia is uric acid. Uric acid is the end product of the process of purine catabolism originating from food scraps, most of which are excreted through the kidneys[1]. The uric acid level in humans is considered normal if it is between 2.6-6 mg dL^{-1}, low if <2.6 mg dL^{-1} and said to be high if the level is > 6.8 mg dL^{-1} [2]. Increased uric acid levels above normal conditions are called hyperuricemia. Hyperuricemia is a metabolic disorder associated with high levels of uric acid in the body, resulting in the deposition of urate crystals in the joints and kidneys (nephrolithiasis), causing inflammation and arthritis[3]. Hyperuricemia also frequently occurs in higher primates including humans due to the inactivation of the uricase gene during primate evolution[4]. Hyperuricemia is thought to develop into gout, kidney disease, hypertension, hyperlipidemia, cancer, diabetes and obesity[5].

Sufferers of uric acid often complain of joint pain at night and in the morning when they wake up. Avoiding pain is one of the basic human needs which is the goal of nursing. The standard drug for hyperuricemia that is widely used is a synthetic uricostatic class, such as allopurinol. The deficiency of uricosuric is that it can cause side effects, including allergic reactions and symptoms of toxicity in various organs and body systems[6]. This underlies the need for the development of safer alternative hyperuricemia therapies. Herbal concoctions have long been used to cure ailments. Herbal therapy is a therapy that utilizes medicinal plants. Treatment using medicinal plants is relatively inexpensive and can be obtained from the natural surroundings or by planting yourself[7].

Leaves salam (*Syzygium polyanthum*) are known to contain tannins, alkaloids, steroids, triterpenoids and flavonoids[8]. This research was conducted to see how giving boiled water from *S. polyanthum* leaves reduces uric acid levels in 10 people with gout sufferers. Before being given the boiled water of *S. polyanthum* leaves, the uric acid level is 7.16 mg dL^{-1}, with the highest uric acid level being 8.2 mg dL^{-1} and the lowest uric acid level being 6.4 mg dL^{-1}. After administration of *S. polyanthum* leaf boiled water, the average uric acid level was 5.76 mg dL^{-1}, with the highest uric acid level being 6.7 mg dL^{-1} and the lowest uric acid level is 4.9 mg dL^{-1}. The average difference in the results of reducing uric acid levels before and after administration of *S. polyanthum* leaf boiled water was 1.40 mg dL^{-1}. It can be concluded that after the administration of boiled water of *S. polyanthum* leaves there was a decrease in uric acid levels, which can be seen from the average difference in uric acid levels before and after administration. The decrease in uric acid levels by utilizing *S. polyanthum* leaves is influenced by the flavonoid content in bay leaves, the use of bay leaves can inhibit the action of the hypoxanthine enzyme so that the formation of uric acid can be inhibited. In addition, *S. polyanthum* leaves are also efficacious as a diuretic which helps excrete uric acid through urine. This is what makes *S. polyanthum* leaves useful for controlling uric acid[9].

Administration of ethanol extract of the herb Putri Malu (*Mimosa pudica*) at a dose of 0.25 g kg^{-1} BW for 1 day and 3 days can reduce uric acid levels by 0.860 mg dL^{-1} and 1.105 mg dL^{-1}. The extract dose of 0.5 g kg^{-1} BW after 1 day and 3 days of administration decreased by 0.658 mg dL^{-1} and 1.400 mg dL^{-1}. The ethanol extract of *M. pudica* herb at a dose of 0.5 g kg^{-1} BW after 1 day and 3 days of administration reduced uric acid levels in the blood induced by FCLJ. The decrease in uric acid levels after administration of *M. pudica* herb ethanol extract for 1 day and 3 days was probably due to the presence of flavonoids from the *M. pudica* herb ethanol extract. From the results of the identification of the flavonoids of the ethanol extract of *M. pudica* herb by ultraviolet absorption spectroscopy and visible light absorption methods. One of them is the addition of $AlCl_3$, causing a spectral shift from 324 nm to 396.50 nm, from the UV absorption spectrum range-it seems to be interpreted that the flavonoids contained in the ethanol extract of *M. pudica* belong to the flavone group[10].

Alternative herbal therapy for hyperuricemia is quite safe from malacca plants (*Salacca zalacca*). In the ethanol extract of *S. zalacca* bark, flavonoid compounds act as antioxidants and anti-inflammatories. Serum malondialdehyde (MDA) levels will return to the normal range when the inflammatory process and tissue damage subside, therefore a compound is needed that can stop the inflammatory process in individuals with hyperuricemia. The content of flavonoids in the skin of *S. zalacca* is able to stop the inflammatory process. The mechanism of action of flavonoids in *S. zalacca* peel extract is to inhibit the lipid peroxidation process at the initiation stage by acting as scavengers against reactive oxygen free radicals and hydroxyl radicals. Flavonoids donate H+ atoms to peroxyl radicals to form flavonoid radicals that react with reactive oxygen (superoxide) to become neutral. This reaction can stop the chain reaction of lipid peroxidation processes[11]. Allopurinol at a dose of 2.52 mg/kg BW/day was still superior to the ethanol extract of *S. zalacca* bark at a dose of 420 mg/kg BW/day in reducing MDA levels and inhibiting kidney damage in hyperuricemic white rats[12].

The *S. polyanthum, M. pudica* and *S. zalacca* are examples of herbal ingredients that have been tested to have the potential to cure hyperuricemia because they contain flavonoids[9-12].

Meanwhile, several ethnic groups in Bengkulu have a tradition of using forest honje (*Etlingera hemisphaerica*) as herbal medicine. The results of the phytochemical tests carried out showed that the leaves of *E. hemisphaerica* are plants that contain flavonoids, alkaloids, saponins and tannins[13]. These compounds are chemical compounds that are anti-inflammatory and have the ability to reduce uric acid in the blood[14]. Based on these facts, this study evaluated the potential of leaves ethanolic extract *E. hemisphaerica* (LE3H) and fruits ethanolic extract *E. hemisphaerica* (FE3H) to restore hyperuricemia in mice.

MATERIALS AND METHODS

This research lasted for 4 months (August-November 2022) at Sumber Belajar Ilmu Hayati (SBIH) Ruyani, Bengkulu, Indonesia[15].

Extract preparation: The identity of the *Etlingera hemisphaerica* (Blume) plant was verified by the Center for Plant Conservation and Botanical Gardens Research, LIPI, Bogor, Indonesia, (http://lipi.go.id/,NumberB-1750/IPH.3./KS/V/2019). The leaves/fruits of *E. hemisphaerica* were collected from Lebong District, Bengkulu Province, Indonesia. The leaves/fruits were cleaned and then sliced into small pieces of 3,000 g/6,260 g fresh leaves/fruits of *E. hemisphaerica*, then dried with the help of wind and ground into a powder of 800 g/1,360 g dried leaves/fruits. The powder was macerated for seven days in 96% ethanol, then concentrated using a rotary evaporator at 50°C and the filtrate was condensed to obtain a concentrated extract[16]. After the ethanol contained in the concentrated extract evaporates, the crude extract can be used as a test material in this study[17].

Phytochemical screening of LE3H and FE3H: Sucrose level of LE3H and FE3H was analyzed quantitatively by High-Performance Liquid Chromatography (HPLC, Gadjah Mada University, Indonesia, https://lppt.ugm.ac.id/id/)[18], while five test parameters (flavonoids, alkaloids, saponins, tannins and phenols) of LE3H and FE3H were analyzed quantitatively. The quantitative analysis was done with UV-vis Spectrophotometry (1800 Shimadzu)[19-23].

Fresh chicken liver juice (FCLJ): The purpose of making fresh chicken liver juice (FCLJ) is for mice to have a high purine diet to increase uric acid levels, achieving a hyperuricemia condition. The composition of this chicken liver juice is Leghorn chicken liver 20 g which was made into juice using up to 100 mL of water. The FCLJ was made by mashing chicken liver weighing 20 g and adding 100 mL of water, then in a blender[10] and then FCLJ is ready to use. Induction of hyperuricemia was carried out by giving FCLJ at a dose of 0.3 mL/20 g BW for seven days.

Dosages: Hyperuricemia in mice was induced by giving 0.3 mL/20 g BW of (FCLJ) for 7 days[10]. Allopurinol is a generic drug used to lower uric acid levels in the blood. This drug works by inhibiting the enzyme xanthine oxidase thereby inhibiting the formation of uric acid and can also inhibit the synthesis of purines. This study used a 0.01 mg g^{-1} body weight (BW) dose of allopurinol as a positive control[10]. According to the report, the three doses of LE3H and FE3H used in this study were 0.13, 0.26 and 0.39 mg g^{-1} BW[19-26].

Group of experimental animals: The first stage (A) used 24 male mice which were divided into 6 groups (A0, A1, A2, A3, A4 and A5). On day (D)1, all groups of mice were satisfied, then the early determination of blood uric acid levels was measured using a glucose, cholesterol and uric acid (GCU) Meter Device. Five mice groups, A1, A2, A3, A4 and A5, were induced by giving 0.3 mL of fresh chicken liver juice (FCLJ) for seven days to achieve hyperuricemia. On day 8, further determination of blood uric acid levels was measured by the GCU Meter Device (Halodoc Building, Jl. HR Rasuna Said Kav. B32-33, South Jakarta 12940). The condition of hyperuricemia in A1 was not neutralized. Meanwhile, hyperuricemia conditions in A2, A3, A4 and A5 were neutralized by giving 0.01 mg g^{-1} body weight (BW) allopurinol, 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H for seven days. On day 15, the final determination of blood uric acid levels was measured by the GCU Meter Device (Table 1).

The second stage (B) used 24 male mice which were divided into 6 groups (B0, B1, B2, B3, B4 and B5). On the day (D) 1, all groups of mice were satisfied, then the early determination of blood uric acid levels was measured using the GCU Meter Device. Five groups of mice, namely B1, B2, B3, B4 and B5 were induced by giving 0.3 mL of fresh chicken liver juice (FCLJ) for seven days to achieve hyperuricemia. On D 8, further determination of blood uric acid levels was measured by the GCU Meter Device. The condition of hyperuricemia in A1 was not neutralized. Meanwhile, B2, B3, B4 and B5 hyperuricemia conditions were neutralized for seven days by giving 0.01 mg g^{-1} body weight (BW) allopurinol, 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H. On day 15, the final determination of blood uric acid levels was measured by the GCU Meter Device (Table 1).

Blood uric acid level determination: Blood uric acid concentration in *M. musculus* was measured using the

Table 1: Research design to determine the potential of leaves ethanolic extract *E hemisphaerica* (LE3H) and fruits ethanolic extract *E hemisphaerica* (FE3H) to restore hyperuricemia in mice

			Research activity, on the day (D)				
Research stage	Experimental animals' group	N	D1 (O)	D2-7 (T)	D8 (O)	D9-14 (T)	D15 (O)
A: The potential of leaves ethanolic extract *Etlingera hemisphaerica* (LE3H) to restore hyperuricemia in mice	A0: Controls, only given DDW	4	Early determination of blood uric acid levels [GCU]	DDW	Further determination of blood uric acid levels [GCU]	DDW	Final determination of blood uric acid levels [GCU]
	A1: 0.3 mL FCLJ	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	DDW	Final determination of blood uric acid levels [GCU]
	A2: 0.3 mL FCLJ+0.01 mg g^{-1} BW allopurinol	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.01 mg g^{-1} BW allopurinol [G]	Final determination of blood uric acid levels [GCU]
	A3: 0.3 mL FCLJ+0.13 mg g^{-1} BW LE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.13 mg g^{-1} BW LE3H [G]	Final determination of blood uric acid levels [GCU]
	A4: 0.3 mL FCLJ+0.26 mg g^{-1} BW LE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.26 mg g^{-1} BW LE3H [G]	Final determination of blood uric acid levels [GCU]
	A5: 0.3 mL FCLJ+0.36 mg g^{-1} BW LE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.36 mg g^{-1} BW LE3H [G]	Final determination of blood uric acid levels [GCU]
B: The potential of fruits ethanolic extract *Etlingera hemisphaerica* (FE3H) to restore hyperuricemia in mice	B0: Controls, only given DDW	4	Early determination of blood uric acid levels [GCU]	DDW	Further determination of blood uric acid levels [GCU]	DDW	Final determination of blood uric acid levels [GCU]
	B1: 0.3 mL FCLJ	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	DDW	Final determination of blood uric acid levels [GCU]
	B2: 0.3 mL FCLJ+0.01 mg g^{-1} BW allopurinol	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.01 mg g^{-1} BW allopurinol [G]	Final determination of blood uric acid levels [GCU]
	B3: 0.3 mL FCLJ+0.13 mg g^{-1} BW FE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.13 mg g^{-1} BW FE3H [G]	Final determination of blood uric acid levels [GCU]
	B4: 0.3 mL FCLJ)+0.26 mg g^{-1} BW FE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.26 mg g^{-1} BW FE3H [G]	Final determination of blood uric acid levels [GCU]
	B5: 0.3 mL FCLJ+0.36 mg g^{-1} BW FE3H	4	Early determination of blood uric acid levels [GCU]	0.3 mL FCLJ [G]	Further determination of blood uric acid levels [GCU]	0.36 mg g^{-1} BW FE3H [G]	Final determination of blood uric acid levels [GCU]

N: Number of animal repetitions, BW: Body weight, FCLJ: Fresh chicken liver juice, LE3H: Leaves ethanolic extract *E. hemisphaerica,* FE3H: Fruit ethanolic extract *E. hemisphaerica,* DDW: Double-distilled water, T: Treatment, O: Observation, G: Administrated by oral gavage, GCU: Glucose, Cholesterol and Uric Acid Meter Device

glucose, cholesterol and uric acid (GCU) Meter Device (https://apotekalkes.com/product/EASY-TOUCH-GCU-181818) Produced by Bioptic Technology with Indonesia Ministry of Health Registration: AKL20101902214 (Halodoc Building, Jl. HR Rasuna Said Kav. B32-33, South Jakarta 12940, Phone: +62 21-5095-9900, Email: help@halodoc.com). Previously, the GCU Meter Device as a uric acid meter was adjusted to the code printed on the strip packaging. After that, blood is dripped on the strip, waited for about ±5 sec to read the blood uric acid level. The level of accuracy shown by the GCU Meter Device is the blood uric acid concentration in units of mg dL^{-1} [27].

Statistical analysis: Multiple comparisons were used to generalize the data from this study, followed by a significant difference test at the 95% confidence level[28].

Ethical statement: This research focuses on the ethics of using animals as well as aspects of human treatment, following the principle of the five freedoms (F), which include (a) freedom from hunger and thirst, (b) freedom from discomfort, (c) free from pain, injury and disease, (d) free from fear and long-term pressure and (e) freedom to express standard patterns of behavior[29]. This study follows the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The working protocol was accepted by the Bengkulu University Animal Experiment Ethics Committee (No. 53/KEH-LPPM/EC/2022, April 18, 2022).

RESULTS

Phytochemical analysis of LE3H and FE3H was carried out simultaneously in the same way. Five of the six test parameters, namely flavonoids, alkaloids, tannins, sucrose and phenol levels in FE3H were higher than in LE3H. Meanwhile, the levels of saponins in LE3H (2.32% w/w) were higher than in FE3H (1.64% w/w) (Table 2).

Giving 0.3 mL of FCLJ for seven days significantly increased (104.23-134.94%) uric acid (A1, A2, A3, A4 and A5) compared to the control (A0), so hyperuricemia was achieved. The condition of hyperuricemia in one group was not neutralized (A1). Administration of 0.01mg g^{-1} BW Allopurinol for seven days reduced hyperuricemia by 54.09% (A2). It was further noted that the administration of doses of 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H for seven days significantly recovered hyperuricemia as much as 54.09, 56.14 and 60.88%, respectively (A3, A4 and A5). The LE3H has the same or higher potential for reducing hyperuricemia than Allopurinol. The results of statistical tests showed that the administration of FCLJ significantly increased uric acid higher than the control, while the administration of LE3H was able to restore hyperuricemia not different from the control (Table 3).

Giving 0.3 mL of FCLJ for seven days significantly increased (30.30-121.78%) uric acid (B1, B2, B3, B4 and B5) compared to the control (B0), so hyperuricemia was achieved. The condition of hyperuricemia in one group is not neutralized (B1). Administration of 0.01mg g^{-1} BW Allopurinol for 7 days reduced hyperuricemia by 55.00% (B2). It was further noted

Table 2: Comparison of phytochemical content of leaf ethanolic extracts *E. hemisphaerica* (LE3H) and fruit ethanolic extract *E. hemisphaerica* (FE3H)

	Results			
Test parameter	LE3H	FE3H	Unit	Method
Total flavonoid	18.14	32.99	% (w/w)	Spektrofotometri UV-vis
Total alkaloid ekuivalen quinine	0.26	2.05	% (w/w)	Spektrofotometri UV-vis
Total saponin from quillaja bark	2.32	1.64	% (w/w)	Spektrofotometri UV-vis
Tannin total ekuivalen tannic acid	7.25	20.89	% (w/w)	Spektrofotometri UV-vis
Total Fenol Ekuivalen Asam Galat	1.64	19.88	% (w/w)	Spektrofotometri UV-vis
Sucrose	0.64	2.96	% (w/w)	HPLC

Table 3: Potential of leaves ethanolic extract *E. hemisphaerica* (LE3H) to restore hyperuricemia in mice

Experimental animals' group	N	Early determination of blood uric acid levels (mg dL^{-1})	Further determination of blood uric acid levels (mg dL^{-1})	Final determination of blood uric acid levels (mg dL^{-1})	LE3H Potential for restoring hyperuricemia (%)
A0: Controls, only given DDW	4	3.55±0.34	3.50±0.22	3.50±0.41[a]	
A1: 0.3 mL FCLJ	4	3.50±0.74	9.05±1.78	10.05±1.21[b]	
A2: 0.3 mL FCLJ+0.01 mg g^{-1} BW Allopurinol	4	3.38±0.43	7.95±0.30	3.15±0.13[a]	54.09
A3: 0.3 mL FCLJ+0.13 mg g^{-1} BW LE3H	4	3.70±0.60	7.45±0.57	3.15±0.10[a]	54.09
A4: 0.3 mL FCLJ+0.26 mg g^{-1} BW LE3H	4	3.55±0.13	7.25±0.26	3.18±0.13[a]	56.14
A5: 0.3 mL FCLJ+0.36 mg g^{-1} BW LE3H	4	3.95±0.65	9.28±1.65	3.63±0.30[a]	60.88

N: Number of animal repetitions, DDW: Double-distilled water, FCLJ: Fresh chicken liver juice, LE3H: Leaves ethanolic extract *E. hemisphaerica* data followed by the same superscript letter(a, b), then the data is not significantly different[28]

Table 4: Potential of fruits ethanolic extract *E. hemisphaerica* (FE3H) to restore hyperuricemia in mice

Experimental animals' group	N	Early determination of blood uric acid levels (mg dL^{-1})	Further determination of blood uric acid levels (mg dL^{-1})	Final determination of blood uric acid levels (mg dL^{-1})	LE3H Potential for restoring hyperuricemia (%)
B0: Controls, only given DDW	4	3.55±0.34	3.50±0.22	3.50±0.40^{c}	
B1: 0.3 mL FCLJ	4	5.05±0.10	6.58±0.75	8.40±1.00^{d}	
B2: 0.3 mL FCLJ+0.01 mg g^{-1} BW Allopurinol	4	4.65±0.60	8.00±0.59	3.62±0.33^{c}	55.00
B3: 0.3 mL FCLJ+0.13 mg g^{-1} BW LE3H	4	3.98±1.08	8.58±1.18	3.40±0.43^{c}	60.37
B4: 0.3 mL FCLJ+0.26 mg g^{-1} BW LE3H	4	5.15±0.23	9.93±0.30	3.75±0.20^{c}	62.24
B5: 0.3 mL FCLJ+0.36 mg g^{-1} BW LE3H	4	4.78±1.19	10.60±1.43	3.65±0.34^{c}	65.57

N: Number of animal repetitions, DDW: Double-distilled water, FCLJ: Fresh chicken liver juice, FE3H: Fruit ethanolic extract *E. hemisphaerica*, data followed by the same superscript letter (c, d), then the data is not significantly different[28]

Table 5: Comparison of the percentage potential of leaves ethanolic extract *E. hemisphaerica* (LE3H) and ethanolic extract *E. hemisphaerica* (FE3H) for restoring hyperuricemia in mice

		Potential for restoring hyperuricemia (%)	
Experimental animals' group	N	LE3H	FE3H
A3/B3: 0.3 mL FCLJ+0.13 mg g^{-1} BW *E. hemisphaerica*	4	54.09	60.37
A4/B4: 0.3 mL FCLJ+0.26 mg g^{-1} BW *E. hemisphaerica*	4	56.14	62.24
A5/B5: 0.3 mL FCLJ+0.36 mg g^{-1} BW *E. hemisphaerica*	4	60.88	65.57

N: Number of animal repetitions, FCLJ: Fresh chicken liver juice, LE3H: Leaves ethanolic extract *E. hemisphaerica* and FE3H: Fruit ethanolic extract *E. hemisphaerica*

noted that the administration of doses of 0.13, 0.26 and 0.36 mg g^{-1} BW LE3H for seven days significantly recovered hyperuricemia as much as 60.37, 62.24 and 65.57%, respectively (B3, B4. and B5). The LE3H has the potential to reduce hyperuricemia higher than Allopurinol. The results of statistical tests showed that the administration of FCLJ significantly increased uric acid higher than the control, while the administration of LE3H was able to restore hyperuricemia not different from the control (Table 4).

The LE3H and FE3H at the same dose showed that FE3H had a higher potential to restore hyperuricemia than LE3H (Table 5).

DISCUSSION

Comparison of phytochemical content of the leaf ethanolic extracts *E. hemisphaerica* (LE3H) and the fruit ethanolic extract *E. hemisphaerica* (FE3H) revealed that five of the six test parameters (flavonoids, alkaloids, tannins, sucrose and phenol) levels in FE3H were higher than in LE3H. Meanwhile, the levels of saponins in LE3H (2.32% w/w) were higher than in FE3H (1.64% w/w). Flavonoids are the most prominent phytochemical content in LE3H and FE3H (Table 2). Flavonoids have been recognized as molecules with great potential as solutions for hyperuricemia and gout at preclinical and clinical levels[30,31]. One non-toxic natural flavonoid, quercetin (QC), in a safe dosage range with antioxidant, anti-apoptotic and anti-inflammatory properties, plays an important role in the treatment of aging-related diseases[32]. The last report states that the QC level in LE3H was 7.47±0.2 µg mL^{-1} [33]. If the presence of QS has been detected in LE3H, then FE3H which contains higher levels of flavonoids is suspected to have QC levels in line with the presence of these flavonoids. It was further proven that the percentage of FE3H potential for restoring hyperuricemia at a dose of 0.13 mg g^{-1} BW was 60.37% (Table 4), while for LE3H at the same dose, it was 54.09% (Table 3).

There were several factors that can trigger an increase in uric acid levels in a person's blood, including often eating foods with high purine content, such as red meat, animal offal and several types of seafood. Fresh chicken liver juice (FCLJ) is an offal animal that contains a lot of purines, so when FCLJ (0.3 mL) was given to mice in gavage for 1 week (A2/Table 3, B2/Table 4) it markedly increased blood uric acid levels compared to control (A0/Table 3, B0/Table 4). Several other studies have also used FCLJ to induce hyperuricemia in test animals[34-36]. Some reports explain that allopurinol is one of the drugs to recover the body from hyperuricemia[37-39]. The facts from this research showed that 0.13 mg g^{-1} BW LE3H works equivalent to 01 mg g^{-1} BW allopurinol in 54.09% restoring hyperuricemia, whereas, at the same dose, 0.13 mg g^{-1} BW FE3H shows a restoring hyperuricemia effect of 60.37% (A3/B3 Table 5). Uric acid is the main substance that causes hyperuricemia (gout), the end product of purine metabolism[40]. It is formed from purines primarily by oxidation catalyzed by xanthine oxidase (XO) of hypoxanthine and xanthine. One of the gout treatment mechanisms is to block the XO enzyme which will inhibit the oxidation of hypoxanthine and xanthine and further inhibit uric acid production[41].

A study was conducted on hyperuricemia mice to assess the effect of five flavonoids genistein, apigenin, QC, rutin and astilbin which showed a significant decrease XO *in vivo*[42].

It was further reported that the uric acid formation was reduced in hyperuricemia mice after oral administration of flavonoid-rich plant extracts such as rutin, QC, kaempferol and apigenin[43]. In another study, morin, myricetin, kaempferol, apigenin and puerarin at 50 and 100 mg/kg body weight elicited a hypouricemic action in hyperuricemia rats. These compounds significantly reduce serum uric acid levels by inhibiting hepatic XO as well[44]. The QC is effective against allergy, inflammation, arteriosclerosis and cancer due to its strong antioxidant properties and metal ion chelating capacity[45,46]. It is one of the most potent flavonoids that can interact by modulating the activity of various enzyme systems including lipoxygenase, phosphodiesterase and tyrosine kinase. The QC is a potent anti-gout compound that exhibits its activity through inhibition of the XO enzyme and exerts strong synergistic activity with kaempferol[47].

The fruit of *E. hemisphaerica* is quite popular among Indonesian people (https://eol.org/pages/1119400), has a distinctive aroma and taste image so that it can be developed as a raw material for the production of fresh drinks[48] and also has anti-hyperuricemic properties.

This preclinical research is limited to showing that the leaf and fruit ethanolic extract of *E. hemisphaerica* can potentially restore hyperuricemia in mice. These preclinical data form the basis that dietary supplementation with the leaf and fruit ethanolic extract may be beneficial in individuals with hyperuricemia problems. If the fresh drink *E. hemisphaerica* with anti-hyperuricemia benefits is successful, this preclinical research will develop into clinical research that has high pharmaceutical and economic value.

CONCLUSION

Leave and fruit ethanolic extract *Etlingera hemisphaerica* can potentially restore hyperuricemia in mice. A dose of 0.13 mg g^{-1} BW LE3H as the anti-hyperuricemic in mice is equivalent to a dose of 0.01 mg g^{-1} BW Allopurinol. The FE3H is more effective than LE3H to reduce uric acid in cases of hyperuricemia in mice. Dietary supplementation with LE3H and FE3H may be beneficial in individuals with hyperuricemia problems.

SIGNIFICANCE STATEMENT

This preclinical research shows that the leaf and fruit ethanolic extract of *Etlingera hemisphaerica* can potentially restore hyperuricemia in mice. Dietary supplementation with the leaf and fruit ethanolic extract may be beneficial in individuals with hyperuricemia problems. The *E. hemisphaerica* has a distinctive taste image and is widely known by the people of Indonesia. It is possible to produce *E. hemisphaerica* fresh drink with anti-hyperuricemia benefits.

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