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The potential of fruit ethanolic extract *Etlingera hemisphaerica* as a solution for hyperglycemia, uremia, and hypercreatininemia in mice (*Mus musculus*)

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ABSTRACT

Background: Leaf ethanolic extract of *Etlingera hemisphaerica* (LE3H) has the potential to restore glucose, triglyceride, and uric acid disorders and reduce mercury toxicity. High levels of glucose (hyperglycemia), urine (uremia), and creatinine (hypercreatininemia) in the blood cause real health problems. Meanwhile, the phytochemical content in fruit ethanolic extract of *E. hemisphaerica* (FE3H) is higher than that of LE3H.

Aim: This study evaluated the potential of FE3H as a solution for hyperglycemia, uremia, and hypercreatininemia in mice.

Methods: Quercetin levels in FE3H were determined by high-performance liquid chromatography. The first (A) stage used 25 male mice divided into five groups. On day (d)1, the body weight (BW) of the mice was weighed. On d2–11, 5-mg/gBW sucrose was given by gavage, and then, on d12, BW and blood glucose level of mice were determined. On d13, 0.52-mg/gBW glibenclamide was given in A2, 0.39-mg/gBW FE3H was given in A3, and 0.52-mg/g BW FE3H was given in A4 by gavage. Control, A0, was only given double-distilled water (DDW). On d14, BW and blood glucose levels of the mice were determined. The second (B) stage used 24 male mice divided into six groups. On d1, B1–B5 were injected intraperitoneally 5-mg/kg BW $HgCl_2$; then, on d3–5, they were given by gavage 0.2-mg/g BW Immunos® in B2, 0.39-mg/gBW FE3H in B3, 0.52-mg/gBW FE3H in B4, and 0.65-mg/gBW FE3H in B5. Controls, B0 and B1, were only given DDW. On d6, the mice were killed by cervical dislocation. The weight of the kidney was determined, and then, the urea and creatinine levels were measured in blood samples from the heart.

Results: FE3H contains 98.92 ± 12.88 µg/g quercetin. Sucrose tends to increase BW, and then, 0.39-mg/g BW FE3H treatment tends to restore BW close to control. Sucrose significantly increased blood glucose, and then, 0.39- and 0.52-mg/gBW FE3H treatment restored significantly blood glucose similar to control. $HgCl_2$ increases kidney weight, and then, 0.65-mg/gBW FE3H treatment tends to restore kidney weight close to control. $HgCl_2$ significantly increased urea and creatinine, and then, 0.52- and 0.65-mg/gBW FE3H treatment significantly restored urea and creatinine similar to the control.

Conclusion: FE3H, which is high in flavonoid quercetin, has the potential to restore hyperglycemia by 47.38%–48.18%, uremia by 29.04%–33.06%, and hypercreatininemia by 49.52%–54.28% in mice.

Keywords: *Etlingera hemisphaerica*, Sucrose, Mercury chloride, Hyperglycemia, Uremia, Hypercreatininemia.

Introduction

Forest honje [*Etlingera hemisphaerica* (Blume) R.M.Sm] was employed as traditional herbs and spices (http://www.theplantlist.org/tpl1.1/record/kew-243067) by several ethnic groups in Bengkulu, Indonesia. Leaves of *E. hemisphaerica* have high polyphenol content, which is good for curing dysentery. Ascorbic acid, minerals, and antioxidants are also present in *E. hemisphaerica*. Several research reports on *E. hemisphaerica* have been published in the form of five papers in reputable international journals and can be accessed online via the following link [https://pubmed.ncbi.nlm.nih.gov/?term=Etlingera+hemisphaerica]. In *Mus musculus* with hyperglycemia and hypertriglyceridemia, 0.39-mg/g body weight (BW) leaf ethanolic extract of *E. hemisphaerica* (LE3H) may potentially lower blood glucose (36.2%) and triglyceride (21.19%) levels (Ruyani *et al.*, 2014).

Researchers looking for herbal medicine found that giving 5-mg/kg BW mercuric chloride ($HgCl_2$) significantly increased the number of white blood cells. However, giving $HgCl_2$ followed by 0.39-

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mg/g BW LE3H may have lowered the number of white blood cells. Because $HgCl_2$ treatment therapy lowered erythrocyte counts while raising leukocyte counts, LE3H therapy was safe for erythrocyte and leukocyte counts. The liver weight was increased by 5-mg/kg BW $HgCl_2$, and the control decreased when LE3H treatment was used at a dose of 0.39 mg/kg BW. Researchers using histology found that $HgCl_2$ treatment made tissues swell and less extracellular space than the control group. However, giving 0.39-mg/g BW LE3H made the symptoms better. The results showed that LE3H protects the livers of mice from the harm induced by $HgCl_2$ (Ruyani *et al.*, 2017).

On the other hand, giving $HgCl_2$ and then 0.39-mg/g BW LE3H treatment kept the number of blood cells close to the control. Leukocytes and erythrocytes decreased when 5-mg/kg BW $HgCl_2$ was given. LE3H treatment could protect this protein profile since the control condition after $HgCl_2$ delivery showed a new 125-kDa protein and caused a 48-kDa protein to be overexpressed. When found in the blood of *M. musculus*, LE3H may help protect against $HgCl_2$ overdose. Because of this, LE3H pills might help protect people who are exposed to $HgCl_2$ (Ruyani *et al.*, 2019).

In an earlier work, LE3H was checked to see if it could protect *M. musculus* from $HgCl_2$'s teratogenic effects. The eight things measured were resorbed egg, dead fetus, living fetus, morphologically normal living fetus, deformed living fetus, the number of Malformed Living Fetus (MLFs), the length of Morphologically Normal Living Fetus (MNLF), and the weight of MNLF. Four of the values for LE3H were significantly different from the controls (50.00%), but seven of the values for $HgCl_2$ were significantly different (87.50%), showing that $HgCl_2$ was much more likely to cause congenital disabilities than LE3H. LE3H changed the teratogenicity of $HgCl_2$ in two ways: it went up by 22.50% and down by 67.50%. Therefore, LE3H lessened the effects of $HgCl_2$ on *M. musculus*, which caused birth defects (Ruyani *et al.*, 2020). Later, studies show that 0.39-mg/g BW LE3H might be a natural substance that can greatly reduce the problems with fetal anatomy and endochondral ossification that are caused by 5-mg/kg BW $HgCl_2$ on *M. musculus* during the time after conception (Ruyani *et al.*, 2021).

Leaves of *E. hemisphaerica* are always easy to find and make great research tools. The community often collects the blooming stage of the plant for different uses, which makes it hard to get to the fruit of *E. hemisphaerica*. We must examine how the phytochemicals in leaves and fruit are similar and different. Many papers (Ruyani *et al.*, 2014; 2017; 2019; 2020; and 2021) have looked into and written about the possibilities of the LE3H. New research suggests that LE3H and FE3H, an ethanolic fruit extract from *E. hemisphaerica*, can help mice with hyperuricemia. For mice, 0.01-mg/g BW allopurinol is the same as 0.13-mg/g BW LE3H when used to treat high blood pressure. The FE3H lowers uric acid better than the LE3H when mice have hyperuricemia (Karyadi *et al.*, 2023). Therefore, we need to study how FE3H can regulate the amounts of glucose, urea, and creatinine in mice.

Materials and Methods

Extract preparation

In the Lebong Regency of Bengkulu Province, Indonesia, fruits of *E. hemisphaerica* were found. The plant's identity was confirmed by the Indonesian Institute of Sciences' Research Center for Plant Conservation and Botanical Gardens in Bogor, Indonesia (http://lipi.go.id/; Number B-1750/IPH.3./3./KS/V/2019). Fresh *E. hemisphaerica* fruit weighing 6.26 kg was collected after being cleaned and cut into small pieces. The fruit was dried in the air for 10 days and then ground up to 1.36 kg of dried fruit. The powder was mixed with 96% ethanol for 7 days, concentrated with a rotating evaporator at 50 ˚C, and the filtrate was cooled down (Sopi *et al.*, 2013). This made an ethanolic extract of *E. hemisphaerica* (FE3H) fruit that was dark brown. Once the ethanol in the concentrated extract has evaporated, the FE3H crude extract can be used as a test sample for this study (Ruyani *et al.*, 2017; 2019; 2020).

Phytochemical screening of FE3H

High-performance liquid chromatography (HPLC; Knauer Smartline) (Macêdo *et al.*, 2021) was used to measure the amount of quercetin in FE3H at Gadjah Mada University in Yogyakarta, Indonesia (https://lppt.ugm.ac.id).

Dosage

The dosage of FE3H (0.39-, 0.52-, and 0.65-mg/g BW) was according to Ruyani *et al.* (2014, 2017, 2019, 2020). A 5-mg/g BW sucrose was given to mice for days (d)2–11 to make them develop hyperglycemia. It was used 0.52-mg/g BW glibenclamide, a sulfonylurea diabetes drug, as a positive control (POM: GKL9520905004A2) was given by gavage. There was one intraperitoneal (IP) injection of 5-mg/kg BW $HgCl_2$ (Merck, Germany; Product No. 104 417) (Chowdhury and Arora, 1982; Saxena and Kumar, 2004; El-Desoky *et al.*, 2013). These are the positive controls for LE3H therapy, and 0.2-mg/g BW Immunos® (POM SD 121542741) was given by gavage from PT Lapi Laboratories (Serang, Indonesia). The Immunos® has 500 mg of echinacea, 50 mg of ascorbic acid, 15 mcg of selenium, and 10 mg of zinc picolinate (Ruyani *et al.*, 2014). This dietary supplement strengthens the immune system against both short- and long-term diseases. In Bengkulu, Indonesia, it was bought at a drugstore.

Group of experimental animals

As an experimental animal, 49 male Swiss Webster mice (*M. musculus*) from the Animal Test Center, School of Life Sciences and Technology (SITH), Bandung Institute of Technology (ITB), Bandung, Indonesia, were employed.

The first (A) stage used 25 male mice aged 6–8 weeks with 25–35-g BW, which was divided into five (A0–A4) groups. On day (d)1, the BW of the mice was weighed. On d2–11, 5-mg/g BW sucrose was given by gavage, and then, on d12, BW and blood glucose level of mice were determined. On d13, 0.52-mg/g BW glibenclamide was given by gavage in A2, 0.39-mg/g BW FE3H was given by gavage in A3, and 0.52-mg/g BW FE3H was given by gavage in A4. Control, A0, was only given double-distilled water (DDW). On d14, BW and blood glucose levels of the mice were determined (Table 1).

In the second (B) stage, 24 male mice aged 6–8 weeks with 25–35-g BW, which was divided into six groups (B0–B5) were used. On d1, B1–B5 were injected IP 5-mg/kg BW $HgCl_2$; then, on d3–5, they were given by gavage 0.2-mg/g BW Immunos® in B2, 0.39-mg/g BW FE3H in B3, 0.52-mg/g BW FE3H in B4, and 0.65-mg/g BW FE3H in B5. Controls, B0 and B1, were only given DDW. On d6, the mice were killed by cervical dislocation (CD), and then, the abdominal and thoracic cavities were dissected. The weight of the kidney was determined, and then, the urea and creatinine levels were measured in blood samples from the heart (Table 1).

Blood glucose level determination

To check the blood glucose level in *M. musculus*, a small amount of blood from the tip of the tail was dripped onto a glucose strip that was then put into a glucometer brand Accu-Chek® (https://www.accu-chek.com/meters). The glucometer was previously set to the code on the glucose strip box. After the blood is on the strip, wait 5 seconds before using the glucometer to check the blood sugar level. The number shown on the glucometer is the blood glucose level of the mice in mg/dl (Andrikopoulos *et al.*, 2008).

Blood urea and creatinine level determination

The mice were killed by CD, the abdomen and chest were dissected, and 0.5 ml of blood was taken with a needle from the heart to determine urea and creatinine levels. The blood samples that had been taken were then put into non-ethylenediamine tetra acid tubes (Microtainer®) To obtain serum, the samples were centrifuged for 15 minutes at a speed of 3,000 rotations per minute (rpm). Serum was immediately initialized from red blood cells for 1 hour after sampling.

The ultraviolet (UV) method with a frequency of 340 nm was used to measure the amounts of blood urea in plasma. Here is how to find out how much urea is in your blood. Before using the BT35i, prepare 0.5 ml of blood, put it into a cloth activator, and spin it at 3,000 rpm for 15 minutes. You will then need to take serum and make blood urea reagent. The sample must then be put into the machine (Syahputra *et al.*, 2021).

Colorimetric testing with a 490-nm frequency was used to measure the amounts of creatinine in blood plasma. If the environment is alkaline, creatinine and picric acid should join to form a yellow–orange complex molecule. To measure blood urea amounts, create a cloth activator and put 0.5 ml of blood in it. Centrifugation at 3,000 rpm for 15 minutes was performed to obtain blood serum. Then, a blood urea reagent was made, and the sample was put into the BT35i device (Gul *et al.*, 2021).

Statistical analysis

Multiple tests (analysis variance, the one-way classification) and the least significant difference (LSD; Waller–Duncan's test) were used to make the data from this study more general (Steel and Torrie, 1980).

Ethical approval

Ethical approval was granted through the local committee of animal care and use at the Committee on the Ethics of Animal Experiments, Department of Medicine within the University of Bengkulu, Indonesia (No. 107/UN30.14.9/ LT/2020, March 31, 2020) before starting this study.

Results

Quercetin is a flavonoid that is found in many fruits and veggies. It is known to be anti-inflammatory, antiviral, and antimicrobial. HPLC was used to measure the amount of quercetin in FE3H and was found to be 98.92 ± 12.88 µg/g (Fig. 1 and Table 2).

Subjects A1–A4 usually gain between 10.07% and 23.62% of their BW after 9 days (d2–11) of 5-mg/g BW sucrose treatment. In addition, testing with 0.39-mg/g BW FE3H and 0.52-mg/g BW FE3H showed that 0.39-mg/g BW FE3H was most effective (1.33%) at restoring BW to a level similar to the control condition (Table 3).

Animals A1–A4 had much higher blood sugar levels than controls after being given 5-mg/g BW sucrose for 11 days (d2–11). According to the results, giving two doses of FE3H was enough to bring blood sugar levels back to the level of the control group (A0). The same effect was observed on diabetes with both FE3H and glibenclamide. FE3H has the potential to restore hyperglycemia by 47.38%–48.18% (Table 4).

The right kidney (B1: 0.49 ± 0.11) and the left kidney (B1: 0.45 ± 0.11) weights on day 6 tended to rise more than those of the control (B0: 0.37 ± 0.05 and 0.36 ± 0.05) after receiving $HgCl_2$ 5-mg/kg BW on day 1. The right and left kidney weights (B2–B5) tended to return to the weight of the control kidney (B0) after the administration of $HgCl_2$ on day 1 and three doses of FE3H on days 3–5. In the instance of $HgCl_2$ poisoning, FE3H and Immunos® displayed a related phenomenon (Table 5 and Fig. 1).

On day 6, levels of urea (B1: 53.03 ± 9.86^b) and creatinine (B1: 1.05 ± 0.26^b) following administration of 5-mg/kg BW $HgCl_2$ were substantially greater than those of the control (B0: 33.53 ± 1.70^a and 0.43 ± 0.10^a). Although three doses of FE3H were administered after the administration of $HgCl_2$ on day 1, the blood levels of urea (B2: 38.70 ± 5.95^a, B3: 37.63 ± 4.52^a, B4: 36.55 ± 6.90^a, and B5: 35.50 ± 6.19^a) and creatinine (B2: 0.55

Table 1. Research design to determine the potential of fruit ethanolic extract *E. hemisphaerica* (FE3H) to restore hyperglycemia, uremia, and hypercreatininemia in *M. musculus*. Standard food and drink were given *ad libitum* by experimental animals.

Research stage	**Experimental animals' group**	***N***	**Hyperglycemia research activity, on day (d)**				
			d1	**d2-11**	**d12**	**d13**	**d14**
			(O)	**(T)**	**(O)**	**(T)**	**(O)**
A. The first stage used 25 male mice, which were divided into five groups.	A0: Controls, only given DDW	5	BW. Blood glucose level	DDW only	BW. Blood glucose level	DDW only	BW. Blood glucose level
	A1: Given sucrose 5-mg/g BW only	5	BW. Blood glucose level	Sucrose 5-mg/g BW [G]	BW Blood glucose level	Sucrose 5-mg/g BW [G]	BW. Blood glucose level
	A2: Given sucrose 5-mg/g BW + glibenclamide 0.52-mg/g BW	5	BW. Blood glucose level.	Sucrose 5-mg/g BW [G]	BW Blood glucose level.	Glibenclamide 0.52-mg/g BW [G]	BW. Blood glucose level
	A3: Given sucrose 5-mg/g BW + FE3H 0.39-mg/g BW	5	BW Blood glucose level	Sucrose 5-mg/g BW [G]	BW. Blood glucose level	FE3H 0.39-mg/g BW [G]	BW. Blood glucose level
	A4: Given sucrose 5-mg/g BW + FE3H 0.52-mg/g BW	5	BW Blood glucose level	Sucrose 5-mg/g BW [G]	BW Blood glucose level	FE3H 0.52-mg/g BW [G]	BW. Blood glucose level
Research stage	Experimental animals' group	N	**Uremia and hypercreatininemia research activity, on day (d)**				
			d1	d3	d4	d5	d6
			(T)	(T)	(T)	(T)	(O)

(Continued)

Research stage	Experimental animals' group	*N*	Hyperglycemia research activity, on day (d)				
			d1 (O)	d2-11 (T)	d12 (O)	d13 (T)	d14 (O)
B. The second stage used 24 male mice, which were divided into six groups.	B0: Control, DDW only	4	DDW only	DDW only	DDW only	DDW only	Weight of kidney. Blood urea and creatinine level
	B1: Induced with $HgCl_2$ 5-mg/kg BW only	4	$HgCl_2$ 5-mg/kg BW [IP]	DDW only	DDW only	DDW only	Weight of kidney. Blood urea and creatinine level
	B2: Induced with $HgCl_2$ 5-mg/kg BW and Immunos® 0.2-mg/g BW	4	$HgCl_2$ 5-mg/kg BW [IP]	Immunos® 0.2-mg/g BW [G]	Immunos® 0.2-mg/g BW [G]	Immunos® 0.2-mg/g BW [G]	Weight of kidney. Blood urea and creatinine level
	B3: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.39-mg/g BW	4	$HgCl_2$ 5-mg/kg BW [IP]	FE3H 0.39-mg/g BW [G]	FE3H 0.39-mg/g BW [G]	FE3H 0.39-mg/g BW [G]	Weight of kidney. Blood urea and creatinine level
	B4: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.52 mg/g BW	4	$HgCl_2$ 5-mg/kg BW [IP]	FE3H 0.52 mg/g BW [G]	FE3H 0.52 mg/g BW [G]	FE3H 0.52 mg/g BW [G]	Weight of kidney. Blood urea and creatinine level
	B5: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.65-mg/g BW	4	$HgCl_2$ 5-mg/kg BW [IP]	FE3H 0.65-mg/g BW [G]	FE3H 0.65-mg/g BW [G]	FE3H 0.65-mg/g BW [G]	Weight of kidney. Blood urea and creatinine level

Note: BW = body weight; $HgCl_2$ = mercuric chloride; FE3H = fruit ethanolic extract E. hemisphaerica; T = treatment; O = observation G=administrated by oral gavage; IP: intraperitoneal injection. DDW = double-distilled water.

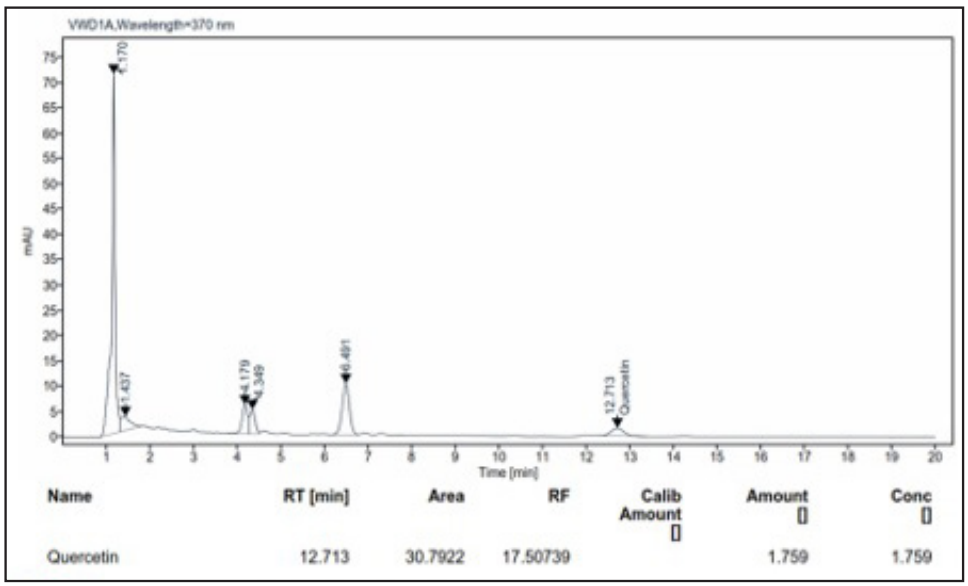

Fig. 1. Amount of quercetin in FE3H was detected by HPLC.

Table 2. Results of quantitative analysis of quercetin levels in the FE3H using HPLC [https://lppt.ugm.ac.id].

No	FE3H sample in cream form	Method	Data acquisition (µg/g)	Quercetin levels X ± SD (µg/g)
1	**Sample A**	**HPLC**	**84.41** 82.92	98.92 ± 12.88
2	Sample B	HPLC	107.70 114.88	
3	Sample C	HPLC	104.48 99.13	

Table 3. Average BW of *M. musculus* on day 1 (d1), day 12 (d12), and day 14 (d14). On day (d)1, BW of the mice was weighed. On d2–11, 5-mg/g BW sucrose was given by gavage, and then, on d12, BW and blood glucose level of mice were determined. On d13, 0.52-mg/g BW glibenclamide was given by gavage in A2, 0.39-mg/g BW FE3H was given by gavage in A3, and 0.52-mg/g BW FE3H was given by gavage in A4. Control, A0, was only given DDW. On d14, BW and blood glucose levels of the mice were determined.

Experimental animal group	*N*	Average weight, on d1 ± SD (g)	Average weight, on d12 ± SD (g) [P]	Average weight, on d14 ± SD (g) [Q]	Weight difference (%) [Q−P]
A0: Controls, only given DDW	5	26.6 ± 2.073	30.8 ± 3.033	30.8 ± 2.300	0.00
A1: Given sucrose 5-mg/g BW only	5	26.6 ± 4.037	32.2 ± 3.033	33.0 ± 3.464	2.42
A2: Given sucrose 5-mg/g BW + glibenclamide 0.52-mg/g BW	5	25.4 ± 3.209	31.4 ± 3.345	29.8 ± 3.033	−5.37
A3: Given sucrose 5-mg/g BW + FE3H 0.39-mg/g BW	5	25.0 ± 2.236	29.6 ± 2.302	30.0 ± 2.449	1.33
A4: Given sucrose 5-mg/g BW + FE3H 0.52-mg/g BW	5	27.8 ± 1.303	30.6 ± 1.341	31.2 ± 1.303	1.92

Note: BW = body weight; $HgCl_2$ = mercuric chloride; FE3H = fruit ethanolic extract *E. hemisphaerica*; T = treatment; O = observation; G = administrated by oral gavage; IP: intraperitoneal injection. DDW = double-distilled water.

Table 4. Average glucose level of *M. musculus* was on day 12 (d12) and day 14 (d14). On day (d)1, BW of the mice was weighed. On d2–11, 5-mg/g BW sucrose was given by gavage, and then, on d12, BW and blood glucose level of mice were determined. On d13, 0.52-mg/g BW glibenclamide was given by gavage in A2, 0.39-mg/g BW FE3H was given by gavage in A3, and 0.52-mg/g BW FE3H was given by gavage in A4. Control, A0, was only given DDW. On d14, BW and blood glucose levels of the mice were determined.

Experimental animal group	*N*	The average blood glucose level, on d12 ± SD mg/dl (P)	The average blood glucose level, on d14 ± SD mg/dl (Q)	Difference (Q−P)	Glibenclamid or FE3H potential for restoring hyperglycemia (%)
A0: The control is only given DDW	5	89.8 ± 12.1[a]	89.8 ± 12.1[a]	0	-
A1: Given sucrose 5-mg/g BW only	5	151.0 ± 19.6[c]	188.8 ± 31.9[c]	37.8	-
A2: Given sucrose 5-mg/g BW + glibenclamide 0.52-mg/g BW	5	173.8 ± 25.8[c]	67.8 ± 10.8[a]	−106.0	60.98
A3: Given sucrose 5-mg/g BW + FE3H 0.39-mg/g BW	5	149.4 ± 7.8[c]	78.6 ± 14.6[a]	−70.8	47.38
A4: Given sucrose 5-mg/g BW + FE3H 0.52-mg/g BW	5	170.6 ± 20.5[c]	88.4 ± 6.8[a]	−82,2	48.18

Note: Different superscript letters in one column show statistically significant differences between A0, A1, A2, A3, and A4, at a significance level of 95% (Steel and Torrie, 1980).

Table 5. Average weight right and left kidneys on day 6 (d6). On d1, B1–B5 were injected IP 5-mg/kg BW $HgCl_2$; then, on d3–5, they were given by gavage 0.2-mg/g BW Immunos® in B2, 0.39-mg/g BW FE3H in B3, 0.52-mg/g BW FE3H in B4, and 0.65-mg/g BW FE3H in B5. Controls, B0 and B1, were only given DDW. On d6, the mice were killed by CD, and then, the abdominal and thoracic cavities were dissected. The weight of the kidney was determined, and then, the urea and creatinine levels were measured in blood samples from the heart.

Experimental animal group	*N*	The average weight of right kidney on d-6 *X* ± SD (g)	The average weight of left kidney on d-6 *X* ± SD (g)
B0: Control, DDW only	4	0.37 ± 0.05	0.36 ± 0.05
B1: Induced with $HgCl_2$ 5-mg/kg BW only	4	0.49 ± 0.11	0.45 ± 0.11
B2: Induced with $HgCl_2$ 5-mg/kg BW and Immunos® 0.2-mg/g BW	4	0.44 ± 0.06	0.42 ± 0.06
B3: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.39-mg/g BW	4	0.35 ± 0.03	0.32 ± 0.03
B4: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.52 -mg/g BW	4	0.41 ± 0.07	0.34 ± 0.05
B5: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.65-mg/g BW	4	0.38 ± 0.06	0.37 ± 0.05

± 0.21[c], B3: 0.53 ± 0.19[a], B4: 0.50 ± 0.32[a], and B5: 0.48 ± 0.17[a]) were comparable to the control (B0) on day 6. When $HgCl_2$ caused elevated blood urea and creatinine levels, FE3H and Immunos® demonstrated a related occurrence. FE3H has the potential for restoring uremia 29.04%–33.06% and hypercreatininemia 49.52%–54.28% (Table 6).

Discussion

A phytochemical study of the fruit ethanolic extract of *E. hemisphaerica* (FE3H) was done using HPLC (Knauer Smartline) and UV-vis spectrophotometry (1800 Shimadzu). The following six test parameters are given in order of their amounts: flavonoids (32.91% w/w), tannins (20.89% w/w), phenol (19.88% w/w), sucrose (2.96% w/w), alkaloids (2.05% w/w), and saponins (1.64% w/w). The phytochemical content of leaf ethanolic extract of *E. hemisphaerica* (LE3H) and FE3H was compared. FE3H had higher amounts of five test parameters: flavonoids, alkaloids, tannins, sucrose, and phenol. In the meantime, LE3H had more saponins than FE3H (2.32% w/w *vs.* 1.64% w/w). The

most important phytochemicals in LE3H and FE3H are flavonoids (Karyadi *et al.*, 2023; Table 7). Flavonoid quercetin is found in many fruits and veggies and is known to have anti-inflammatory, antiviral, and antimicrobial properties (Petrillo *et al.*, 2022). This study used HPLC to measure the amount of quercetin in FE3H and found it to be 98.92 ± 12.88 µg/g (Table 2 and Fig. 1). Also, it was said that quercetin is a strong antioxidant that can grab reactive oxygen species, reactive nitrogen species, and reactive chlorine species. This quercetin can bind to transition metal ions and work as a reducing agent (Carrillo-Martinez *et al.*, 2024). The literature shows that blood glucose (Yarahmadi *et al.*, 2021), urea (Balkrishna *et al.*, 2023), and creatinine (Vrbjar *et al.*, 2023) levels are related to the presence of quercetin.

Technically speaking, hyperglycemia means high blood glucose. Diabetes happens when the body does not have enough insulin or cannot use it correctly. The 9-day (d2–11) 5-mg/g BW sucrose treatment changed the blood glucose levels of *M. musculus* (Tables 3 and 4). *Mus musculus* had high blood sugar levels after this treatment, which is called hyperglycemia. High-sucrose diets can cause hyperglycemia in mice

Table 6. Average blood of urea and creatinine level on day 6 (d-6). On d1, B1–B5 were injected IP 5-mg/kg BW $HgCl_2$; then, on d3–5, they were given by gavage 0.2-mg/g BW Immunos® in B2, 0.39-mg/g BW FE3H in B3, 0.52-mg/g BW FE3H in B4, and 0.65-mg/g BW FE3H in B5. Controls, B0 and B1, were only given DDW. On d6, the mice were killed by CD, and then, the abdominal and thoracic cavities were dissected. The weight of the kidney was determined, and then, the urea and creatinine levels were measured in blood samples from the heart.

Experimental animal group	*N*	The average blood urea level on d6 $X \pm$ SD (mg/dl)	Immunos®, or FE3H potential for restoring uremia (%)	The average blood creatinine level on d6 $X \pm$ SD (mg/dL)	Immunos® or FE3H potential for restoring hypercreatininemia (%)
B0: Control, DDW only	4	33.53 ± 1.70^{a}	-	0.43 ± 0.10^{c}	-
B1: Induced with $HgCl_2$ 5-mg/kg BW only	4	53.03 ± 9.86^{b}	-	1.05 ± 0.26^{d}	-
B2: Induced with $HgCl_2$ 5-mg/kg BW and Immunos® 0.2-mg/g BW	4	$38.70 \pm 5.9^{5}a$	27.02	$0.55 \pm 0.2^{1}c$	47.62
B3: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.39-mg/g BW	4	37.63 ± 4.52^{a}	29.04	0.53 ± 0.19^{c}	49.52
B4: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.52-mg/g BW	4	36.55 ± 6.90^{a}	31.07	0.50 ± 0.32^{c}	52.38
B5: Induced with $HgCl_2$ 5-mg/kg BW and FE3H 0.65-mg/g BW	4	35.50 ± 6.19^{a}	33.06	0.48 ± 0.17^{c}	54.28

Note: Different superscript letters in one column show statistically significant differences between B0, B1, B2, B3, B4, and B5 at a significance level of 95% (Steel and Torrie, 1980).

Table 7. Comparison of phytochemical content of leaf ethanolic extracts *E. hemisphaerica* (LE3H) and fruit ethanolic extract *E. hemisphaerica* (FE3H) (Karyadi *et al.*, 2023).

Test parameter	Results		Unit	Method
	LE3H	FE3H		
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

(Burchfield *et al.*, 2018). Extremely high blood glucose levels, above 180–200 mg per deciliter (mg/dl), usually cause diabetes symptoms. Clodi *et al.* (2023) stated that in critical illness, hyperglycemia is associated with increased mortality. Based on the currently available evidence, intravenous insulin therapy should be initiated when blood glucose is above 180 mg/dl. After initiation of insulin therapy, blood glucose should be maintained between 140 and 180 mg/dl. Sucrose-induced hyperglycemia can cause diabetes and obesity, but a plant product or the drug glibenclamide can stop these problems (Ngueguim *et al.*, 2016). In this study, glibenclamide (0.52-mg/g BW) treatment led to similar blood glucose levels in the treated group compared to the control group (Table 4), and BW tended to drop (Table 3). FE3H (0.39 or 0.52-mg/g BW) also showed that glibenclamide can stop sucrose from raising blood sugar levels (Table 4). There is an old glibenclamide drug that includes the sulfonylurea molecule and is very important for treating type 2 diabetes (T2D) mellitus. By stopping ATP-sensitive K^+ channels, the drug works to depolarize cells and cause insulin to be released (Luzi and Pozza, 1997). Evidence shows that FE3H can reverse hyperglycemia. Glibenclamide and FE3H have similar effects on hyperglycemia, and glibenclamide has the potential to restore hyperglycemia by 60.98% (Table 4).

The three main plant chemicals that make up FE3H are flavonoids, tannins, and phenols (Karyadi *et al.*, 2023). Table 2 shows that quercetin is a powerful flavonoid with many positive effects. It lowers blood pressure, fights hyperlipidemia and hyperglycemia, and stops cancer and other diseases by being antiviral, anticancer, anti-inflammatory, and antimicrobial (Hosseini *et al.*, 2021). There are also claims that flavonoids can help people with diabetes, but more studies are needed to understand how they can treat diabetes (Al-Ishaq *et al.*, 2019) fully. Tea from the red honeybush plant (*Cyclopia genistoides*) has no caffeine or much tannin. The different honeybush tea extracts, especially the aqueous and ethyl acetate extracts, slowed down the breakdown of lipids and sugars linked to T2D, lowered blood sugar, and controlled oxidative damage to the pancreas (Xiao *et al.*, 2020). Researchers found that the water-based solution of *Raphia hookeri* leaves can act as an antioxidant and stop enzymes from breaking down carbohydrates because it contains phenolic compounds. Free radicals can cause oxidative stress in pancreatic cells, which may be helped by the extract. It may also lower blood sugar levels, which are important for treating T2D. However, it is strongly suggested that more clinical trials and *in vivo* studies can be done (Dada *et al.*, 2017). The phytochemical parts of FE3H—flavonoids, tannins, and phenol—have been found to help lower blood sugar. FE3H has the potential to restore hyperglycemia by 47.38%–48.18% (Table 4).

"Urine in the blood," or uremia, is most common in people with end-stage renal disease and chronic kidney disease. On the other hand, if kidney function is lost quickly, it could also be due to severe renal damage. Urea hurts many types of cells in direct and indirect ways. Polyneuropathy is a typical sign of uremia, especially when renal replacement therapy is started too late (Zemaitis *et al.*, 2023). For example, uremia can also lead to neurological problems. The kidney is the organ that mercury ions are most likely to damage. Blocking the urinary tract can also stop mercury ions from building up in the kidney tissue. In the same tests, this decrease is less strong than with inulin, however. Based on these results, gamma-glutamyl transpeptidase (gam-GT), an enzyme found in the kidneys, may help remove mercury from the tubule lumen. The death caused by mercury chloride and the effects on the buildup of organic ions in kidney slices were worse when nonprotein sulfhydryl was low because glutathione levels dropped quickly and production was stopped (Berndt *et al.*,1985). Male rats of the Long Evans breed were kept alive for a long time after mercuric chloride ($HgCl_2$) caused uremia. Over time, the intramolecular structure of muscle glycogen changed, but the amount of glycogen in the muscles did not change significantly (Mannan and Rahman, 1977). In this research, kidney weights tended to rise more after being given 5-mg/kg BW of $HgCl_2$ on day 1 (B1) compared to the control group (B0). In addition, giving $HgCl_2$ and then treating the kidney weight with Immunos® (B2) and FE3H (B3–B5) tends to get closer to the state of control kidney weights (B0). The left and right kidneys behaved similarly, though the right kidney usually weighed more than the left kidney (Table 4 and Fig. 2). Compared to controls, giving $HgCl_2$ raised blood urea levels by a large amount. After treatment with $HgCl_2$ and FE3H, blood urea levels returned to where they were in the control group (Table 5). This shows that $HgCl_2$ leads to uremia (Levine *et al.*, 2003; Levine and Saltzman, 2003). In the meantime, both Immunos® and FE3H work to stop uremia. Immunos® has the potential to restore uremia by 27.02% (Table 6). Reports say that flavonoids change some parts of nitrogen metabolism in animals with experimental uremia. According to research, flavonoids robinine and hyperine lowered the amount of free ammonia in the rat brain and raised the amount of glutamine amide nitrogen and protein amide nitrogen. Robinine also slightly reduced urea synthesis and arginase activity in liver slices from rats that had their kidneys removed (Sokolova and Liubartseva, 1979). In treating uremia and hypercreatinemia (Hsieh *et al.*, 2013), quercetin, a natural antioxidant, works much better. Another finding said that puerarin, an isoflavone, can stop vascular hardening in uremic rats by reducing inflammation (Liu *et al.*, 2019). A substance called RG-tannin can lower the amounts of urea and creatinine in mice (Yokozawa *et al.*, 1991). Zingerone, a phenolic alkenone found

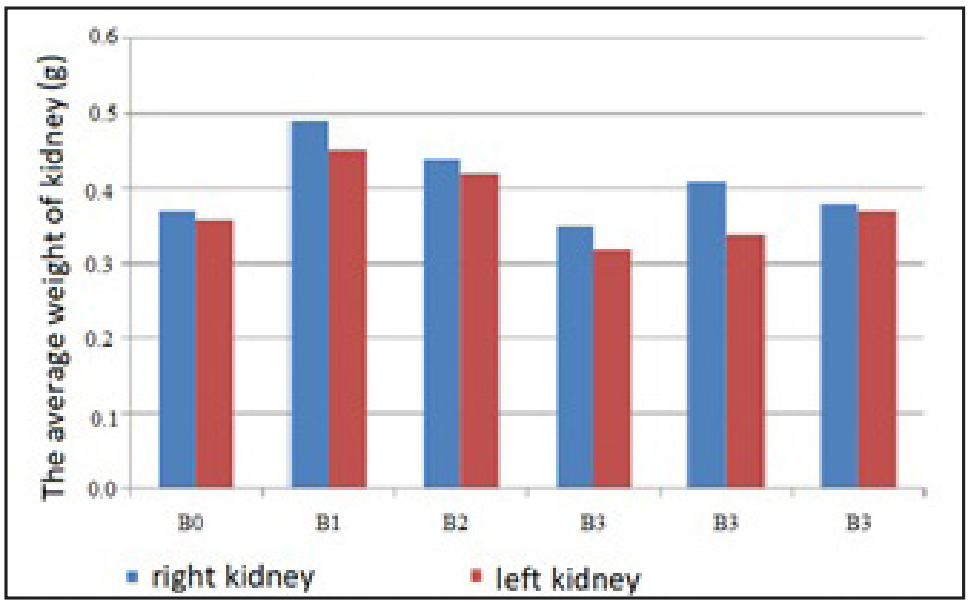

Fig. 2. Average weight right and left kidneys on day 6 (d-6). On d1, B1–B5 were injected IP 5-mg/kg BW $HgCl_2$; then, on d3–5, they were given by gavage 0.2-mg/g BW Immunos® in B2, 0.39-mg/g BW FE3H in B3, 0.52-mg/g BW FE3H in B4, and 0.65-mg/g BW FE3H in B5. Controls, B0 and B1, were only given DDW. On d6, the mice were killed by CD, and then, the abdominal and thoracic cavities were dissected. The weight of the kidney was determined, and then, the urea and creatinine levels were measured in blood samples from the heart.

in ginger, was used to treat kidney damage caused by cecal closure and puncture surgery in mice, and it was tested by measuring blood urea nitrogen and serum creatinine (Lee *et al.*, 2019). Scientists say that cyclophosphamide lowers the functions of the kidneys, liver, and antioxidant enzymes. This makes blood urea nitrogen and creatinine levels rise greatly. Meanwhile, saponins might protect the liver and kidneys from the harmful effects of cyclophosphamide (Golmohammadi *et al.*, 2023). Phytochemicals in FE3H, such as flavonoids, tannins, phenol, and saponins, have been shown to help lower uric acid levels. Flavonoids exhibit the most promising potential (Karyadi *et al.*, 2023) (Table 7). FE3H has the potential to restore uremia by 29.04%–33.06% (Table 6).

Being busy or moving makes muscles make creatinine, a waste product. The kidneys will control how much creatinine is in the blood. Depending on a person's age, gender, daily actions, and BW, their normal creatinine levels may be different. Normal amounts of creatinine in the body for adult men are between 0.6 and 1.2 mg/dl, and for adult women, they are between 0.5 and 1.1 mg/dl. Creatinine levels should be normal, but if they are too high or too low, it could mean that the kidneys are not working properly. In people with hypercreatininemia (>1.3 mg/dl), levels of creatinine are higher than usual (Akrom *et al.*, 2017; Manna *et al.*, 2005). Treatment with $HgCl_2$ raised creatinine levels much more than the control group (Table 6), and kidney weights were also generally higher (Table 5 and Fig. 2). Many studies have shown that $HgCl_2$ can cause hypercreatininemia (Moreira-Rodrigues *et al.*, 2010; Chan *et al.*, 2020; Ijaz *et al.*, 2021; Goel *et al.*, 2023). This fact fits with those results. According to this study, hypercreatininemia can be improved by 47.62% when Immunos® is given as a supplement. Immunos® is a supplement that boosts the immune system against short- and long-term infections. It is well-known outside of the supplement industry. The potential and process for the immune system are thought to be similar to those of FE3H (Table 6).

FE3H may help lower hypercreatininemia, and flavonoids play the biggest part (Karyadi *et al.*, 2023), especially when quercetin is present (Table 2). Multiple study reports must be used to back up this claim. Quantitative measures of biochemical, such as blood urea nitrogen and serum creatinine, show that quercetin completely stops $HgCl_2$-induced acute kidney damage. In particular, quercetin greatly lowered the buildup of Hg in the kidneys (Shin *et al.*, 2015). Quercetin stopped the mercurial-induced oxidation of glutathione. Findings show that quercetin's ability to protect mitochondria from mercurial-induced dysfunction is linked to removing oxidant species made when methylmercury (MeHg) or $HgCl_2$ is present. There was the first demonstration of quercetin's ability to protect against mercurial-induced toxicity, pointing to its ability to block mercurial-dependent hydrogen

peroxide production as a possible molecular method of protection (Franco *et al.*, 2007). Human-derived liver cells are damaged by DNA damage and oxidative stress caused by $HgCl_2$ and MeHg. The flavonoid lessens these effects. Although the amounts of metals and flavonoids used in this study were based on human exposure, results suggest that quercetin may protect people from the harmful effects of the metal (Barcelos *et al.*, 2011). Scientists also found that quercetin lowered the levels of several substances in the blood, including malondialdehyde, serum/plasma creatinine, blood urea nitrogen, urine protein, urine albumin, and superoxide dismutase (Hu *et al.*, 2022). Quercetin greatly lowered the BW, blood glucose, creatinine, and blood urea nitrogen levels of diabetes mice (Zhu *et al.*, 2024). As a potential drug for treating diabetic nephropathy, quercetin makes clinical prediction and therapy easier (Feng *et al.*, 2022). Results from earlier research agree with this, showing that FE3H has the potential for restoring hypercreatininemia 49.52%–54.28% (Table 6). More research should be done on the useful natural substances in the ethanol extract of *E. hemisphaerica* using the right proteome methods (Ruyani *et al.*, 2023).

Conclusion

The ethanolic extract of fruit *E. hemisphaerica* (FE3H) that was high in flavonoid quercetin has the potential for restoring hyperglycemia by 47.38%–48.18%, uremia by 29.04%–33.06%, and hypercreatininemia by 49.52%–54.28% in mice.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

AR: initiator of research idea, recipient of research funding, finalization of publication paper, and corresponding author; ES and RE: *E. hemisphaerica* fruit extract preparation, hyperglycemia research activity, and data collection; DPP and O: uremia and hypercreatininemia research activity, and data collection; RE and O: generalization through statistical tests; DP: research project administrator, and drafter of report. All authors have read, reviewed, and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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