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Modulation effects of Etlingera elatior ethanol extract as anti-inflammatory on chronic kidney disease in mice with hypertension and diabetes

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ABSTRACT

The incidence of diabetes increased significantly around the world in accordance with lifestyle and change in eating behaviour. *Streptozotocin-Nicotinamide* (STZ-NA) is capable of inducing Diabetes Mellitus type 2 in experimental animals for insulin resistance. In this research, we inspect the therapeutic potential of *Etlingera elatior* ethanol extract (EEEE) on diabetes associated with diabetic nephropathy and hypertension complications in mice models. Diabetes and hypertension are induced in mice using STZ 45 mg/kgBB and NA 110 mg/kgBB, followed by unilateral ureter ligation (UUO) for 4 weeks after a week of STZ-NA induction. The EEEE solution was given in the last 4 weeks with doses of 200, 400, 600, and 800 mg/kgBB. The results of this study prove the effect of vanillic acid on improving systolic blood pressure, plasma creatinine, plasma glucose, albuminuria and reducing the inflammatory marker high sensitivity C-reactive protein (hs-CRP). Histopathology of kidney is under investigation for being part of diabetes hypertension pathology. Treatment using EEEE 600 and 800 mg/kgBB for 4 weeks in experimental mice results in the decrease of plasma glucose, systolic blood pressure, plasma creatinine, albuminuria, and hs-CRP, including the restoration of kidney histology significantly compared to 200 and 400 mg/kgBB doses. This result concludes that EEEE offers modulation effects on

diabetes hypertension control by reducing blood glucose rate, blood pressure rate, kidney defect, and inflammation markers.

Keywords: *Diabetic nephropathy, Ethanol extract of Etlingera elatior fruit, Anti-inflammation*

INTRODUCTION

Chronic kidney disease (CKD) is a rampant health issue around the world with increasing prevalence with each passing year. The causes of CKD resulting in hemodialysis treatment in 2015 are hypertension 44%, diabetes 22%, primary glomerulopathy (GNC) 8%, chronic pyelonephritis 7%, lupus nephropathy/Systemic Lupus Erythematosus (SLE) 1%, polycystic kidney disease 1%, gout nephropathy 1%, others 8%, and undefined 3%.[1] Diabetes mellitus and hypertension are the two biggest correlating causes of CKD.[2,3] Both are affected by life style and genetic factors, and their prevalence is increasing along with the prevalence of CKD each year.[4] Because of this, the progressivity of CKD caused by hypertension and diabetes, especially non-insulin-dependent diabetes mellitus (diabetes type 2; DM2), needs to be controlled.

Research shows that oxidative stress decreases and inflammation will hamper the CKD development caused by hypertension and diabetes.[5] Oxidative stress causes excess ROS production, which will increase proinflammatory cytokines, one of which is Interleukin 6 (IL-6). Interleukin 6 will stimulate hepatocyte cells to synthesize high-sensitivity C-reactive protein (hs-CRP).[6,7] Oxidative stress and inflammation have significant positive correlation with the level of kidney failure; hence, a biological agent with anti-inflammatory activity can be used for CKD management.[8]

The beneficial parts of Etlingera elatior (E. elatior) proven by research are flowers, leaves, rhizome, and stem; however, there is still only limited research on the E. elatior fruit.[9] Therefore, the E. elatior fruit will be inspected for the anti-inflammatory potential including its beneficial role in reducing CKD progressivity.[9–11]

MATERIALS AND METHODS

Materials

E. elatior fruit ethanol extract

E. elatior fruits were dried and subjected to maceration process using six litres of ethanol solvent 96% to get Etlingera elatior ethanol extract (EEEE).[12–14] The E. elatior fruit was obtained from Langkaplancar district, Pangandaran regency, West Java.

Chemicals

The substances induced to model were Streptozotocin (STZ) and Nicotinamide (NA) from Nacalai Tesque Inc. Vanillic acid from Sigma-Aldrich was for DPPH *(α,α-diphenyl-β-picrylhydrazyl) inspection. Reagen latex CRPHS* (Cardiac C-Reactive Protein (latex) High Sensitive) from Roche was for blood hs-CRP inspection.

Experimental animals

The experimental animals were healthy wistar rats aged 8 weeks and weighed 180–200 g. The mice were bred and taken from Laboratory of Experimental Animal Development, Faculty of Pharmacy, Gadjah Mada University. The entire experiment was reviewed and agreed by ethical commissions of Sebelas Maret University. The experimental mice were randomly divided into four groups in controlled room temperature (20–26°C) with relative humidity (40–70%) and light dark

cycle lighting 12:12 hours. The mice were acclimatized in the environment for a week and provided with food and ad libitum water.

Methods

Induction of diabetic hypertension

The mice were induced with Streptozotocin-Nicotinamide (STZ 45 mg/kg BB-NA 110 mg/kg BB) and the following week went through unilateral ureter ligation (UUO) to develop CKD mice model with hypertension and diabetes. Samples were divided into four groups with different extract doses, 200 mg/kgBB, 400 mg/kgBB, 600 mg/kgBB, and 800 mg/kgBB. The development of diabetes hypertension is confirmed by measuring fasting plasma glucose rate and systolic blood pressure (tail cuff method). Mice with higher plasma glucose rate than 200 mg/dl (11.1 mmol/l) and systolic blood pressure higher than 135 mmHg after ligation were chosen for further experiments.

Experimental design

EEEE was given orally to each group with different doses using a gastric probe once a day for 4 weeks continuosly. The extract was dissolved in NaCMC (Sodium carboxymethyl cellulose) 0.5% and given in 2 mL volume for each mice. Other than EEEE, mice were given pellets as food and ad libitum pump water as drink. Weight, tension, and glucose rate were measured each week within the experiment. The examination of albuminuria and creatinine rate in blood was done on the fourth and fifth weeks, followed by examination of hs-CRP rate in the last week. The four groups were evaluated for anti-inflammatory activity that improves diabetes status, hypertension, and kidney function.

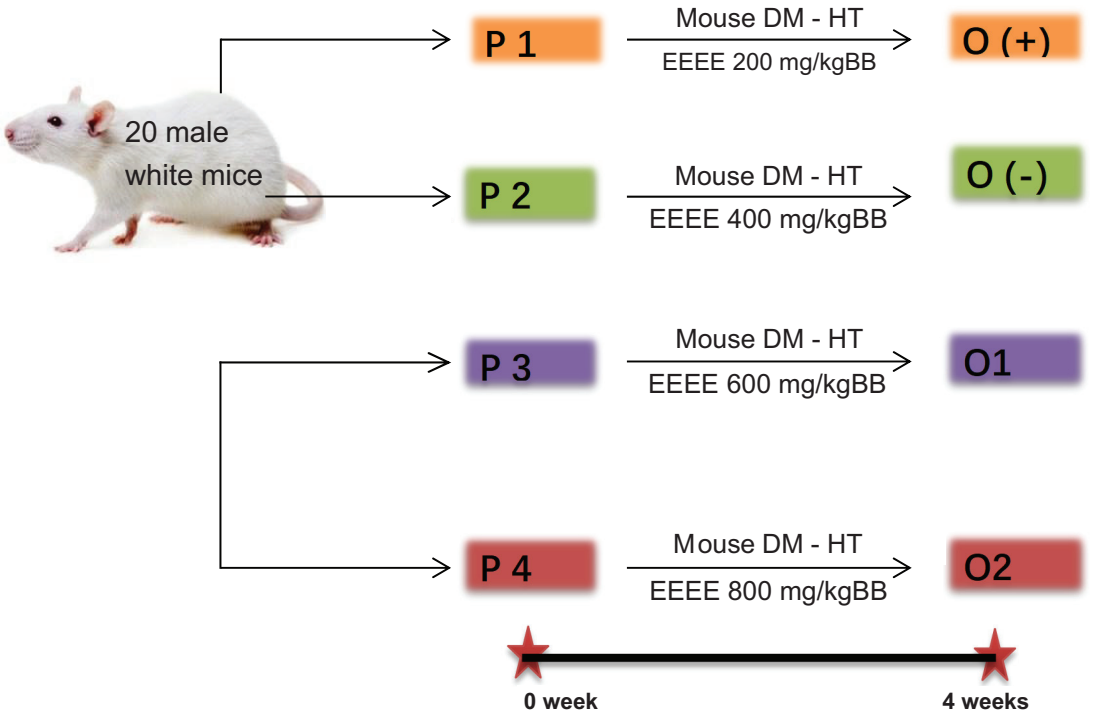

FIG 1. Experimental design. Group 1 (P1) – EEEE 200 mg/kgBB, Group 2 (P2) – EEEE 400 mg/kgBB, Group 3 (P3) – EEEE 600 mg/kgBB, and Group 4 (P4) – EEEE 800 mg/kgBB. EEEE, Etlingera elatior ethanol extract.

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After that, the four groups were compared to determine the effective dose solution of the experiment.

Estimation of blood glucose

Measurement of blood glucose rate with glucose oxidase enzyme (GOD) gives D-gluconic acid and hydrogen peroxide. Hydrogen peroxide will produce quinoneimine colorant in red. The color intensity is compatible with glucose concentration in samples, measured with spectrophotometer in 505 nm wavelength equivalent to blood glucose rate in mg/dL of samples.[15]

Blood pressure measurement

Systolic blood pressure is examined with the tail cuff method using the blood pressure analyzer tool for experimental animals. In prior, each mouse was put inside a retainer and kept in serene environmental heat temperature for 5–10 minutes. This procedure is repeated for a week to habituate the mice with the retainer.

Estimation of creatinine

Determination of creatinine through the Jaffe reaction that occurs between picric acid and creatinine under alkaline conditions using colorimetric analysis method.[16]

Determination of albuminuria

Albuminuria examination is done on the fourteenth day (H14), twenty-eighth day (H28), and fifty-sixth day (H56) by collecting mice urine in individual metabolic cages for 24 hours using enzyme linked immune-sorbent assay (ELISA) method.

Estimation of hs-CRP

The testing technique of hs-CRP ELISA can measure systemic inflammation in the early stage accurately.

RESULTS

Effects of EEEE on body weight changes

Table 1 shows that EEEE effects body weight changes of experimental mice in different doses. The EEEE results in a decrease in body weight in experimental mice due to the effects of diabetes. The extract treatment using 800 mg/kgBB dose significantly shows optimum dose ($p < 0.05$).

195.28 ± 3.81^{a} (5th week (W5) — P2) = Significant as compared to P1 ($P < 0.05$; ANOVA followed by Duncan Multiple Range Test (DMRT)), 183.00 ± 3.67^{b} (2nd week (W2) – P3) = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT), 206.80 ± 4.43^{b} (5th week (W5) – P3) = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT), 177.00 ± 3.78^{b} (2nd week (W2) – P4) = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT), 191.85 ± 3.76^{c} (4th week (W5) – P4) Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT), 199.00 ± 3.46^{c} (4th week (W5) – P4) Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT).

EEEE, Etlingera elatior ethanol extract.

Effect of EEEE on plasma glucose

The effect of EEEE on blood glucose rate in experimental animals is shown in Table 2. Experimental mice were treated using EEEE on

TABLE 1. Effects of E. elatior ethanol extract on body weight.

Parameters	**P1**	**P2**	**P3**	**P4**
2nd week (W2)	175.71 ± 4.02	170.57 ± 3.50	183.00 ± 3.67^{b}	177.00 ± 3.78^{b}
4th week (W4)	186.42 ± 3.69	188.57 ± 3.52	201.80 ± 3.56	191.85 ± 3.76^{c}
5th week (W5)	190.28 ± 4.38	195.28 ± 3.81^{a}	206.80 ± 4.43^{b}	199.00 ± 3.46^{c}

Group 1 (P1) – EEEE 200 mg/kgBB, Group 2 (P2) – EEEE 400 mg/kgBB, Group 3 (P3) – EEEE 600 mg/kgBB, and Group 4 (P4) – EEEE 800 mg/kgBB. Values are means ± SD of five rats from each group.

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TABLE 2. Effect of EEEE on plasma glucose.

Parameters	P1	P2	P3	P4
2nd week (W2)	276.81 ± 3.19	278.74 ± 1.66	266.09 ± 3.97	262.85 ± 3.21[a,b,c]
4th week (W4)	133.86 ± 2.12	124.28 ± 2.52[a]	113.98 ± 3.63[a,b]	107.09 ± 2.15[a,b,c]
5th week (W5)	117.70 ± 2.27	114.15 ± 2.60[a]	104.25 ± 3.95[a]	89.90 ± 1.23[b,c]

Group 1 (P1) = EEEE 200 mg/kgBB, Group 2 (P2) = EEEE 400 mg/kgBB, Group 3 (P3) = EEEE 600 mg/kgBB, and Group 4 (P4) = EEEE 800 mg/kgBB. Values are means ± SD of five rats from each group. EEEE, Etlingera elatior ethanol extract.

[a] = Significant as compared to P1 ($P < 0.05$; ANOVA followed by DMRT).

[b] = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT).

[c] = Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT).

doses of 400, 600, and 800 mg/kgBB; this significantly reduces blood glucose rate in the fourth week. In comparison of different doses, the optimum dose was 800 mg/kgBB in the fifth week.

Effect of EEEE on blood pressure levels

Systolic blood pressure of experimental mice after receiving EEEE in different doses is shown in Figure 2. Experimental mice receiving EEEE in doses of 400, 600, and 800 mg/kgBB experienced decreasing blood pressure significantly in the fourth week. The lowest blood pressure happened while taking the 800 mg/kgBB dose of EEEE.

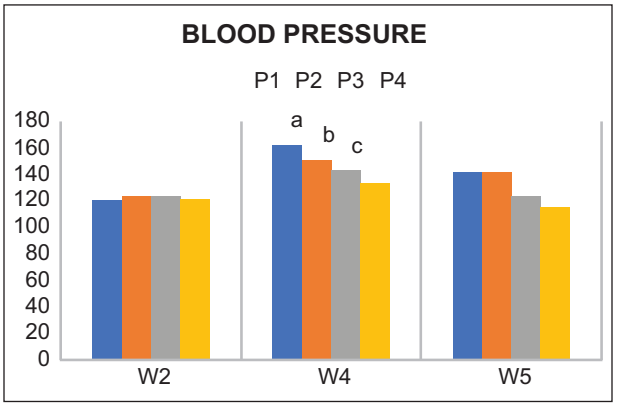

FIG 2. Effect of EEEE on systolic blood pressure levels in normal and diabetic hypertensive rats. Group 1 (P1) = EEEE 200 mg/kgBB, Group 2 (P2) = EEEE 400 mg/kgBB, Group 3 (P3) = EEEE 600 mg/kgBB, and Group 4 (P4) = EEEE 800 mg/kgBB. EEEE = Etlingera elatior ethanol extract.

[a] = Significant as compared to P1 ($P < 0.05$; ANOVA followed by DMRT).

[b] = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT).

[c] = Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT).

Effect of EEEE on renal markers

Rate of renal markers is shown in Table 3. Rate of creatinine and albuminuria of experimental mice decreases significantly after receiving EEEE with doses of 400, 600, and 800 mg/kgBB ($p < 0.05$), but there is no significant difference with the 200 mg/kgBB dose.

Effect of EEEE on hs-CRP

E. elatior fruit ethanol extract will decrease hs-CRP rate in blood on doses of 200, 400, and 800 mg/kgBB ($p < 0.05$).

Effect of EEEE on the histopathology of kidney

Hematoxylin and eosin stain of kidney sections were showed on Figure 4a and b. STZ-NA induction followed by ligation changes kidney tissue morphology. After treatment with EEEE, those changes are normalized. Oil red 'O' stain of liver tissue is represented in Figure 4c.

TABLE 3. Effects of EEEE on renal markers in diabetic hypertensive rats.

Albuminuria				
Parameters	**P1**	**P2**	**P3**	**P4**
2nd week (W2)	25.15 ± 0.47	24.90 ± 0.35	24.72 ± 0.52	24.76 ± 0.45
4th week (W4)	24.00 ± 0.48	22.52 ± 0.37[a]	22.25 ± 0.21[a]	22.30 ± 0.49[a]
5th week (W5)	20.03 ± 0.37	20.60 ± 0.36	24.25 ± 0.69[a,b]	20.04 ± 0.37[c]
Creatinine				
Parameters	**P1**	**P2**	**P3**	**P4**
2nd week (W2)	2.86 ± 0.06	2.80 ± 0.10	2.82 ± 0.12	2.79 ± 0.11
4th week (W4)	1.93 ± 0.06	1.60 ± 0.09[a]	1.40 ± 0.16[a,b]	1.03 ± 0.03[a,b,c]
5th week (W5)	1.32 ± 0.05	1.10 ± 0.03[a]	0.87 ± 0.03	0.96 ± 0.03[a,b,c]

Group 1 (P1) = EEEE 200 mg/kgBB, Group 2 (P2) = EEEE 400 mg/kgBB, Group 3 (P3) = EEEE 600 mg/kgBB, and Group 4 (P4) –=EEEE 800 mg/kgBB. Values are means ± SD of five rats from each group. EEEE=Etlingera elatior ethanol extract.

[a] = Significant as compared to P1 ($P < 0.05$; ANOVA followed by DMRT).

[b] = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT).

[c] = Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT).

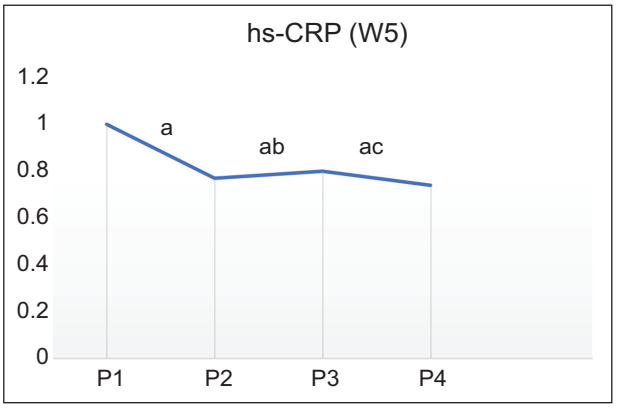

FIG 3. Effects of EEEE on hs-CRP in the plasma of diabetic hypertensive rats.

Group 1 (P1) = EEEE 200 mg/kgBB, Group 2 (P2) = EEEE 400 mg/kgBB, Group 3 (P3) = EEEE 600 mg/kgBB, and Group 4 (P4) = EEEE 800 mg/kgBB. EEEE=Etlingera elatior ethanol extract.

[a] = Significant as compared to P1 ($P < 0.05$; ANOVA followed by DMRT).

[b] = Significant as compared to P2 ($P < 0.05$; ANOVA followed by DMRT).

[c] = Significant as compared to P3 ($P < 0.05$; ANOVA followed by DMRT).

DISCUSSIONS

Although the model is not an animal model with DM2 and other complications, this STZ-NA model is proven to be the closest to DM2 mice models. STZ causes damage to the pancreas B cell, while NA protects some of the insulin-secreting cells. STZ is transported to B cell through glucose transporter GLUT2 and then causes damages. The severity of diabetes depends on the given STZ-NA dose. Insulin secretion response to glucose is weakened due to STZ-NA induction in mice as a result of the decrease of B cell amounts. The results of various experiments show that this diabetes model is useful for the study of different aspects of diabetes.[17,18]

This research discloses that induced STZ-NA mice undergo fasting blood glucose rate escalation. All groups given EEEE show that their fasting blood glucose rate is almost normal and plasma glucose decrease is significant with doses of 400, 600, and 800 mg/kgBB in the fourth week. Significant decrease in the last week is because of using the 800 mg/kgBB ($p < 0.05$) dose.

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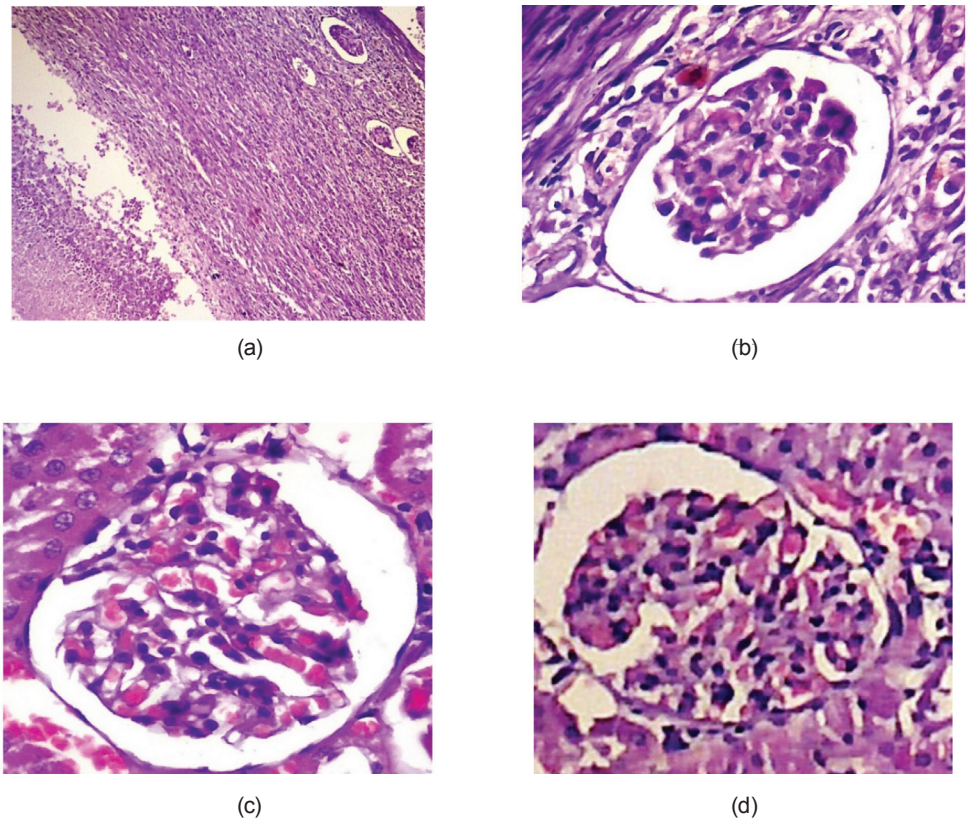

FIG 4. (a) Morphology changes in mice with EEEE 200 mg/kgBB dose results in the thinning of cortex with medulla dilatation; (b) existing changes on glomerulus with moderate mesangial cell expansion after receiving EEEE 400 mg/kgBB dose; (c) no mesangial expansion with minimum glomerulus changes after receiving EEEE 600 mg/kgBB dose; (d) no changes on glomerulus; only minimum inflammation after receiving EEEE 800 mg/kgBB dose. EEEE, Etlingera elatior ethanol extract.

Hypertension is closely related to kidney failure, as a result of some mechanisms such as hyperinsulinemia, sympatic activation increase, oxidative stress, endotel dysfunction, and renin-angiotensin system activation due to inflammation increase. Yang and Li[19] stated that UUO will induce fibrosis interstitial causing end stage renal failure. Mice given UUO will produce ROS (reactive oxygen species), which plays a centric role in apoptosis, fibrosis, and inflammation due to obstruction induction formation. Ureter obstruction results in angiotensin receptor type 1 (AT1R) in kidney, NF B, monocyte chemotactic protein 1 (MCP-1), and fibronectin expression escalation. Angiotensin II through AT1R receptor bond will activate NFkB and hs-CRP, thereby stimulating inflammation and fibrosis, which are closely related to hypertension.[20,21] Our study shows that the experimental mice undergo increase in blood pressure. The hypertension condition is reducing after EEEE cure with doses of 400, 600, and 800 mg/kgBB.

Creatinine is a leftover metabolism product, which is mostly filtered from kidney and secreted in urine.[22–24] Our finding shows that creatinine rate is influenced by EEEE treatment with doses of 400, 600, and 800 mg/kgBB. Post-treatment creatinine levels in the 400 mg/kg BW group slightly increased, although not significantly compared to

before treatment but still significantly different from the 200 mg/kg BW dose, so it can be concluded that the ethanolic extract of E. elatior fruit is able to normalize blood creatinine levels in plasma by being excreted through urine.

Albuminuria is a key feature of diabetic nephropathy; therefore; we inspect EEEE effects on albuminuria development and compare them based on the EEEE doses of 400, 600, and 800 mg/kgBB to reach the benefit of EEEE. In the 600 mg/kgBB dose group, the EEEE effect is the most significant to reduce albuminuria after 4 weeks of therapy on experimental mice ($p > 0.05$).

The research result shows that giving EEEE at doses of 200, 400, 600, and 800 mg/kgBB has an effect on hs-CRP rate. Undertaking STZ-NA induction followed by UUO in mice results in glomerulosclerosis and kidney tubulus degeneration. Necrosis and apoptosis can trigger inflammation marked by hs-CRP increase detected in low concentration. After giving EEEE to experimental mice at doses of 200, 400, 600, and 800 mg/kgBB, the hs-CRP level is proven to decrease within 3 weeks. This shows that EEEE possess anti-inflammatory activity.

UUO is admitted as a representative method to build kidney fibrosis model.[2,25] The UUO model in 24 hours experiences significant decrease of kidney blood stream and glomerular filtration rate in which interstitial inflammation will happen in 2–3 days. Tubular dilatation, tubular atrophy, and fibrosis will happen on the seventh day.[26] Dilatation of cortex and thinning of medulla happen in the 200 mg/kgBB dose group of EEEE mice. Diffuse mesangial expansion is a unique sign of diabetic nephropathy.[27] Mesangial matrix expansion will reduce capillary surface area that contributes to proteinuria and kidney function failure.[28] The EEEE dose of 400 mg/kgBW still showed mesangial cell expansion as in diabetic nephropathy, but no histologic features such as unilateral ureteral obstruction were found. Administration of EEEE at a dose of 600 mg/kgBW only showed minimal glomerular changes without mesangial expansion, whereas at a dose of 800 mg/kgBW only minimal inflammation was observed.

CONCLUSIONS

The experimental animals that are induced with STZ-NA followed by UUO represent proper diabetes and hypertension animal models. Increased production of high-level free radical oxygen is related to glucose oxidation and nonenzymatic protein glycation that contribute to the development of diabetes complications. This research proves that EEEE possesses anti-hyperglycemia and anti-hypertension effects, resulting in significant recovery of plasma glucose, blood pressure, kidney failure, and also anti-inflammation activity.

REFERENCES

1. Indonesian Renal Registry. 8th Report of Indonesian Renal Registry. Tim Indonesian Renal Registry. 2015; p 45. https://www.indonesianrenalregistry.org/data/INDONESIAN%20RENAL%20REGISTRY%202015.pdf
2. Nogueira A, Pires MJ, and Oliveira PA. Pathophysiological mechanisms of renal fibrosis: a review of animal models and therapeutic strategies. In Vivo. 2017; 31(1): 1–22. https://doi.org/10.21873/invivo.11019
3. National High Blood Pressure Education Program Working Group report on hypertension in diabetes. Hypertension. 1994 Feb; 23(2): 145–158; discussion 159–160. https://doi.org/10.1161/01.HYP.23.2.145
4. Hill NR, Fatoba ST, Oke JL, et al. Global prevalence of chronic kidney disease – a systematic review and meta-analysis. PLoS One. 2016; 11(7): e0158765. https://doi.org/10.1371/journal.pone.0158765
5. Kao MPC, Ang DSC, Pall A, et al. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J Hum Hypertens. 2010; 24(1): 1–8. https://doi.org/10.1038/jhh.2009.70
6. Purwanto B. Hipertensi (patogenesis, kerusakan target organ, dan penatalaksanaan). Surakarta: Uns Press; Surakarta. 2012. pp: 3-5, 8-25, 59-60

7. Tucker PS, Scanlan AT, and Dalbo VJ. Chronic kidney disease influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. Oxid Med Cell Longev. 2015; 2015: 806358. https://doi.org/10.1155/2015/806358
8. Meenakshi Sundaram SP, Nagarajan S, and Manjula Devi AJ. Chronic kidney disease—effect of oxidative stress. Chin J Biol. 2014; e216210. https://doi.org/10.1155/2014/216210
9. Isyanti M, Andarwulan N, and Faridah DN. Karakteristik Fisik dan Fitokimia Buah Kecombrang (Etlingera elatior (Jack) R.M. Sm). Warta Industri Hasil Pertanian. 2019; 36(2): 96–105. https://doi.org/10.32765/wartaihp.v36i2.5267
10. Fristiohady, A., Wahyuni, W., Ilyas, M., Mentarry, B., Ode, L., Purnama, M. J., Sangadji, F., Malaka, M.H., Malik, F., Hong, J., Wibawa, A., Yodha, M., & Sahidin, S. Nephroprotective effect of extract Etlingera elatior (Jack) R.M. Smith on CCl 4 - induced nephrotoxicity in rats. *Current Research on Biosciences and Biotechnology*. 2020; 1. 62–65. https://doi.org/10.5614/crbb.2019.1.2/DAPM6106
11. Nasution J, Riyanto R, and Chandra RH. Kajian Etnobotani Zingiberaceae Sebagai Bahan Pengobatan Tradisional Etnis Batak Toba Di Sumatera Utara: Ethnobotany of Zingiberaceae as a traditional herbal medicine of Batak Toba Ethnic in North Sumatera. Medkon. 2020; 25(1): 98–102. https://doi.org/10.29244/medkon.25.1.98-102
12. Leorita M, Mardikasari SA, Wahyuni W, et al. Aktivitas Antioksidan dan Toksisitas Akut Ekstrak Etanol Buah, Daun, Batang dan Rimpang Tanaman Wualae (Etlingera elatior (Jack) R.M. Smith). Pharmauho: Jurnal Farmasi, Sains, dan Kesehatan [Internet]. 2019 [cited 2022 Jun 3]; 4(2). Available from: http://ojs.uho.ac.id/index.php/pharmauho/article/view/6263
13. Sahidin S, Salsabila S, Wahyuni W, et al. Potensi Antibakteri Ekstrak Metanol dan Senyawa Aromatik dari Buah Wualae (Etlingera elatior). J Kimia Valensi. 2019; 5(1): 1–7. https://doi.org/10.15408/jkv.v5i1.8658
14. Ramasamy S, Mazlan N, Ramli N, et al. Bioactivity and Stability Studies of Anthocyanin-Containing Extracts from Garcinia mangostana L. and Etlingera elatior Jack (Aktiviti Biologi dan Kajian Kestabilan Ekstrak mengandungi Antosianin daripada Garcinia mangostana L. dan Etlingera elatior Jack). Sains Malaysiana. 2016; 45:559–565. https://www.researchgate.net/publication/303458813
15. Dumitriu I, Gurzu B, Elena C, et al. Validation of GOD/PAP method for quantitative determination of glucose concentration in human serum. Rev Rom Med Labor. 2011; 19: 85–100.
16. Delanghe, J. R., & Speeckaert, M. M. Creatinine determination according to Jaffe-what does it stand for?. NDT plus, 2011; 4(2): 83–86. https://doi.org/10.1093/ndtplus/sfq211
17. Ghasemi A, Khalifi S, and Jedi S. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiol Hung. 2014; 101(4): 408–420. https://doi.org/10.1556/APhysiol.101.2014.4.2
18. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med (Maywood). 2012; 237(5): 481–490. https://doi.org/10.1258/ebm.2012.011372
19. Yang T, and Li C. Role of COX-2 in unilateral ureteral obstruction: what is new? Am J Physiol Renal Physiol. 2016; 310(8): F746–F747. https://doi.org/10.1152/ajprenal.00498.2015
20. Atılgan D, Parlaktas BS, Uluocak N, et al. Effects of melatonin on partial unilateral ureteral obstruction induced oxidative injury in rat kidney. Urology Annals. 2012; 4(2): 89. https://doi.org/10.4103/0974-7796.95552
21. Wu Z, Zhu A, Takayama F, et al. Brazilian green propolis suppresses the hypoxia-induced neuroinflammatory responses by inhibiting NF-κB activation in microglia. Oxid Med Cell Longev. 2013; 2013: 906726. https://doi.org/10.1155/2013/906726
22. Chinnappan SM, George A, Thaggikuppe P, et al. Nephroprotective effect of herbal extract Eurycoma longifolia on paracetamol-induced nephrotoxicity in rats. Evid Based Complement Altern Med. 2019; 2019: e4916519. https://doi.org/10.1155/2019/4916519
23. Ezejiofor AN, and Orisakwe OE. Nephroprotective effect of Costus afer on lead induced kidney damage in albino rats. Int J Physiol Pathophysiol Pharmacol. 2019; 11(2): 36–44.

24. Vaidya VS, Waikar SS, Ferguson MA, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. Clin Transl Sci. 2008; 1(3): 200–208. https://doi.org/10.1111/j.1752-8062.2008.00053.x
25. Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. Am J Physiol Renal Physiol. 2016; 311(1): F145–F161. https://doi.org/10.1152/ajprenal.00164.2016
26. Yang HC, Zuo Y, and Fogo AB. Models of chronic kidney disease. Drug Discov Today Dis Models. 2010; 7(1): 13–19. https://doi.org/10.1016/j.ddmod.2010.08.002
27. Bilous RW. The pathology of diabetic nephropathy. In: Alberti K, Zimmet P, DeFronzo RA, and Keen H, editors. International textbook of diabetes mellitus. Chichester: Wiley; 1997; pp. 1349–1362.
28. Ziyadeh FN. The extracellular matrix in diabetic nephropathy. Am J Kidney Dis. 1993; 22(5): 736–744. https://doi.org/10.1016/S0272-6386(12)80440-9

J Popul Ther Clin Pharmacol Vol 29(4):e140–e149; 2 December 2022.