



Can Ethanol Extract of *Etligeria elatior* Fruit Prevent Inflammations on Diabetic Nephropathy Mice Models?

Teky Widyarini¹, Dono Indarto², Soetrisno³, Bambang Purwanto⁴

^{1,2,3,4}Faculty of Medicine, Universitas Sebelas Maret, Surakarta 57126, Indonesia

*Corresponding author: Teky Widyarini, Faculty of Medicine, Universitas Sebelas Maret, Jalan Ir. Sutami 36 Surakarta, Jawa Tengah, Indonesia 57126, Email: twidyarini@gmail.com

Submitted: 18 January 2023; Accepted: 23 February 2023; Published: 19 March 2023

ABSTRACT

Objective: This research aimed to compare ethanol extract of *Etligeria elatior* (*E.elatior*) effects given in diabetes, pre diabetes, and post diabetes period.

Materials and Methods: This research used post-test only control group design. The first five mice group was given extract in pre DM period, the next group was given extract in post DM period, and the last group was given after ligation. Unilateral ureter ligation will be given to diabetic mice after one week. Evaluation of body weight, tension, glucose, albuminuria, and creatinine was in the fourth and fifth week of experiment. In the last week, High Sensitive C Reactive Protein (hsCRP) was examined.

Results: The results of the research showed that ethanol extract of *E.elatior* significantly reduce creatinine and hsCRP rate when the extract was given in pre diabetic and DM period ($p < 0,05$). There is no significant difference of urine albumin rate based on extract giving period. Mice receiving extracts are proven to decrease necrotic and inflammation condition in kidney histologically.

Conclusion: ethanol extract of *E.elatior* fruit possesses anti-hyperglycemic and anti-hypertension activities as well as hinders weight loss. The extract is beneficial for nephroprotective with natural antioxidant and anti-inflammation substances inside of it.

Keywords: *diabetic nephropathy, ethanol extract of E.elatior fruit, nephroprotective, anti-inflammation, antioxidant*

INTRODUCTION

Diabetic nephropathy is one of micro vascular complications in diabetes mellitus (DM), the main reason of mortality and morbidity in diabetes^{1,2}. Diabetic nephropathy is marked with albuminuria and the decrease of Estimated Glomerular Filtration Rate (eGFR) without other symptoms of kidney failures^{3,5}.

Inflammation is a body protection mechanism involving host cell, blood vessels, protein, and

other mediators against infections and other dangerous stimulants [6,7]. Inflammation may occur on diabetes in consequence of hyperglycemia activating pro-inflammatory cytokines such as Tumor Necrosis Factor-Alfa (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Interleukin-18(IL-18) that trigger leukocytes recruitment on inflammation area by resulting Reactive Oxygen Species (ROS) [8,9].

Chronic hyperglycemia on diabetes will induce sustainable ROS and activate pro-inflammatory cytokines [10–12]. High Sensitive C Reactive Protein (hsCRP) as the most stable inflammation marker will be formed by hepatocytes due to IL6 stimulation resulting in endothelium damage [12–16]. Because of that, inflammation process has important impact in diabetic nephropathy and can be a therapy target [11,17,18].

Etlingera elatior (*E.elatior*) or kecombrang is Indonesian plant with rich bioactive compounds such as flavonoid, phenol, glycoside, saponins, tannin, steroid, and terpenoid [19–22]. *Etlingera elatior* fruit itself can work as anti-bacteria, anti-fungi, and immuno-modulator [23–26]. Therefore, we are interested in investigating ethanol extract of *E.elatior* effects as a candidate of inflammation resistor, observed from hsCRP marker on diabetic nephropathy mice models by comparing the extract giving period.

MATERIALS AND METHODS

Materials

Materials to use in this research are Streptozotocin (STZ) and Nicotinamide (NA) from Nacalai Tesque, Inc. product. The *E.elatior* fruit is from Langkaplancar district, Pangandaran city, West Java. Other materials are Ethanol 96% from Merck, Vanillic acid from Sigma-Aldrich, and DPPH radical from Sigma-Aldrich. In hsCRP blood inspection, the substances to use are latex reagen Cardiac C-Reactive Protein High Sensitive from Roche. In blood glucose inspection, the substances to use are standard

solution of glucose 100 mg/dL and glucose reagent kit from DiaSys. The tool to measure glucose level activity and hsCRP serum is spectrophotometer. Extract producing process uses chemical glass, measuring cup, test tube, and Erlenmeyer from Pyrex®; pipette, analytic scale from Precisa®; blender from Philips; rotary vacuum evaporator from Buchi®; incubator from Froilabo®; spectrophotometer UV± Vis 20D from Thermo®; and vortex from Stuart®.

Method

The implemented laboratory experimental research design is post-test only control group design. The study was approved by Research Ethics Committee, Faculty of Medicine Universitas Sebelas Maret (39/UN27.06.6.1/KEP/EC/2021).

Sample collection and extraction procedure

Etlingera elatior identification

Ripe *E.elatior* fruit at three months age from flower bud phase was determined by biological department in MIPA Faculty of Universitas Muhammadiyah Surakarta.

Etlingera elatior fruit extraction

Etlingera elatior fruit was clean washed, dried, and triturated, then follow maseration process using six litres of ethanol 96% [22,27]. The result of maseration process is then separated to gain ethanol extract of *E.elatior* fruit [24,28,29].

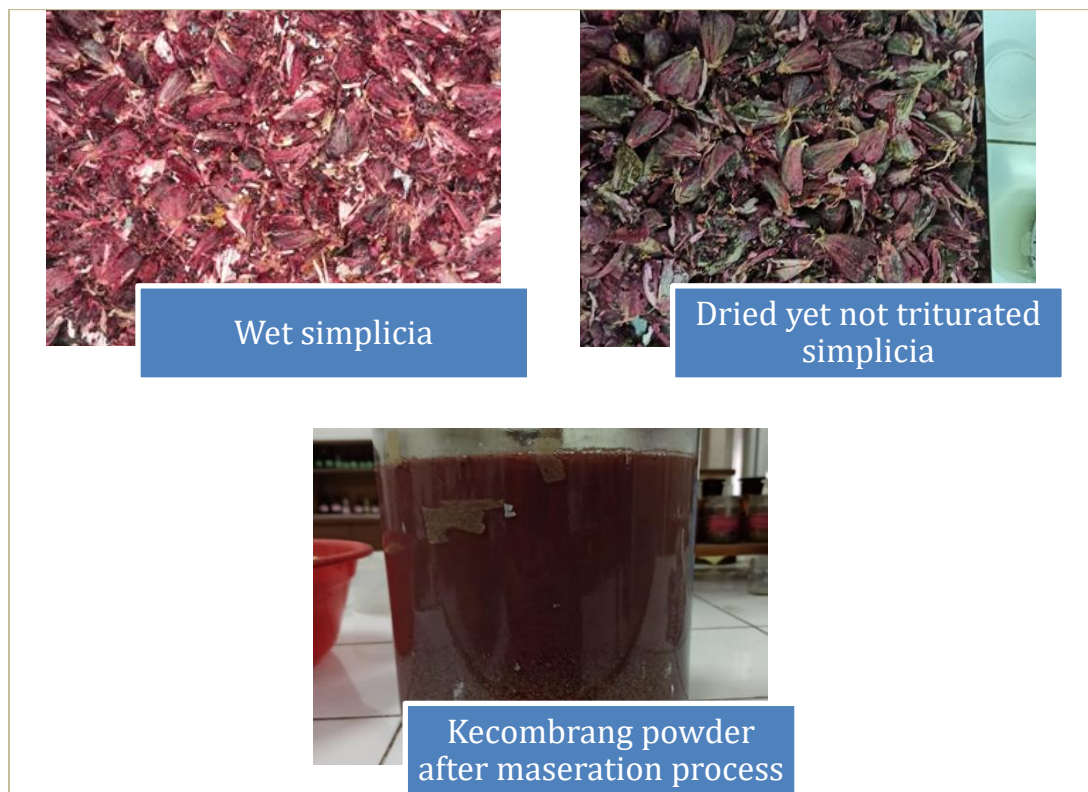


FIGURE 1: Sample of *E. elatior* fruit collection and extraction procedures.

Antioxidant activity test using DPPH method

Antioxidant activity test using DPPH method was done based on [30]. The measurement of absorbance at 517nm wavelength using spectrophotometer UV-Vis twice. After that Inhibition Concentration 50% (IC50) value is determined, the sample concentration that dampen DPPH free radical as much as 50%. IC50 is the extract concentration that dampen the DPPH activity as much as 50%.

Experimental animal

Fifteen male white mice (*Rattus norvegicus*) with 120-200 grams weight in Pengembangan Hewan Coba Fakultas Farmasi Universitas Gadjah Mada Laboratory were acclimatized and given standard diet for seven days before experiment [31].

Induction of nephropathy mice models in DM type 2

After one week of adaptation, mice are randomly divided into four groups: given extract before DM group (n=5), given extract after DM group

(n=5), given extract after ligation group (n=5), and normal group. The normal group was not given any extract, their tissue was the only thing taken at week five as well as other groups. On experiment day, white mice were injected with NA 110 mg/kg BB and STZ 45 mg/kg BB. Glucose blood and body weight were measured after 72 hours or seven days after NA/STZ injection [32]. After a week of STZ-NA induction, ureteral unilateral ligation (UUO) was conducted. Body weight and tension were measured on the first, second, and fourth week. Body weight, tension, glucose, albuminuria, and creatinine were measured on week three and five. On the last week, body weight, tension, glucose, albuminuria, creatinine, hsCRP, and histopathology were measured. The normal group with no extract given is exclusively used as the comparison for histopathology without treatment.

Blood and Tissue Samples

At the end of the treatment period, mice were anesthetized by intraperitoneal injection of ketamine (45 mg/kg) after fasted overnight and

then sacrificed by cervical dislocation. The serum samples were prepared by collecting the blood from the retroorbital vein in anticoagulant-free tubes followed by centrifugation at 3000× g for 10 min. The tissue samples from kidney were quickly removed, cleaned in ice-cooled saline, blotted dry, and weighed. This procedure was carried out right after blood sampling. The tissue samples from kidney were preserved in buffered neutral formalin at a 10% concentration for histopathological and immunohistochemical analyses. A second weighted component was stored in a freezer at -80 °C for future research.

Statistical Analysis

Biological parameter result is declared as mean ± standard deviation. The difference statistical

analysis among average values of three groups was conducted using F-test, followed by Student t-test or Aspin-Welch t-test. The difference is significant when <0,05.

Data of body weight, tension, glucose, albuminuria, creatinine, and hsCRP were analyzed using parametric test One Way Analysis of Variance (Anova) and Repeated Anova. One Way Anova test is used to find out existing difference of average rate values between the groups on specific time. Repeated Anova is used to analyze how big the rate change from time to time in specific group. The result is statistically meaningful when p<0,05. Data analysis was done using Statistical Product and Service Solutions (SPSS) program 24.0 version for Windows.

RESULTS

Antioxidant activity test result of *E.elatior* fruit

TABLE I: IC50 Value

No.	Sampel	IC50 (ppm)	Persamaan	r2
1	Ekstrak etanol kecombrang	5,079	$y = 2,4479x + 37,568$	0,9985
2	Ekstrak metanol kecombrang	5,445	$y = 1,8978x + 39,666$ dan $r2 = 0,9663$	0,9663
3	Vanilic acid	8,996	$y - 3,5571x + 18$ dan $r2 = 0,9934$	0,9934

Description: IC50 = inhibitory concentration 50%

In vivo anti-hyperglycemic activity from *E.elatior* extract and its effect on body weight and tension

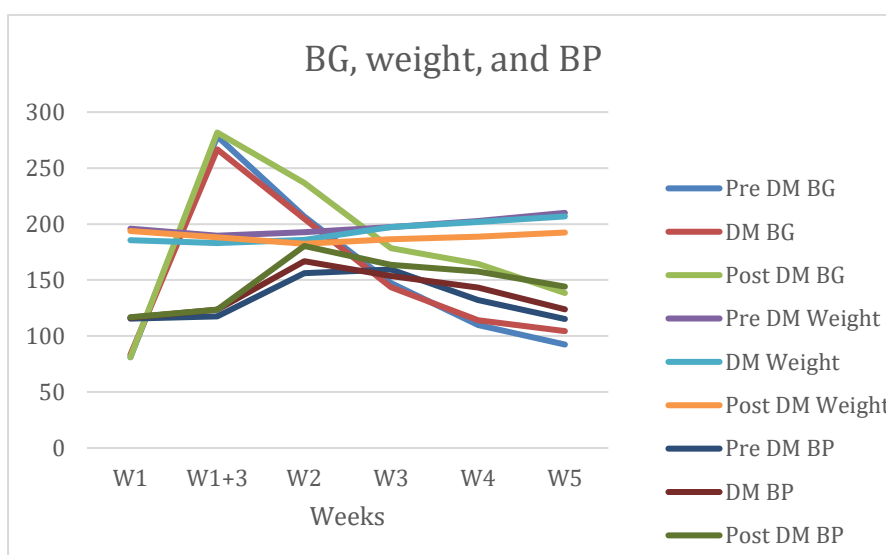


FIGURE 2: Effects of *E.elatior* extract on blood glucose, body weight, and tension.

Description: BG = Blood Glucose, BP = Blood Pressure, W = week

Figure 2 shows that ethanol extract of E.elatior fruit significantly decrease blood glucose rate, as when extract was given before DM and when DM ($p < 0,05$). In other side, glucose rate difference is shown significantly ($p < 0,05$) if extract is given when DM ($143,69 \pm 2,54$ mg/dl) in comparison to pre DM ($147,30 \pm 1,16$ mg/dl).

Ethanol extract of E.elatior fruit that was evaluated on diabetic nephropathy mice model can prevent weight loss significantly ($p < 0,05$). What's interesting is no significant weight difference in each week, especially week four to five, both when extract was given before DM and when DM.

Tension was significantly decreasing due to ethanol extract of E.elatior fruit adduction ($p < 0,05$), especially if extract was given on diabetic and pre-diabetic condition, especially in week five.

In general, if extract was given after diabetic nephropathy occurred, there is no significant difference in body weight from the beginning of extract giving to the end of research, however there is still a meaningful difference of tension and blood glucose rate ($p < 0,001$).

Kidney protection effect of E.elatior fruit ethanol on diabetic nephropathy mice

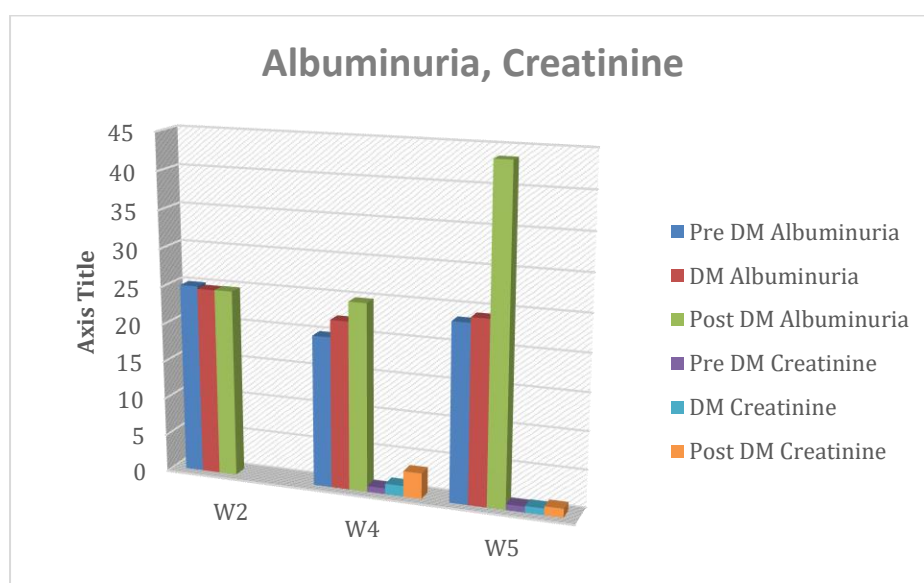


FIGURE 3: Albuminuria and Creatinine activities during week 2, 4, and 5.

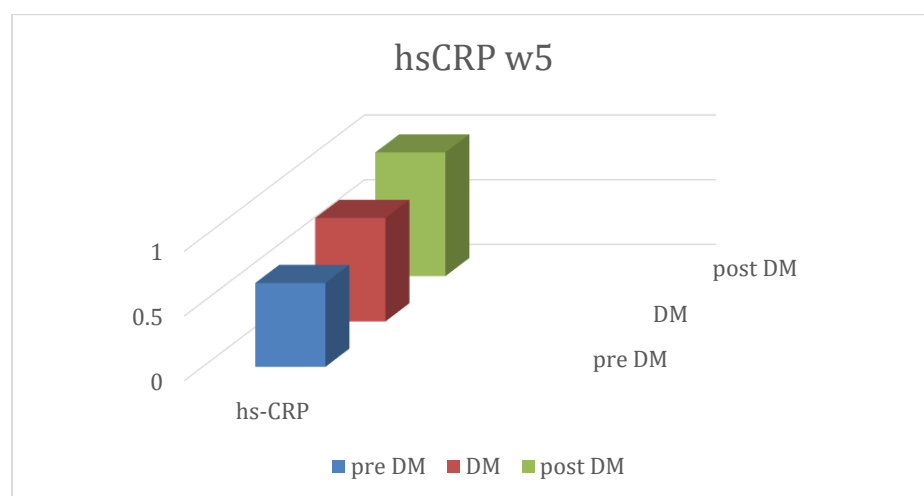


FIGURE 4: hsCRP activities during week 5.

There is significant difference of creatinine level reduction and hsCRP in week five if the extract was given on pre diabetic and DM in comparison to post DM ($p < 0,05$), with the lowest decrease on pre diabetic extract adduction. Albumin urine level is lower if the extract was given when DM though no significant difference.

Histopathology

Kidneys were collected for histopathology inspection and comparing them to control group. The control group is the normal group with no *E.elatior* ethanol extract adduction after ligation to show pathological markers such as thinning cortex (atrophy) with dilatation and necrosis medulla. The group of mice with extract given in

pre DM period shows architecture, glomerulus size, membrane basement thickness, cortex, and medulla in relatively normal condition with minimum inflammation cells. Otherwise in mice group with extract given in DM period shows glomerulus with slight difference and no clear sign of mesangial cell expansion. All three pre DM, DM, and after ligation extract adduction prove to lessen necrotic condition and the decrease of inflammation cells infiltrations in cortex and medulla. Therefore, antioxidant compounds and fatty acid in plants may help in chronic blood glucose rate refinement, oxidative stress due to AGEs resistance, and other related consequences, including effectively resist diabetic nephropathy development.

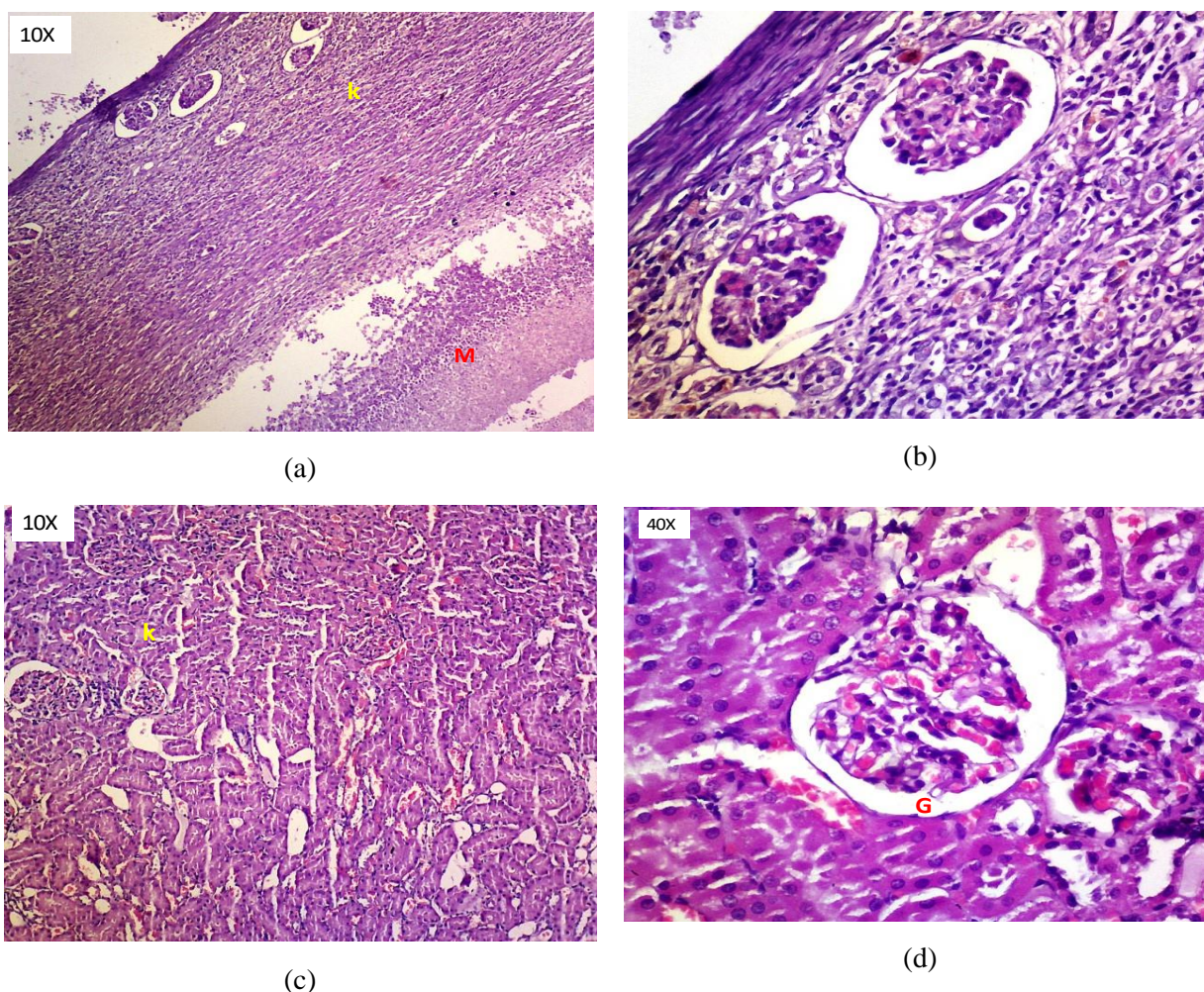


FIGURE 5: (a) Histopathology of normal mice group with no *E.elatior* ethanol extract; (b) Histopathology of mice group with extract given after ligation; (c) Histopathology of mice group with extract given in post DM period; (d) Histopathology of mice group with extract given in DM period

DISCUSSION

Vanillic Acid (VA) compound possess strong antioxidant and anti-inflammation action, easy to dissolve in polar solvent such as methanol and ethanol [33,34]. *Etligeria elatior* fruit holds VA as the most active compound [24]. The research results in the antioxidant activity of *E.etlangior* fruit with IC50 value of 5,079 mg/L higher than methanol extract with value of 5,445 mg/L, and VA with with IC50 value of 8,996 mg/L.

Diabetic nephropathy patient treatment with non-selective antioxidant like vitamin E and C failed to repair diabetic complication by reason of both vitamins detain non-selective ROS source [18]. *Etligeria elatior* fruit contains bioactive compound with antioxidant potentials and ability to seize free radicals [22,26,35]. In this research, we utilise DM type 2 (DM2) mice models induced with NA-STZ and ligated to investigate the renoprotective effects of *E.etaliior* ethanol extract. After a week of hyperglycemia, UO was conducted, the diabetic mice will go through kidney disfunction and hispathology change, related to inflammation rate marker increase and oxidative. Theraphy using ethanol extract of *E.elatior* fruit results in significant reduction of albuminuria as well as increase of other renal functional parameters.

The benefit of *E.elatior* ethanol extract is further evaluated in DM2 mice models in vivo study that evidently reduce blood glucose and tension level depending on extract giving period. The extract also prevents weight loss of mice without significant difference if compared each week. The result clearly shows that ethanol extract of *E.elatior* fruit owns positive effects to hyperglycemia reduction, tension, and weight loss prevention.

This research proves that consuming ethanol extract of *E.elatior* fruit significantly reduce blood glucose rate on DM2 mice induced with STZ-NA. Several research show that rimpang extract and *E.elatior* extract can reduce blood glucose rate on DM2 mice models. Rimpang and *E.elatior* extracts can hinder α -amylase and α -glucosidase as well as hold anti-hyperglycemic on DM2 mice models [36,37]. Research about anti-hyperglycemic effect from ethanol extract of *E.elatior* remains very limited.

Ethanol extract of *E.elatior* adduction prevents weight loss on diabetic mice, hence their body weight during experiment was consistent, so that if the body weight is compared each month there will be no significant difference ($p>0,05$). Weight loss on DM mice model caused by insulin deficiency catabolic effect that triggers muscle downsizing and structural protein degradation on diabetes patients [31].

Hypertension is both causing and consequence factor of Chronic Kidney Disease (CKD). This research shows that UO will implicates on significant blood tension increase. In line with [38,39], UO causes apoptosis, fibrosis, and inflammation formation. Anti-inflammation and antioxidant activity of *E.elatior* ethanol extract manage to resist blood tension increase [31].

Several mechanisms play important roles in diabetic nephropathy pathogenesis. Hyper filtration and glucose absorption in kidney induce AGE accumulation, oxidative stress, and inflammation [40]. Oxidative stress increase cytokine pro inflammation, hsCRP, by activating transcription nucleus factor $\text{NF}\kappa\beta$. Because of that, we explore ethanol extract of *E.elatior* effects with anti-inflammation and antioxidant activity when given to diabetic nephropathy mice. It results in dampening inflammation and oxidative stress. Inverse correlation between GFR and inflammation is clearly proven. Chronic Renal Insufficiency Cohort (CRIC) study results of inflammation biomarker ($\text{IL-1}\beta$, antagonist receptor IL-1 , IL-6 , $\text{TNF-}\alpha$, CRP and fibrinogen), in reverse to functional renal size and correlating positively with albuminaria [41,42].

Fibrosis interstitial mechanism in CKD associates with proteinuria, ROS, vasoactive substance, tubular hypertrophy, hypermetabolism, and endotel disfunction [43]. Other than that, according to [44], UO causes various kidney failures, such as glomerulosclerosis, tubular apoptosis, and interstitial fibrosis [45]. This research shows that ethanol extract of *E.elatior* fruit hinder glomerulosclerosis and inflammation, where the best result shows on both extract giving in the beginning of diabetes and pre DM period.

CONCLUSION

Strong antioxidant capacity in ethanol extract of *E. elatior* fruit possess great impact in anti-diabetes and anti-hypertension effects. It holds anti-hyperglycemic activity on DM2 mice models. Diabetes complication causes weight loss, however ethanol extract of *E. elatior* fruit can detain the happening weight loss. The antioxidant activity of *E. elatior* ethanol extract reduces blood tension increase. Giving *E. elatior* ethanol extract will reduce inflammation marked by hsCRP decrease. Nephroprotective activity of *E. elatior* ethanol extract repairs creatinine and albuminuria rate. By observing kidney histopathology, ethanol extract of *E. elatior* restore normal functional structure of kidney. All these concludes that *E. elatior* is a natural source of antioxidant and anti-inflammation with curing diabetes patients and diabetic nephropathy prevention potentials. This research unfolds the usage of extracts on pre diabetic period that will expand more beneficial results in clinical trial.

REFERENCES

- Haller H, Ji L, Stahl K, Bertram A, Menne J. Molecular Mechanisms and Treatment Strategies in Diabetic Nephropathy: New Avenues for Calcium Dobesilate—Free Radical Scavenger and Growth Factor Inhibition. *BioMed Research International* 2017; 2017:e1909258.
- Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int Suppl* 2018; 8(1):2–7.
- Lim AK. Diabetic nephropathy – complications and treatment. *Int J Nephrol Renovasc Dis* 2014; 7:361–81.
- Pugliese G, Penno G, Natali A, Barutta F, Di Paolo S, Reboldi G, et al. Diabetic kidney disease: new clinical and therapeutic issues. Joint position statement of the Italian Diabetes Society and the Italian Society of Nephrology on “The natural history of diabetic kidney disease and treatment of hyperglycemia in patients with type 2 diabetes and impaired renal function.” *J Nephrol* 2020; 33(1):9–35.
- Singh D, Winocour P, Farrington K. Oxidative stress in early diabetic nephropathy: Fueling the fire. *Nature reviews Endocrinology* 2011; 7:176–84.
- Kumar R, Chhatwal S, Arora S, Sharma S, Singh J, Singh N, et al. Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase- lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. *Diabetes Metab Syndr Obes* 2013; 6:49–56.
- Baratawidjaja KG, Rengganis I. *Imunologi dasar / Karnen Garna Baratawidjaja, Iris Rengganis*. 8th ed. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia; 2010. 721 p.
- Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovasc Ther* 2012; 30(1):49–59.
- Wright CB, Sacco RL, Rundek TR, Delman JB, Rabbani LE, Elkind MSV. Interleukin-6 is associated with cognitive function: the Northern Manhattan Study. *J Stroke Cerebrovasc Dis* 2006; 15(1):34–8.
- Giridharan S, Srinivasan M. Mechanisms of NF- κ B p65 and strategies for therapeutic manipulation. *J Inflamm Res* 2018; 11:407–19.
- Lee TF, Drake SM, Roberts GW, Bersten A, Stranks SN, Heilbronn LK, et al. Relative Hyperglycemia Is an Independent Determinant of In-Hospital Mortality in Patients With Critical Illness. *Critical Care Medicine* 2020; 48(2):e115–22.
- Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol* 2019; 11(3):45–63.
- Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in Immunology* [Internet]. 2018 [cited 2022 Jun 3]; 9. Available from: <https://www.frontiersin.org/article/10.3389/fimmu.2018.00754>
- Purwanto E. Korelasi Jumlah Netrofil, Limfosit, dan Monosit dengan Kadar Albumin Urin pada Pasien DM Tipe 2 dengan Mikroalbuminuria. *Biomedika* [Internet]. 2009 [cited 2022 Jun 3]; 1(1). Available from: <https://journals.ums.ac.id/index.php/biomedika/article/view/287>
- Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; 54(6):1615–25.
- Ghayur A, Margetts PJ. Transforming growth factor-beta and the glomerular filtration barrier. *Kidney Res Clin Pract*. 2013; 32(1:3–10.
- Gnudi L, Coward RJM, Long DA. Diabetic Nephropathy: Perspective on Novel Molecular Mechanisms. *Trends Endocrinol Metab*. 2016; 27(11):820–30.
- Gorin Y, Cavaglieri RC, Khazim K, Lee DY, Bruno F, Thakur S, et al. Targeting NADPH oxidase with a novel dual Nox1/Nox4 inhibitor attenuates renal pathology in type 1 diabetes. *Am J Physiol Renal Physiol* 2015; 308(11):F1276–1287.
- Chan EWC, Lim YY, Tan SP. Standardised herbal extract of chlorogenic acid from leaves of *Etligeria elatior* (Zingiberaceae. *Pharmacognosy*

- Res 2011; 3(3):178–84.
20. Wijekoon MMJO, Bhat R, Karim AA. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* Jack. inflorescence. *Journal of Food Composition and Analysis* 2011; 24(4):615–9.
 21. Koraag ME, Anastasia H, Isnawati R, Octaviani O. Efikasi Ekstrak Daun Dan Bunga Kecombrang (*Etlingera elatior* Terhadap Larva *Aedes Aegypti*. *Aspirator* 2016; 8(2):63–8.
 22. Isyanti M, Andarwulan N, Faridah DN. Karakteristik Fisik dan Fitokimia Buah Kecombrang (*Etlingera elatior* (Jack R.M. Sm. *Warta Industri Hasil Pertanian* 2019; 36(2):96–105.
 23. Ghasemzadeh A, Jaafar HZE, Rahmat A, Ashkani S. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of *Etlingera elatior* (Jack R.M.Sm grown in different locations of Malaysia. *BMC Complementary and Alternative Medicine* 2015; 15(1):335.
 24. Sahidin S, Salsabila S, Wahyuni W, Adryan F, Imran I. Potensi Antibakteri Ekstrak Metanol dan Senyawa Aromatik dari Buah Wualae (*Etlingera elatior*. *Jurnal Kimia Valensi* 2019; 5(1):1–7.
 25. Wahyuni, Grashella SHL, Fitriah WOI, Malaka MH, Fristiody A, Imran, et al. Antibacterial and antioxidant potentials of methanol extract and secondary metabolites from Wualae rhizome (*Etlingera elatior*. *Current Research on Biosciences and Biotechnology* 2019; 1(1):13–6.
 26. Nasution J, Riyanto R, 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.
 27. Suwadi, Pujiyono, et al. "Legal comparison of the use of telemedicine between Indonesia and the United States." *International Journal of Human Rights in Healthcare* ahead-of-print (2022).
 28. Leorita M, Mardikasari SA, Wahyuni W, Malaka MH, Sartinah A, Sahidin S. 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>
 29. Ramasamy S, MAZLAN N, RAMLI N, Rasidi W, Manickam S. Bioactivity and Stability Studies of Anthocyanin-Containing Extracts from *Garcinia mangostana* L. and *Etlingera elatior* Jack. 2016: 45:559–65.
 30. Burda S, Oleszek W. Antioxidant and Antiradical Activities of Flavonoids. *J Agric Food Chem* 2001; 49(6):2774–9.
 31. Stevani H. *Praktikum Farmakologi*. Jakarta: Kemenkes RI; 2016.
 32. Potârniche AV, Dreanca A, Sarpataki O, Sevastre B, Marcus I. Experimental model of Streptozotocin-Nicotinamide induced Diabetes Mellitus type II in Sprague-Dawley rats : Step by step protocol and the encountered issues. *Revista Română de Medicină Veterinară* 2018; 28.
 33. Kim SJ, Kim MC, Um JY, Hong SH. The Beneficial Effect of Vanillic Acid on Ulcerative Colitis. *Molecules* 2010; 15(10):7208–17.
 34. Handayani H, Sriherfyna FH, Yunianta Y. Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan : Pelarut Dan Lama Ekstraksi [In Press Januari 2016]. *Jurnal Pangan dan Agroindustri* [Internet]. 2016 [cited 2022 Jul 23]; 4(1. Available from: <https://jpa.ub.ac.id/index.php/jpa/article/view/327>
 35. Naufalin R, Rukmini H. Potensi Bunga Kecombrang Sebagai Pengawet Alami Pada Tahu Dan Ikan. *Semnas Seminar Nasional Pusat Penelitian Pangan, Gizi dan Kesehatan*, 8-9 Oktober 2010. 2010 Oct 8;
 36. Srey C, Sontimuang C, Thengyai S, Ovatlarnporn C, Puttarak P. Anti a-glucosidase, anti a-amylase, anti-oxidation, and anti-inflammation activities of *Etlingera elatior* rhizome. *Journal of Chemical and Pharmaceutical Research* [Internet]. 2014 [cited 2022 Jul 23]; 6(12). Available from: <https://www.jocpr.com/abstract/anti-aglucosidase-anti-aamylase-antioxidation-and-antiinflammation-activities-of-etlingera-elatior-rhizome-8635.html>
 37. Nor NAM, Noordin L, Bakar NHA, Ahmad WANW. Evaluation of antidiabetic activities of *Etlingera elatior* flower aqueous extract in vitro and in vivo. *J App Pharm Sci* 2020; 10(8):043–51.
 38. Yang T, Li C. Role of COX-2 in unilateral ureteral obstruction: what is new? *Am J Physiol Renal Physiol* 2016; 310(8):F746–7.
 39. Susanto A, Putranto W, Dewi RTK, Purwanto B. Unilateral Ureteral Obstruction as a Model of Kidney Fibrosis and Increasing of Systolic Blood Pressure in Mice. *Indonesian Journal of Kidney and Hypertension* 2019; 2(3):20–5.
 40. Lim AKH, Tesch GH. Inflammation in diabetic nephropathy. *Mediators Inflamm* 2012; 2012:146154.
 41. Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. *Biochimica et Biophysica Acta (BBA - Gene Regulatory Mechanisms)* 2010; 1799(10):775–87.
 42. Akchurin OM, Kaskel F. Update on inflammation in chronic kidney disease. *Blood Purif* 2015; 39(1–3):84–92.

43. Liu T, Zhang L, Joo D, Sun SC. NF- κ B signaling in inflammation. *Sig Transduct Target Ther* 2017; 2(1):1–9.
44. Lee J, Bae EH, Ma SK, Kim SW. Altered Nitric Oxide System in Cardiovascular and Renal Diseases. *Chonnam Med J* 2016; 52(2):81–90.
45. 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):F145-161.