

Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extracts in alloxan-diabetic rats

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Article history

Received: 7 January 2014
Received in revised form:
7 February 2014
Accepted: 8 February 2014

Keywords

Eleutherine palmifolia (L.)
Merr.
Bawang dayak
Diabetes
Antioxidant
Traditional medicines

Abstract

Bawang Dayak (*Eleutherine palmifolia* L. Merr.) is traditionally used to cure diabetes mellitus and other diseases by Dayak tribes in Kalimantan Island, Indonesia, despite there is no scientific reports on its anti-diabetic activity both *in-vitro* or *in-vivo*. The study aimed to evaluate the ability of aqueous (EPA) and ethanolic extracts (EPE) of *Eleutherine palmifolia* L. Merr. bulbs to control hyperglycemic condition in alloxan-induced diabetic rats. Treatment with 100 mg/kg EPA or EPE for 28 days: (1) maintained the body weight of diabetic rats similar to those of non diabetic rats, (2) significantly reduced blood serum glucose level as compared to untreated-diabetic rats, (3) significantly had higher blood serum insulin level as compared to untreated-diabetic rats, and (4) significantly had lower blood serum total cholesterol and LDL levels compared to untreated diabetic rats. The data of 1H and 2D NMR spectra of EPE revealed the existence of eleutherinoside A, eleuthoside B, and eleutherol previously reported to be present in this plant. The results of this study justify the traditional use of *Eleutherine palmifolia* L. Merr. bulb in the management of diabetes mellitus among Dayak tribe in Kalimantan Island, Indonesia. The anti-diabetic actions of the plant is suggested by inhibiting alpha-glucosidase which could decrease postprandial blood glucose level, and also by repairing the damage of pancreatic beta cells, thus enhancing the insulin secretion directly.

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Introduction

Diabetes mellitus type 2 (DM) is a disease involving chronic carbohydrate, fat, and protein metabolism disorder due to the lack of insulin secretion, various level of resistance to insulin action, or both. The manifestation of the disease is characterized particularly by hyperglycemia (WHO, 2006). DM has become an epidemic in both developed and developing countries mainly due to a sedentary life style and unhealthy diets (Roglic *et al.*, 2005). Indonesia is on 7th position among the world top ten countries with the highest diabetes mellitus incident after China, India, USA, Brazil, Rusia and Mexico with diabetic population at age 20-76 years reached 7.6 millions in 2012 (IDF, 2013). Total Indonesian population affected by DM was 8.4 millions in 2000 and is predicted to be 21.3 millions in 2030 (Wild *et al.*, 2004). Most of the consequences of diabetes mellitus is macrovascular

and microvascular complications, such as coronary heart disease and cataracts. Deaths from coronary heart disease and stroke in the diabetic population is 2-4 times greater than non diabetic population (Bell, 1994). The treatment goals for patients with diabetes have evolved significantly over the last 80 years, from preventing imminent mortality, to alleviating symptoms, to the now recognized objective of normalization or near normalization of glucose levels with the intent of forestalling diabetic complications. The Diabetes Control and Complications Trial has conclusively demonstrated that tight glucose control in patients with type 1 diabetes significantly reduces the development and progression of chronic diabetic complications, such as retinopathy, nephropathy, and neuropathy (DCCT, 1993). Long-term follow-up of these patients demonstrated beneficial effects on macrovascular outcomes in the Epidemiology of Diabetes Interventions and Complications Study (Cleary *et al.*, 2006). Ironically, late diagnosis and

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improper treatment are the main cause for diabetes related death incident in developing countries.

Eleutherine palmifolia (L.) Merr. (EP, Iridaceae), or Bawang Dayak (local name) is a well-known plant among Dayak tribe living in Kalimantan Island, Indonesia. The origin of Eleutherine plant is from South America. Others species from this genus for examples are *E. americana*, *E. bulbosa*, *E. plicata* and *E. latifolia*. They are cultivated and naturalized in Africa, Malaysia, Indonesia (Kalimantan and West Java) and the Philippines (Luzon, Leyte, Negros, Mindanao) (zipcodezoo.com, 2011). The plant has a good adaptation capability to grow on various types of climate and soil. Dayak tribe uses the plant to cure various type of illness such as cancer, high blood pressure, diabetes mellitus, cholesterol, and ulcers (Kuntorini and Nugroho, 2010; Arung *et al.*, 2011). The most common traditional preparation is by boiling 7 cloves of EP bulb in three glasses of water until reduced by half. The water is then taken one to three times daily.

There is only a few studies regarding EP bioactivities and its chemical constituents. Shibuya *et al.* (1997) reported the presence of eleuthoside A, B and C from water soluble fraction of EP methanolic extract, as well as eleutherol, eleutherin, and iso-eleutherin from ethyl acetate soluble fraction of EP methanolic extract. Li *et al.* (2009) comprehensively reported fifteen naphthalene derivatives from EP which ten among them showing inhibitory effect on Wnt/b-catenin signaling. The Wnt/b-catenin signaling pathway plays key roles in cell morphology, motility, proliferation, and differentiation. However, abnormal activation of this pathway may lead to the formation of tumors (Mori *et al.*, 2011). Subramaniam *et al.* (2012) reported antibacterial activity of EP ethanolic extract against several pathogenic bacteria. Arung *et al.* (2009) reported that EP methanolic extract exerted melanin production inhibition in B16b melanoma cells without significant toxicity, therefore potential to be used as whitening agent in cosmetic products. Ieyama *et al.* (2011) reported alpha-glucosidase inhibitory activity of three naphthalene derivatives in methanolic extract of *Eleutherine americana* which were eleutherinoside A, eleuthoside B, and eleutherol. Eleutherinoside A was found to be the most active one with IC_{50} of 0.5 mM, while the other two showed less than 50% inhibition at concentration of 1mM.

Looking to a scarcity of scientific reports on EP anti-diabetic activity, in the present study we evaluated the anti-diabetic activity of EP bulb aqueous (EPA) and ethanolic extracts (EPE) in alloxan-induced diabetic rats. The decision to study ethanolic and aqueous extracts was taken because

the two solvents are less toxic than other organic solvents, thus further application of the extracts as functional food ingredients can be more acceptable. We also investigated anti-hyperlipidemic capacities *in-vivo* of the extracts since diabetic condition tends to elaborate LDL-cholesterol oxidation which could lead to diabetic macrovascular complication such as coronary heart disease.

Materials and Methods

Collection of plant material

The fresh plant of EP was collected from traditional market at Air Hitam village, Samarinda, East Kalimantan, Indonesia. The plant was identified as *Eleutherine palmifolia* (L.) Merr. by Dr. Joeni Setijo Rahajoe from Herbarium Bogoriense, Indonesian Institute of Sciences, and the voucher specimen was kept in The Department of Processing Technology of Forest Product, Samarinda State Agricultural Polytechnic, Samarinda 75131, Indonesia.

Chemicals, drugs, and analytical kits

Absolute ethanol was from Merck (EMSURE[®], Merck Darmstadt, Germany). Alloxan monohydrate was obtained from Sigma Chemicals, (Detroit, MI, USA). Glucometer (Accucheck[®]) was bought from Roche (Germany). Ultra sensitive rat insulin elisa kit (Biorbyt[®]) was bought from Biorbyt (Cambridge, UK). Lipid profile test kits (Fluitest[®]) was from Analyticon Biotechnologist (Lichtenfels, Germany). Serum creatinine, albumin, SGPT and SGOT test kits (AMS[®]) from Advanced Medical Supplies UK Ltd (BT42 1 FL, UK) while Glibenclamide was from Indofarma (Jakarta, Indonesia).

Plant material extraction

One kilogram of EP bulbs were cleaned, crushed, and soaked with 4 L of water to obtain EPA. The solution was sonicated for 30 minutes followed by shaking at room temperature for 2 hours. After centrifugation at 3000 rpm, the extract was filtered with Whatman[®] No 1. The filtrate was then freeze dried overnight. The same procedures were repeated using ethanol as extraction solvent to obtain EPE.

Experimental animals

This study was conducted in accordance with the Guide for Care and Use of Laboratory Animals and had ethical clearance approval from Ethical Clearance Committee, The Ministry of Health, Republic of Indonesia (RI). Male Sprague Dawley rats age 2 months (180-200 g) were provided by Food and Drugs Control Agency, Republic of Indonesia.

The animals were kept under standard laboratory conditions (22°C, 12 h light and dark cycle) and fed with standard laboratory animal feed (AOAC, 2005). Ransoom and water were given *ad libitum*.

Diabetes induction

Sprague Dawley rats were made diabetic by intraperitoneal injection of alloxan monohydrate (110 mg/kg, dissolved in physiological NaCl solution). Diabetes status was confirmed by measuring blood glucose levels at day 4 after alloxan injection. Rats with blood glucose level above 200 mg/dL were considered to be diabetic and were used for the study.

Experimental design

Experimental rats were randomly divided into 7 groups consisted of 6 rats each: DC = untreated-diabetic group, DEA=100 mg/kg EPA treated-diabetic group, DEE = 100 mg/kg EPE treated-diabetic group, DG = 10 mg/kg glibenclamide treated-diabetic group, NDC= untreated non-diabetic group, NDEA= 500 mg/kg EPA treated-non diabetic group, NDEE = 500 mg/kg EPE treated-non diabetic group. DC and NDC groups were given 1 ml of distilled water daily. The extracts were dissolved in distilled water and were administered to experimental rats orally by gavage for 28 days. All groups were sacrificed humanely on the last day of the treatment by ketamine injection. The blood was collected and the serum was separated immediately, and then stored for further biochemical investigations.

Analytical procedures

Blood serum glucose analysis was carried out using Accucheck® glucometer (Roche, Germany). Serum insulin was measured with rat insulin Elisa Kit (Biorbyt, UK). Triacylglycerol (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL), and high density lipoprotein-cholesterol (HDL) were analyzed with Fluitest® commercial kit (Analyticon Biotechnologist, Lichtenfels, Germany). Serum albumin, creatinine, GPT and GOT were measured with AMS® commercial kit from Advanced Medical Supplies UK Ltd (BT42 1 FL, UK).

NMR measurement

The NMR measurement and data analysis were performed according to Kim *et al.* (2010). NMR spectra were recorded on a 500-MHz Bruker DMX 500 Spectrometer (Bruker, Karlsruhe, Germany). Each extract was dissolved in methanol- d_4 . All NMR experiments were performed at 25°C. Chemical shifts (δ) are given in ppm, and coupling constants (J) are

reported in Hz. The resulting spectra were manually phased and baseline corrected, and calibrated to methanol- d_4 at 3.33 ppm, using XWIN NMR (version 3.5, Bruker).

Statistical analysis

The results were expressed as a mean \pm SD. The statistical analysis was carried out using one-way ANOVA followed by DMRT posthoc test. P value < 0.05 was considered to be statistically significant.

Results

Effect of EP extracts administration on the body weight and glycemic controls of diabetic and non diabetic rats

The effect of EPA and EPE administration on the body weight of diabetic and non diabetic rats is given in Figure 1. At day 28 the body weight of diabetic untreated rats decreased significantly while the body weight of other groups increased significantly. It was shown that the body weight increment of diabetic treated groups were similar to those of NDC group. It was also shown that EPA and EPE intake did not decrease the body weight of non diabetic rats.

The effect of EP extracts administration on blood serum glucose level is presented in Figure 2. The blood serum glucose levels of diabetic rats were significantly higher than those in normal rats but at day 28 there was a significant improvement in the blood glucose levels of extracts treated-diabetic rats. Administration of EP bulb extracts was shown to did not effect the blood glucose level of non diabetic rats.

The serum insulin levels of experimental rats is presented on Figure 3. At day 28, the diabetic rats showed significantly lower serum insulin level than normal rats, but extracts treated-diabetic rats showed significantly higher serum insulin levels than diabetic control rats, although those levels were still significantly lower than those of normal rats.

Effect of EP extracts administration on lipid profile

The levels of serum TC, LDL, HDL, and TG of all groups are presented in Table 1. The levels of TG and HDL were not significantly different among all experimental groups. Serum LDL and TC levels between untreated-diabetic group and other experimental groups were found to be significantly different. Administration of EP extracts to diabetic rats was found to significantly lower serum TC and LDL levels as compared to glibenclamide treated diabetic rats. EP extracts administration to non diabetic rats gave no significantly different effect

Table 1. The levels of serum lipida profile of experimental rats

Group	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Triglycerides (mg/dl)
DC	134.65 ± 17.44 ^a	130.05 ± 18.91 ^a	85.80 ± 6.45 ^a	52.25 ± 4.11 ^a
DEA	62.55 ± 11.53 ^{cd}	32.70 ± 7.92 ^c	63.70 ± 24.08 ^a	54.75 ± 12.44 ^a
DEE	55.03 ± 15.92 ^d	41.78 ± 10.72 ^c	66.25 ± 20.66 ^a	56.15 ± 9.25 ^a
DG	90.45 ± 6.25 ^{bc}	77.33 ± 10.75 ^b	79.85 ± 12.36 ^a	63.65 ± 12.85 ^a
NDC	89.95 ± 14.96 ^{bc}	57.10 ± 8.36 ^{bc}	78.00 ± 4.87 ^a	75.25 ± 19.36 ^a
NDEA	83.90 ± 7.64 ^{bcd}	59.98 ± 19.56 ^{bc}	72.60 ± 7.66 ^a	77.33 ± 18.71 ^a
NDEE	95.80 ± 23.94 ^b	55.13 ± 17.30 ^{bc}	72.78 ± 3.80 ^a	73.18 ± 19.62 ^a

DC = diabetic control group, DEA = EPA treated-diabetic group, DEE = EPE treated-diabetic group, DG = glibenclamide treated-diabetic group, NDC = non diabetic control group, NDEA = EPA treated-non diabetic group, NDEE = EPE treated-non diabetic group. Values are expressed as mean ± SD. Means in the same column with different superscripts are significantly different ($p < 0.01$) using DMRT.

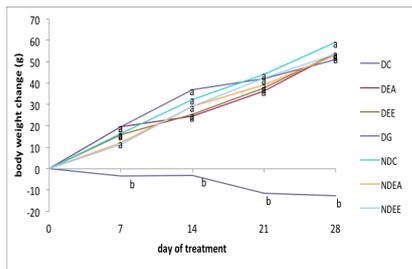


Figure 1. The body weight changes of experimental rats. DC = diabetic control group, DEA = EPA treated-diabetic group, DEE = EPE treated-diabetic group, DG = glibenclamide treated-diabetic group, NDC = non diabetic control group, NDEA = EPA treated - non diabetic group, NDEE = EPE treated-non diabetic group. Values not sharing common superscript differ significantly at $p < 0.05$ using DMRT.

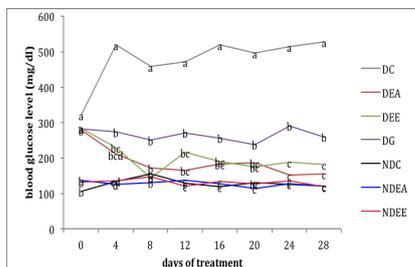


Figure 2. The blood serum glucose levels of experimental rats. DC = diabetic control group, DEA = EPA treated-diabetic group, DEE = EPE treated-diabetic group, DG = glibenclamide treated-diabetic group, NDC = non diabetic control group, NDEA = EPA treated - non diabetic group, NDEE = EPE treated-non diabetic group. Values not sharing common superscript differ significantly at $p < 0.05$ using DMRT.

of TC and LDL levels as compared to non diabetic untreated rats.

Effect of EP extracts administration on kidney and liver function marker

The levels of serum albumin, creatinine, GOT and GPT of all groups are summarized in Table 2. Serum albumin level of diabetic control rats is significantly lower than those of non diabetic groups. EPE treated diabetic rats had significantly higher level of serum albumin than untreated ones. Administration of this extract to diabetic rats resulted in significantly

Table 2. The levels of serum creatinine, albumin, GPT and GOT of experimental rats

Groups	Creatinine (mg/dl)	Albumin (g/dl)	GOT (U/l)	GPT (U/l)
DC	1.18 ± 0.12 ^a	2.71 ± 0.33 ^c	74.90 ± 7.82 ^a	31.85 ± 2.50 ^a
DEA	0.96 ± 0.07 ^{ab}	4.16 ± 0.54 ^{ab}	55.15 ± 17.35 ^a	25.15 ± 1.50 ^a
DEE	0.72 ± 0.24 ^b	4.69 ± 1.02 ^{ab}	51.98 ± 7.80 ^a	29.49 ± 4.78 ^a
DG	0.97 ± 0.07 ^{ab}	3.01 ± 0.85 ^{bc}	51.45 ± 18.57 ^a	36.00 ± 8.80 ^a
NDC	0.93 ± 0.05 ^{ab}	5.34 ± 0.19 ^a	49.80 ± 5.24 ^a	29.88 ± 1.45 ^a
NDEA	0.91 ± 0.27 ^{ab}	4.06 ± 0.99 ^{abc}	47.88 ± 18.27 ^a	26.20 ± 7.10 ^a
NDEE	0.99 ± 0.18 ^{ab}	3.80 ± 0.91 ^{abc}	50.50 ± 10.86 ^a	26.25 ± 4.35 ^a

DC = diabetic control group, DEA = EPA treated-diabetic group, DEE = EPE treated-diabetic group, DG = glibenclamide treated-diabetic group, NDC = non diabetic control group, NDEA = EPA treated-non diabetic group, NDEE = EPE treated-non diabetic group. Values are expressed as mean ± SD. Means in the same column with different superscripts are significantly different ($p < 0.01$) using DMRT.

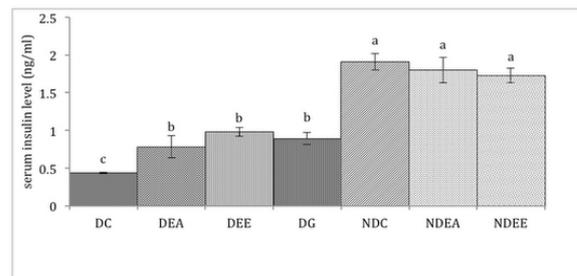


Figure 3. Serum insulin levels of experimental rats. DC = diabetic control group, DEA = EPA treated-diabetic group, DEE = EPE treated-diabetic group, DG = glibenclamide treated-diabetic group, NDC = non diabetic control group, NDEA = EPA treated - non diabetic group, NDEE = EPE treated-non diabetic group. Values not sharing common superscript differ significantly at $p < 0.01$ using DMRT.

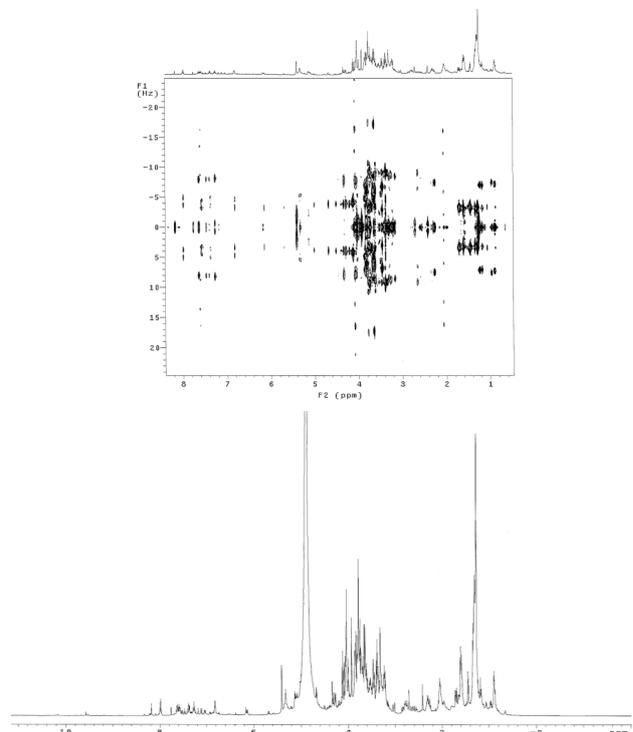


Figure Supplement 1. ¹H and J-resolved NMR spectra of EPE

lower serum creatinine level than the untreated ones. There were no significant differences on the levels of serum GPT and GOT value of EP treated group with untreated ones.

NMR measurement

¹H NMR measurement was conducted for both EPA and EPE. The ¹H and 2D NMR spectra can be found in supplementary data (Figure S1, available upon request). It was shown in the spectra that EPE has more signals in aromatic area as compared to EPA. Further EPE *J*-resolved NMR analysis and by comparing the spectra with previous data (Shibuya *et al.*, 1997; Ieyama *et al.*, 2011), the presence of eleutherinoside A, eleuthoside B, and eleutherol was indicated. Some of characteristic signals for these naphthalene derivatives are doublets between δ 6.00 – 8.00 ($J = 8$ Hz), double of doublets between δ 7.40 – 7.50 ($J = 7.6, 7.9$ Hz), and singlets between δ 7.60 to 8.00. Multiplets located between δ 3.00 – 5.00 indicated that the compounds are present as glycosides.

Discussion

According to Prabhakar and Doble (2008), the anti-diabetic activity of medicinal plants is mediated by modulation or inhibition several possible pathways, i.e.: glycolysis and Krebs cycle, gluconeogenesis, hexose monophosphate shunt, glycogen synthesis from unused glucose, glycogenolysis, digestion and absorption of carbohydrate, or acting as insulin mimetic compounds. Ieyama *et al.* (2011) reported that the aqueous-methanolic extract of *Eleutheria americana* bulb showed α -glucosidase inhibitor activity with the IC_{50} of 0.5 mM. In our preliminary study, EPA and EPE were shown to have *in-vitro* α -glucosidase inhibitor activity as well (data not shown). The inhibition of this enzymes catalytic activity lead to the retardation of glucose absorption and the decrease in postprandial blood glucose level (Dwek *et al.*, 2002). The results of this study (Figure 2 and Figure 3) revealed that EPA and EPE glycemic control mode of action is not only by α -glucosidase inhibition in gastro intestinal tracts. Increasing beta cells insulin secretion in Langerhans islet or repairing the beta cells damage is other possible mechanism. To confirm this, further observation on the histology of pancreatic beta cells is required.

An increase in oxidative stress level, alteration and disturbance in glucose and lipid metabolism are important risk factors for diabetes and cardiovascular disease (Kumar *et al.*, 2010). Insulin deficiency in diabetes mellitus leads to accumulation of lipids such as total cholesterol in diabetic patient. This is because of insulin deficiency increases free fatty acid mobilization from adipose tissue, resulting in an increase of LDL (Latha and Daisy, 2011). Therefore, one of common complication in diabetes mellitus

patient is dyslipidemia. Furthermore, high levels of total cholesterol and LDL-cholesterol in blood are the major coronary risk factors (Tchobroutsky 1978). According to Witztum and Steinberg (1991), oxidation of LDL-cholesterol has been implicated as one of the main reason of human atherosclerosis. In this study, aqueous extract and ethanolic extracts of *E. palmifolia* could improve lipid profile by reducing serum total cholesterol and LDL-cholesterol levels.

Diabetic control rats on this study had a decrease in serum albumin level. Lacking of insulin secretion or its sensitivity implies to cells glucose uptake inhibition. It leads to protein and fat catabolism as energy source alternative for the body cells (Almdal and Vilstrup, 1988). This is the reason of the body weight and albumin level decrease in diabetic rats (Latha and Daisy, 2010; Swanston-Flat *et al.*, 1990; Bakris, 1997). The decrease of albumin is also due to micro-albuminuria which is an important clinical marker of diabetic nephropathy (Mauer *et al.*, 1981). The results of the present study showed that EPA and EPE treated diabetic rats had a significant higher serum albumin level as compared to untreated diabetic ones. The role of *E. palmifolia* in preventing nephropathy diabetic complication was indicated by the higher serum albumin level and the lower serum cratinine level in treated diabetic rats. The study also showed that EP administration had no adverse effect on the rat liver. It can be seen from the serum GOT and GPT levels among the high dose-EP treated non diabetic rats and untreated non diabetic rats which were not significantly different.

The result of NMR measurement indicated the presence of eleutherinoside A, eleuthoside B, and eleutherol in EPE (NMR spectra is provided as supplemental material and available upon request). According to Ieyama *et al.* (2011), those three naphthalene derivatives had α -glucosidase inhibition activity. Thus, the active compounds responsible for anti-diabetic activity in EPE are possibly eleutherinoside A, eleuthoside B and eleutherol. In this study, both extracts showed similar anti-diabetic activity *in-vivo*. Based on efficiency and safety reasons, it is recommended to choose EPA for further application as functional food.

Conclusion

This study demonstrated that aqueous and ethanolic extracts of *E. palmifolia* were able to improve blood serum glucose and serum insulin levels in diabetic rats. The extracts were able to prevent diabetes complications through its anti-hyperlipidemic activities. It also demonstrated renal

protective effects, thus diabetic nephropathy can be prevented. This study gave scientific evidence for the traditional use of *E. palmifolia* bulb to cure diabetes among Dayak tribe, Kalimantan Island, Indonesia. Further study on histopathology of pancreatic beta cells is required to elucidate the better explanation of *E. palmifolia* extracts hipoglycemic mode of action.

Acknowledgement

This study is fully funded by Indonesian Danone Institute Foundation. The views expressed herein are those of the individual authors, and do not necessarily reflect those of Indonesian Danone Institute Foundation.

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