Effect of *Ardisia elliptica* Thunb. on Diabetes Mellitus Type 2 Rat Models

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Abstract. *Ardisia elliptica* is one of Indonesian medicinal plants known as Lampeni. Some studies proved that *Ardisia elliptica* has activities as antibacterial, antiviral, antiplatelet, antiplasmodial and antiproliferative in cancer cell line. Its leaves contain some phytochemical compounds such as α-amyrin, β-amyrin and Bergenin. The aim of this study was to investigate the effect of *Ardisia elliptica* ethanolic extract (AEE) on Spraque Dawley rats induced by high fat diet (HFD) and streptozotocin (STZ). These models represent DM type 2 conditions. Animals were divided into six groups, namely normal group, negative control group, positive control group (treat with Metformin) and three level doses of AEE (20 mg/200 gBW; 40 mg/200 gBW and 80 mg/200 gBW). All animals were fed with HFD every day. After acclimatization, animals were induced by STZ 6 mg/200 gBW intraperitoneally. Samples were given 7 days after induction until day 21. Blood glucose level were analysed at day 0, 4, 7, 10, 14 and 21 after sample treatment. Histopathological analyses were done for pancreas organ. The result showed that at day 4, AEE start on 20 mg/200 gBW could decrease blood glucose level significantly ($p < 0.05$). After 21 days treatment, AEE on 80 mg/200 gBW gave the best effect by decrease blood glucose level until 39%, while on Dose 1 (20 mg/200 gBW) could decrease 22% and on Dose 2 (40 mg/200 gBW) could decrease 28%. Histopathological analyses of pancreas organ showed that AEE could increase the number of β-cell, 48% on Dose 1, 53% on Dose 2 and 37% on Dose 3. This study concluded that *Ardisia elliptica* has potency to treat Diabetes Mellitus Type2.

Keywords: *Ardisia elliptica* · Diabetes Mellitus Type2 · High Fat Diet · Streptozotocin · Pancreas

1 Introduction

Diabetes mellitus (DM) is one of the chronic metabolic diseases with a high prevalence in the world, where the pancreas cannot produce insulin or the body cannot use the insulin produced effectively. Some of the consequences of DM that often occur is an increased

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risk of heart disease and stroke, neuropathy (damage to nerves), diabetic retinopathy, kidney failure, and a high risk of death. International Diabetes Federation (IDF) gives a projection of about 415 million adults suffer from DM and is expected to increase to 642 million people by 2040. Type 2 DM is the most common type found and the prevalence about 90–95% of the number of other DM patients [1]. DM treatment takes a long time so it is expected that DM drugs have high safety in long-term use in addition to being efficacious. Another alternative treatment is to use medicinal plants, which known safer and empirically have been widely used locally for DM. One of the medicinal plants which have the potential to treat DM is *Ardisia elliptica* Thunb. synonyms with *Ardisia humilis* Vahl. and *Ardisia Solanaceae* Roxb [2].

Ardisia is a genus in the family of Myrsinaceae. Approximately 400 to 500 species of Ardisia exists in the tropical regions of East and Southeast Asia, Americas, Australia and the Pacific Islands [3]. In this experiment, we used a traditional Indonesian herb, *Ardisia elliptica* Thunberg (local name: Lampeni), which is already cultivated in a herb farm in B2TP-Lampung, Indonesia. Empirically, the leaves are used as a medicine for diarrhea or scurvy. Many studies have already been done to investigate the pharmacological effect of this plant, such as activity as antiplatelet [4, 5], antioxidant and antidiarrheal activities [6] and also the potency as anti-Inflammatory activity [7]. Our previous research showed that Lampeni has potency as antihyperglicemia through alpha-glucosidase inhibitory activity [8].

Ardisia elliptica leaves contain phytochemical compounds such as α-amyrin, β-amyrin, bauerenol, rapanone, 5-(Z-heptadec-4'-enyl) resorcinol, and 5-pentadecylresorcinol [3]. Another research showed that α-amyrin and β-amyrin have activity as antihyperglicemia [9]. These compounds prevent steatosis and insulin resistance in high-fat diet-induced mouse model via the AMPK-mTORC1-SREBP1 signaling mechanism [10]. This experiment was conducted to investigate the effect of Indonesian *Ardisia elliptica* ethanolic extract (AEE) on Spraque Dawley rats induced by high fat diet (HFD) and streptozotocin (STZ) which represent DM type 2 models.

2 Materials and Methods

2.1 Animals

As many as 30 male *Spraque Dawley* rats, 2–3 months, 110–120 g/kgBW, were purchased from The Indonesian National Agency of Drug and Food Control (BPOM Indonesia). Animals were caged in polycarbonate individual cage, each 5 rats, in an environment control room maintained at 23 +2 °C, 60–70% relative humidity, 12:12 light/dark cycle. During experiment, beside samples, animals were fed with standard food and drink. Bedding (pet wood shaving) in every cage was changed twice a week. Experiment was approved by Ethics Committee for Health Research, Medical Faculty, University of Indonesia – Cipto Mangunkusumo Hospital Jakarta, Indonesia, with No. 17-03-0211.

2.2 Plant Extraction

Ardissia elliptica leaves were collected from a herb farm in B2TP – Lampung. Leaves were dried in oven at 45 °C until moisture <10%. As much as 2 kg of dry leaves

were ground and extracted with ethanol food grade 70%, then filtered and evaporated with a rotary evaporator (Axiovert®) and obtained 210 g *Ardisia elliptica* ethanolic extract (AEE). Extract was dissolved into CMC-Na 0.5% and divided into three doses 20 mg/200 gBW; 40 mg/200 gBW and 80 mg/200 gBW).

2.3 Experimental Design

After acclimatization for 1 week, animals were divided into six groups, each consists of 5 rats, namely normal group, negative control group, positive control group (treat with Metformin) and three level doses of AEE (20 mg/200 gBW; 40 mg/200 gBW and 80 mg/200 gBW). All animals were fed with High Fat Diet (HFD) every day, except Normal Control Group. HFD was formulated by mixing laying hen pellets, pork fat, duck egg yolk and glucose. After 9 weeks, animals (except normal group) were induced by 6 mg/200 gBW Streptozotocin (STZ) (Sigma Aldrich®, Germany) intraperitoneally. Samples were given after 7 days of induction per-oral using gastric canula for 21 days. Fasting Blood Glucose (FBG) Level were analysed at day 0, 4, 7, 10, 14 and 21 at samples treatment, GlucoDR™ Test Meter. Blood was collected from plexus retro-orbitalis using capillary pipes after fasting for 12 h (overnight). On day 21, all animals were terminated and each pancreas was isolated for histopathological analyses.

2.4 Tissue Processing and Histological Analysis

All pancreas was isolated immediately after termination and was fixed in buffered formalin for 48 h. pancreas were removed, embedded in paraffin and stained with hematoxylin-eosin (HE staining) for histological analysis. Preparate observation was done under Olympus® BX4 fluorescence microscope. The area and number of Langerhans islet, as well as the number of β-cells were measured to evaluate the effect of *AEE* on the pancreas.

2.5 Statistical Analysis

Statistical analyses were performed using Kruskall wallis or one-way analysis of variance (ANOVA), which each followed by Mann Whitney test and LSD post hoc test. The distribution of data was checked for normality by the Kolmogorov-Smirnov and Saphiro Wilk test. Numeric data for each A-series P value of <0.05 was regarded as significant. Statistical analysis was performed by the SPSS.016 software.

3 Results

3.1 Effect of AEE on FBG

During experiment, animal body weights were weighed twice a week. Figure 1 showed that all animals' weight in treated groups increased, except Normal Control Group. These may be due to the administration of HFD. After administration of HFD for 9 weeks, all animals except Normal Control Group, were induced with STZ and caused decrease of body weight start on week 10.

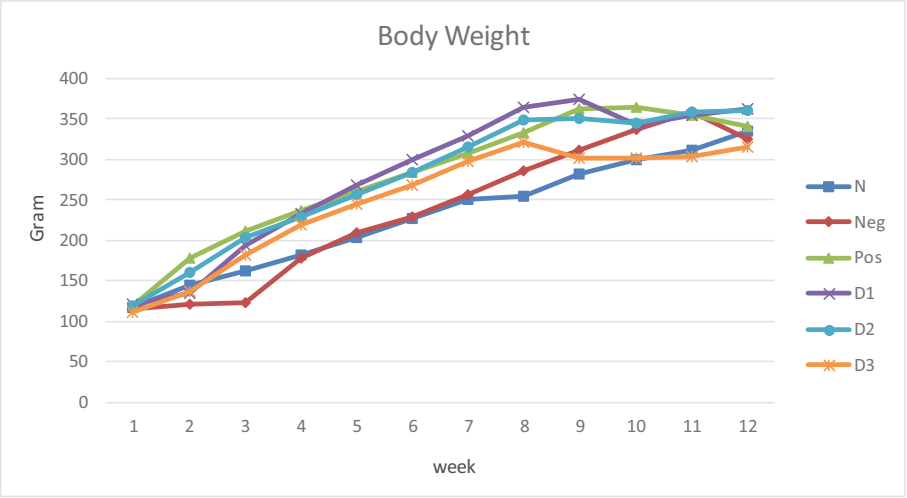


Fig. 1. Animals body weight

Table 1. Blood Glucose Level from all groups during experiment on day 0, 4, 7, 10, 14 and 21

Group	Blood Glucose Level after STZ induction (mg/dL) P < 0,05					
	Day 0	Day 4	Day 7	Day 10	Day 14	Day 21
Normal Control	100 ± 4.2^{a}	97.8 ± 18.4^{a}	99.4 ± 7.6^{a}	94 ± 7.8^{a}	86.2 ± 11.3^{a}	114 ± 17.8^{a}
Negative Control	331 ± 16.1^{b}	393.2 ± 48.9^{c}	380.2 ± 41.1^{b}	340 ± 45.6^{b}	264.8 ± 72.7^{b}	362 ± 14.5^{b}
Metformin 9 mg/200 gBW	493.8 ± 67^{c}	391.8 ± 28.4^{c}	160 ± 55.9^{d}	$248.4 \pm 126.4^{b,d}$	216.8 ± 111.2^{b}	$244 \pm 113.1^{b,d}$
AEE 20 mg/200 gBW	316.2 ± 87.2^{b}	230.0 ± 53.4^{b}	253.8 ± 110.9^{d}	$245.8 \pm 126.1^{b,d}$	254.8 ± 131^{b}	$247.8 \pm 134.3^{b,d}$
AEE 40 mg/200 gBW	307.8 ± 124.9^{b}	222.8 ± 78.5^{b}	236 ± 81.5^{d}	191.4 ± 84.5^{d}	223 ± 107.2^{b}	239.6 ± 107.4^{d}
AEE 80 mg/200 gBW	$383.6 \pm 92.6^{b,c}$	241.4 ± 112^{b}	232.6 ± 145.7^{b}	221.8 ± 84.4^{d}	257.6 ± 111.6^{b}	223.2 ± 110.9^{d}

FBG levels were analyzed before samples administration to check the diabetic models. In diabetic groups, FBG increased significantly ($P < 0.05$) than Normal Control Group. During samples administration, FBG was analyzed on day 4, 7, 10, 14 and 21. The results of analyses were shown in Table 1. On day 4, three groups of AEE treated could decrease FBG Level significantly ($P < 0,05$) than Negative Control Group. We also analyzed the percentage decrease in FBG level, showed in Table 2 and Fig. 2. Percentage was calculated by comparing with blood glucose level on day 0 in each group.

3.2 Effect of AEE on Pancreas

Histopathological analyses results are shown in Fig. 3 and Fig. 4. In Normal Control Group, Langerhans Islet defined well surrounded by the exocrine portion of the pancreas (Fig. 3.A). Pancreas of untreated groups showed damage and distortion endocrine cells (Fig. 3.B), where the islet of Langerhans shrank and cell necrosis. Meanwhile the AEE

Table 2. The percentage of decrease in blood glucose level during experiment on day 4, 7, 10, 14 and 21

Group	Percentage decrease in blood glucose level				
	Day 4	Day 7	Day 10	Day 14	Day 21
Normal Control	2%	1%	6%	15%	-16%
Negative Control	-19%	-15%	-3%	20%	-9%
Metformin 9 mg/200 gBW	21%	68%	50%	56%	51%
AEE 20 mg/200 gBW	27%	20%	22%	20%	22%
AEE 40 mg/200 gBW	28%	23%	38%	28%	22%
AEE 80 mg/200 gBW	37%	39%	42%	33%	42%

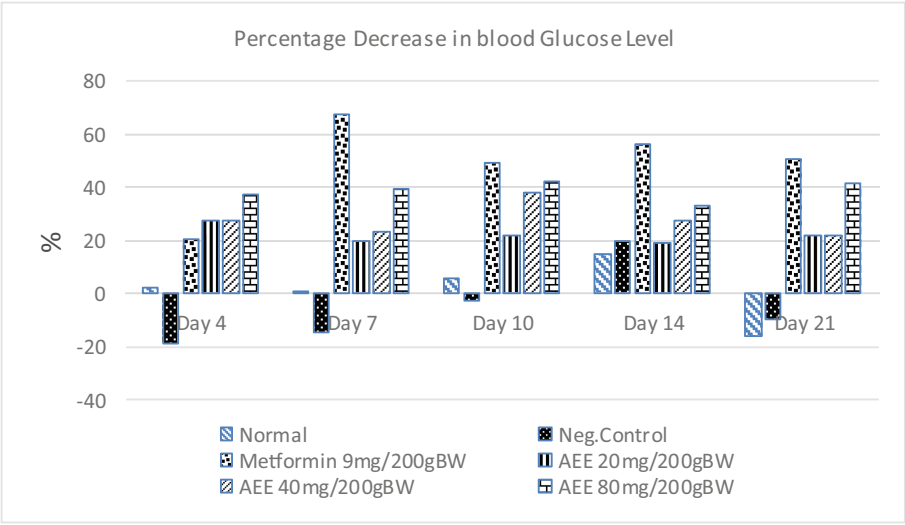


Fig. 2. The percentage decrease in FBG Level during experiment on day 4, 7, 10, 14 and 21

treated groups showed morphological repairment of the islets of Langerhans. Quantitatively, pancreatic repair can be seen from the number of β-Cells, number and area of the Islets of Langerhans in Fig. 4.

4 Discussion

Animal model of DM type 2 was made by inducing the rat with HFD and low doses of STZ. Figure 1 showed that rats body weights were increased along HFD administration till week 9. The administration of HFD will increase fat adipose tissue, especially under the skin and in cavities stomach, leading to weight gain [11]. After STZ administration at week 9, all animal's body weight in treated groups were decrease at week 10. We

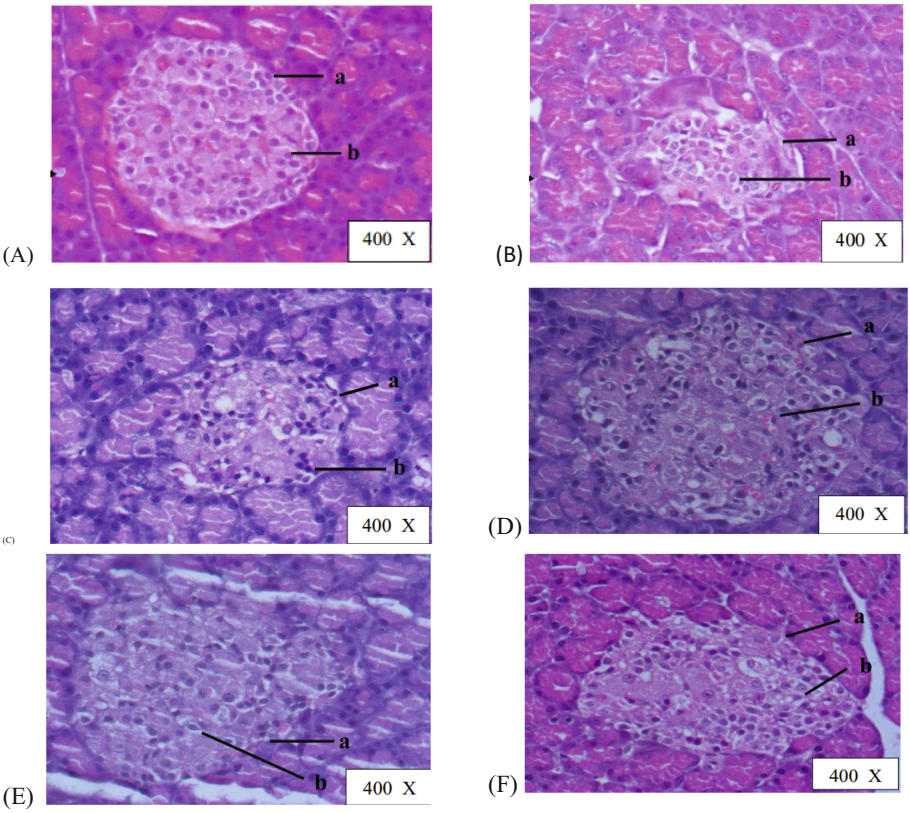
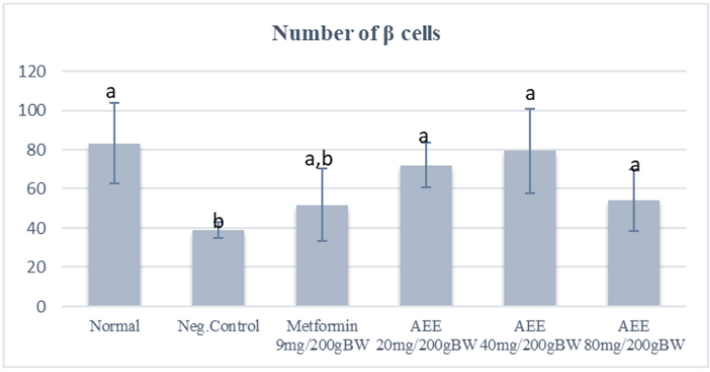


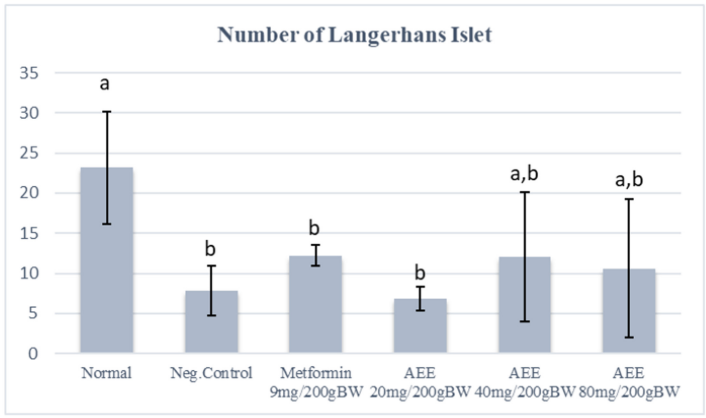
Fig. 3. Histopathological analysis of pancreas in normal and HFD-STZ induced diabetic rat. H&E staining. 400x magnification. Islet of the Langerhans (a) and β-cell (b). (A) Normal Control; (B) Negative Control; (C) Positive Control Metformin; (D) AEE 20 mg/200 gBB; (E) AEE 40 mg/200 gBB; (F) AEE 80 mg/200 gBB.

also identified polyuria and polydipsia which are another symptom of hyperglycemia in rats. So, it was predicted that rats already in DM condition. Low doses of STZ can stimulate the insulin resistance which is one of the factors of DM type 2 [11]. In Table 1 showed that on 7 days after STZ induction (or day 0 of samples treatment), all FBD were increased significantly ($P < 0,05$) than Normal Control Group. This because of STZ accumulates in β-cells via the glucose transporter 2 (GLUT 2) and change the alkyl group on guanine which causes DNA damage of β-cells and decrease insulin production [12].

FBG levels on day 4 till day 21 fluctuated in the range of 200–300 mg/dl, the glucose level value shows that test animals were still in a state of hyperglycemia though lower than FBG at day 0. According to [13], glucose levels in adult rats (2–4 months) normal are 50–135 mg/dl. The fluctuations that occur are due to the effect of stress as a result of treatment, because the treated groups were still given PTL during the sample treatment. Inability of the body to break down glucose into energy will encourage the body to break



a.



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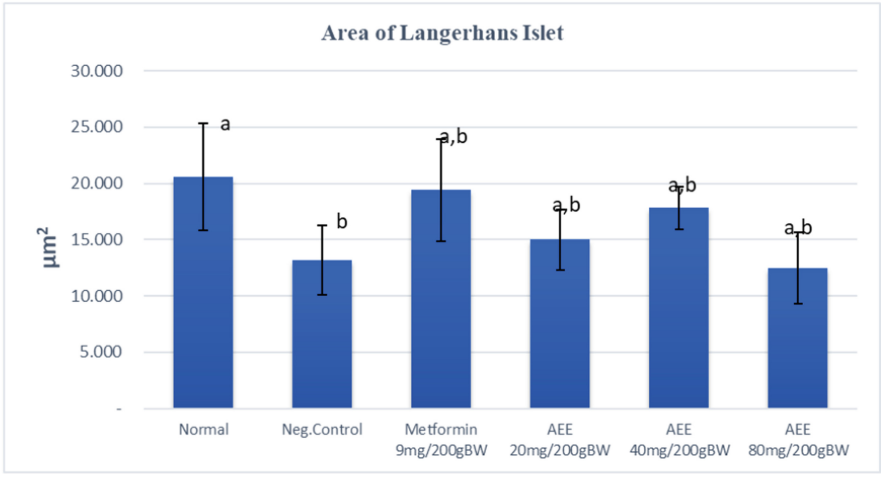

c.

Fig. 4. Histopathological analyses of pancreas. a. Mean Number of β-Cells; b. Mean Number of Langerhans Islet; c. Average Area of Langerhans Islet.

down fat and protein as glucose so that glucose levels will remain high due to the presence of formation of glucose from fat consumed through the process of gluconeogenesis.

AEE can decrease FBG significantly ($P > 0.05$) start on 4th day after treatment in treated groups than Negative Control Group. AEE starting from dose 20 mg/200 gBW can decrease FBG 27%–37% better than Metformin (21%) after administrated for 4 days, though on the next analyses till 21 days, metformin still better in decreasing FBD. The best effect of AEE is shown in day 10 and day 21, where AEE dose 80 mg/200 gBW can decrease FBD 42% significantly different ($P > 0.05$) than Negative Control. At the end of experiment, at day 21, AEE at dose 20 mg/200 gBW and dose 40 mg/200 gBW also can decrease FBG significantly ($P > 0.05$) than negative control. The antihyperglycemic effect of AEE caused by the active compounds α-amyrin and β-amyrin, the group of triterpenoids contain in leaf of *Ardisia elliptica*. Lee (2010) showed that the triterpenoid in *Ardisia elliptica* leaves have biological activity to stimulate glucose uptake by muscle cells and act as insulin (insulinotropic) [14]. Receptors Activated insulin then stimulates glucose transporter 4 (GLUT 4) to facilitate the entry of glucose molecules into body cells, especially muscles. Previous studies confirmed that α and β-amyrin has analgesic, antiinflammatory, anticonvulsant, antidepressive, gastroprotective, hepatoprotective, antipancreatitic, anticholytic, antihyperglycemic and hypolipidemic activities [15]. The other phytochemical compounds that may also affect the decrease in glucose levels is saponin, which works with several mechanisms include accelerating the release of insulin from cells, inhibits gluconeogenesis, inhibits the activity of the -glucosidase enzyme, inhibits mRNA expression of glycogen phosphorylase and glucose-6-phosphate, and increase GLUT4 expression [16].

Histopathology of pancreas in STZ induced-rats (Negative Control Group) showed damage of the islet of Langerhans and necrosis of β-cells. STZ induces DNA fragmentation in cells which will cause a decrease in nucleotides cellular and components such as NAD+ resulting in necrosis of β-cells [17, 18]. STZ also produces diabetes in rodents through severe oxidative stress by free radical formation and through inhibition of DNA synthesis. It involves partial to complete destruction of the islets and reduction of the diameter of islets, vacuolation and necrosis of the β-cells [19, 20].

Repair of cells occurred in the AEE treatment groups, identified from morphology of the islets of Langerhans and β-cells. In Fig. 4.a, it is shown that the three doses of AEE treatments give good repairment of the number of β-cells not significantly different ($p < 0.05$) with Normal Control. AEE treatments also give repairment of the number and area of Langerhans islet significantly ($P < 0.05$) close to Metformin (Positive control). AEE could increase the number of β-cell, 48% on Dose 20 mg/200 gBW, 53% on Dose 40 mg/200 gBW and 37% on Dose 80 mg/200 gBW.

5 Conclusion

Our results demonstrated that AEE at a minimum dose of 20 mg/200 gBW was able to decrease Fast Blood Glucose significantly ($p < 0.05$). Furthermore, it was also deemed to carry a tissue-repairing properties as shown by the elevated number of β-cell as well as the broadened area of the Islets of Langerhans, rendering it promising to be harnessed as an antidiabetic agent.

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